COMPARATIVE STUDY OF FYM AND AZOTOBACTER ON THE GROWTH, YIELD, QUALITATIVE TRAITS AND PHYTOCHEMICAL ASPECTS OF CRUCIFEROUS VEGETABLES AT COLD DESERT REGION AND PLAIN AREA

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY

in

Horticulture (Vegetable Science)

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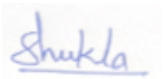
2025

DECLARATION

I, hereby declared that the presented work in the thesis entitled **"Comparative Study of FYM and *Azotobacter* on the Growth, Yield, Qualitative Traits and Phytochemical Aspects of Cruciferous Vegetables at Cold Desert Region and Plain Area"** in fulfilment of degree of **Doctor of Philosophy (Ph.D.)** is outcome of research work carried out by me under the supervision of Dr. Monisha Rawat, Assistant Professor, Department of Horticulture, School of Agriculture, Lovely Professional University, Punjab, India and Dr. Shweta Saxena, Scientist 'F', Medicinal and Aromatic Plant Division, Defence Institute of High Altitude Research, Defence Research and Development Organization (DIHAR-DRDO), Leh-Ladakh, India. In keeping with general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigator. This work has not been submitted in part or full to any other University or Institute for the award of any degree.



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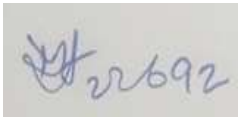

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PARENTS

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LIST OF ABBREVIATION

%	:	Percentage
&	:	And
°C	:	Degree centigrade
°E	:	Degree East
°N	:	Degree North
^{0}B	:	Brix
µg/L	:	Microgram per liter
µL	:	Micro liters
µM	:	Micro molar
µmol/L	:	Micromole per liter
ABTS	:	2,2'-azino-bis(3- ethylbenzthiazoline-6-sulphonic acid
$AlCl_3$	:	Aluminum chloride
ANOVA	:	Analysis of Variance
ACI	:	Anthocyanin content index
Ca	:	Calcium
CCI	:	Chlorophyll content index
CD	:	Curd diameter
cm	:	Centimeters
Cu	:	Copper
DAD	:	Diode-Array-Detector
DIHAR	:	Defence Institute of High Altitude Research
DPE	:	Dry powdered extract
DPPH	:	2,2-diphenyl-1-picrylhydrazyl
DRDO	:	Defence Research and Development Organization
DW	:	Dry weight
eg.	:	Example
et al.	:	Et alibi
(Fe (III) - TPTZ)	:	Ferric 2, 4, 6-tripyridyl-s-triazine
FC	:	Folin–ciocalteu reagent
Fe	:	Iron

$FeCl_3$	:	Ferric chloride
$FeSO_4$	:	Ferrous sulfate
FRAP	:	Ferric reducing antioxidant power
FW	:	Fresh weight
FYM	:	Farm yard manure
g	:	Grams
GAE	:	Gallic acid equivalent
H_2O	:	Water
HA	:	High Altitude
HCl	:	Hydrochloric acid
HC	:	Head compactness
HD	:	Head diameter
HNO_3	:	Nitric acid
HPLC	:	High Pressure Liquid Chromatography
i.e.	:	That is
IAA	:	Indole acetic acid production
K	:	Potassium
kg	:	Kilogram
L or l	:	Liter
LA	:	Low Altitude
LR	:	Leaf area
LAC	:	Leaf anthocyanin content
LCC	:	Leaf chlorophyll content
LL	:	Leaf length
LW	:	Leaf width
m	:	Meters
M	:	Molar
max	:	Maximum
mg	:	Milli gram
Mg	:	Magnesium
mL	:	Millilitre
Mn	:	Manganese
MSL	:	Mean sea level

MW	:	Molecular weight
N	:	Nitrogen
Na	:	Sodium
Nitrate	:	NO_3-
NL	:	No. of leaves
NNL	:	No. of non-wrapper leaves
O_2	:	Oxygen
P	:	Phosphorus
$p \leq 0.0001$	:	Significance at 1% level
$p \leq 0.05$	:	Significance at 5% level
PGPR	:	Plant growth promoting rhizobacteria
pH	:	Power of hydrogen
PH	:	Plant height
ppm	:	Parts per million
PSB	:	Phosphate solubilization bacteria
PSM	:	Phosphorus solubilizing microorganisms
PM	:	Poultry manure
PS	:	Plant spread
RBD	:	Randomized block design
RD	:	Root diameter
RE	:	Rutin trihydrate equivalents
RL	:	Root length
RP	:	Reversed phase
Rpm	:	Rotation per minute
RT	:	Room temperature
S	:	Sulfur
SD	:	Standard deviation
SPSS	:	Statistical Package for the Social Studies
SL	:	Stem length
TCC	:	Total carbohydrate content
TE	:	Trolox equivalent
Temp	:	Temperature
TFC	:	Total flavonoid content.

TKN	:	Total kjeldahl nitrogen
TAC	:	Total antioxidant capacity
OC	:	Organic carbon
TPC	:	Total polyphenolic content.
TPTZ	:	2,4,6-Tri(2-pyridyl)-s-triazine
TSS	:	Total soluble solid
UV	:	Ultra violet
viz.	:	Varifactors Namely
VAM	:	Vesicular-arbuscular mycorrhiza
VC	:	Vermicompost
Zn	:	Zinc

Abstract

ABSTRACT

Bio-organic farming is essential for environmental sustainability, as it promotes plant growth and quality under abiotic stress conditions. Abiotic stress under extreme environmental conditions exposes food crops to tremendous stress which hinders their normal growth and nutritional status as well. However, with a view of promoting environmental and ecological sustainability, organic farming is encouraged these days over chemical countermeasures for abiotic stressors.

The purpose of selection of these two stations (Leh and Chandigarh) for carrying out present experimental study is that most of the fresh food supply to meet food requirement of army personnel and local natives of Ladakh is met by supplies sourced from Chandigarh and nearby locations which levies huge air-transportation costs along with loss of nutritional quality of the fresh produce. This study explores the much desired simple yet implementable agri-interventions for growing ample nutritionally rich food crops under extreme environmental conditions of technologically backward areas of Leh-Ladakh.

Furthermore, it was also hypothesized that bio-organic cultivation of brassica vegetables at higher altitudes, compared to lower elevations, would result in greater production of crops and valuable bioactive phyto-compounds due to the harsh environmental circumstances at higher elevations. To validate this, the current investigation was undertaken to examine the impact of farm yard manure (FYM) and *Azotobacter* on the morphological, biochemical and phytochemical profile of *Brassica oleracea* L. vegetables such as cabbage, cauliflower, knol-khol and radish grown at two different altitudes, *i.e.* HA- high altitude [3340 meters above mean sea level (MSL) at Leh-Ladakh, India] and low altitude [321 meters above mean sea level (MSL) at Chandigarh, India]. The samples grown at HA were compared with those grown at LA in terms of growth patterns, morphological parameters, nutritional composition, phyto-chemical composition as well as anti-oxidant benefits. The effect of bio-organic treatments and altitudinal variation on all these aspects was studied to understand the role of *Azotobacter* on extreme environment in enriching phytocompounds and prospective benefits of consuming locally grown nutrition rich brassica vegetables as a functional food for people living at high altitudes.

It was noticed that T_3 treatment (FYM+*Azotobacter*) exhibited superior crop growth and yield attributes as compared to the control (without treatment). At 90 days after transplanting (DAT) *i.e.* cabbage and cauliflower or 60 days after sowing (DAS) and DAT *i.e.* radish and knol-khol, T_3 treatment demonstrated increased plant height, leaf length, leaf width, leaf area, plant spread, stem diameter, leaf chlorophyll content, and leaf anthocyanin content at HA, whereas higher number of leaves and greater plant height and leaf area (knol-khol and radish) were observed at LA. Further, T_3 treatment at HA cultivated brassica vegetables showed higher yield *i.e.* cabbage (494.75±4.97 q/ha), cauliflower (259.05±10.34 q/ha) and radish (390.64±4.65 q/ha) than LA grown cabbage (302.06±11.31 q/ha) cauliflower (209.05±0.72 q/ha) and radish (308.13±8.53 q/ha) respectively. Whereas, Knol-khol produced in LA had a significantly higher yield (137.60-fold) than that cultivated at HA.

The application of FYM+*Azotobacter* to HA soil led to notable enhancements in its chemical composition, exhibiting substantial increments in organic carbon (26.98%), nitrogen (19.12%), phosphorus (30.54%), potassium (4.52 %), sulfur (37.89 %), and manganese (46.72 %) compared to LA. In contrast, LA soil displayed higher levels of zinc (86.21%), iron (55.86 %), magnesium (51.16%), and copper (26.76 %). The nutrient profiling of bio-organic treated cruciferous vegetablesfrom high and low altitudes revealed significant variations ($p \leq 0.05$). The application of treatment T_3 resulted in higher total carbohydrate content (73.52±0.27 µg/g) in cabbage, total soluble solids (9.15±0.07 $^{\circ}$B), titratable acidity (0.37±0.02 %), total protein (19.06±0.19 g/100 g) and dietary fiber (11.06±0.09 %) in knol-khol at HA grown vegetable whereas, maximum crude fat (1.21±0.01 %) was recorded in cauliflower at LA. Nitrogen (3103.98±19.37 mg/100 g) and manganese (6.28±0.07 mg/100 g) were maximum in knol-khol, the highest ash content (15.21±0.05 %), potassium (6275.00±54.49 mg/100 g) and sodium (556.84±14.74 mg/100 g) was observed in radish at HA Whereas, more in magnesium (461.87±4.82 mg/100 g) was found in knol-khol, iron (17.61±0.45 mg/100 g), copper (2.35±0.04 mg/100 g) and zinc (6.47±0.22 mg/100 g) in radish at LA. Additionally, the maximum anions content was found at HA grown cruciferous vegetable *i.e.* nitrate (1879.45±14.01 mg/kg) and phosphate (978.09±5.65 mg/kg) content in radish and sulphate content (335.21±5.43 mg/kg) in cauliflower as compared to those grown at LA.

Further, hydro-methanolic extract of HA grown Brassicaceae vegetable (cabbage) had the highest value of total phenolic content (TPC) *i.e.* 9.56±0.15 µg GAE/mg, total flavonoid content (TFC) *i.e.*14.48±0.41 µg RE/mg, and antioxidant potential as expressed using two assays, *viz.* DPPH (85.97±0.24%) and FRAP (30.77±0.46 µg TE/mg) in comparison to LA grown samples. Signature phytocompound analysis results using RP-HPLC-DAD analysis of phytocompound *i.e.* kaempferol was found maximum at HA in cabbage (0.92±0.02 µg/mg) followed by cauliflower (0.81±0.01 µg/mg) and radish (0.73±0.01 µg/mg) as compared to LA cabbage (0.66±0.01 µg/mg), cauliflower (0.59±0.02 µg/mg) and radish (0.32±0.01 µg/mg) respectively. However, the maximum accumulation of indole-3-carbinol content was measured at HA grown cauliflower (1.31±0.01 µg/mg), radish (1.01±0.03 µg/mg), knol-khol (0.91±0.02 µg/mg) and cabbage (0.65±0.02 µg/mg) than low altitude grown cauliflower (0.40±0.01 µg/mg), radish (0.85±0.02 µg/mg), knol-khol (0.74±0.01 µg/mg) and cabbage (0.52±0.00 µg/mg). Furthermore, higher sulforaphane content was found at HA grown cabbage (8.94±0.24), radish (4.48±0.04 µg/mg), cauliflower (4.11±0.02 µg/mg) and knol-khol (3.24±0.06 µg/mg) respectively as compared to LA cultivated Brassicaceae vegetables.

Overall, this study concludes that combination of organic manure and biofertilizerhelped to achieve enhanced soil fertility, productivity, nutritional and mineral composition as well as key bioactive phytocompounds of cruciferous vegetables at harsh environment of high altitudes region. In this context, brassica vegetablescould be grown as a functional food at HA and in remote areas where seasonal fluctuation and technological backwardness limit the availability of fresh vegetables. Therefore, we suggest growing cruciferous vegetables with increased nutritional value for local consumption at high altitudes using organic manure in conjunction with biofertilizers.

Chapter -1

CHAPTER-1
INTRODUCTION

The Himalayas, called the 'king of mountains,' are ancient mountain range located in India. The 'Trans Himalayan' or 'Tibet Himalayan region' situated in the north of the Great Himalayas and includes the mountain ranges, namely Karakoram, Ladakh, Zanskar and Kailash. Among them, Ladakh Mountain range is considered as the most significant mountain range (Dame and Nussar, 2011). Its unique environment is marked by extreme temperatures (-25°C to 22°C), low precipitation (< 100 mm) (mostly snow), high winds, sparse vegetation, thin air with intense UV radiation, and a fragile ecosystem due to which the region has limited resources (Stobdan *et al.,* 2018). Additionally, in Ladakh unfavourable climate causes a drop in agricultural activity to just three to four months annually. Because of which the availability of fresh and nutrition rich food at high altitude region (3500 m mean sea level) is limited (Dame *et al*., 2011). Ladakh is the only union territory of India which has three international boundaries with three different countries i.e. Pakistan China, and Afghanistan. Hence, a large number of military troops are being stationed in the Ladakh region, due to the geo strategic nature of the location. The region remains cut-off (from low altitude region) for more than six months in a year posing daunting challenges to local population and military personnel in meeting the nutritional requirements by getting fresh vegetables and food for maintaining their highest level of mental and physical fitness so that they can survive under adverse climatic conditions (Kumar *et al*., 2022b). Till now, the essential nutritional support was being provided from locally available resources, as timely supply of fresh vegetables from low altitude regions (320 m mean sea level) is not always possible due to logistics constrains. Moreover, the crops and vegetables grown at low altitude regions are chemically treated by using chemical fertilizers, pesticides, insecticides, herbicides, *etc.* in order to increase the productivity of the crop. The extensive application of hazardous pesticides and artificial fertilizers on food crops, also impacts the environment which includes the loss of topsoil, decreases the fertility of soil, leads to surface and ground water contamination and damage the genetic diversity (Bhandari, 2014; Horrigan *et al*., 2002). Thus, by considering the above factors, we needed to focus on enhancing the nutritive and phytochemical value of food crops grown at high

altitude region of Ladakh as well as low altitude regions, which could only be accomplished through organic farming to promote self-sufficiency and food security.

Organic farming is an agricultural method that prioritizes nature supported practices; by decreasing the use of synthetic compounds such as growth regulators, pesticides, fertilizers, genetically modified organisms (Reddy *et al.,* 2004; Swapna *et al*., 2016). As an alternative, it also emphasizes on strategies like crop rotation, the use of crop remains, green manures, animal manures, legumes, organic waste, and bio fertilizers (Nurhidayati *et al*., 2016**)**. These approaches are employed to preserve and improve soil quality, provide essential nutrients to plants, and manage insect infestations, weeds, and other pests while maintaining soil productivity and structure (Altieri *et al*., 2012; Muneret *et al*., 2018). In organic farming, it is important to constantly working on improving the health of soil that has rich organic matter and has all the nutrients that the plants need (Das *et al*., 2016). Numerous methods for soil quality improving (Improvement of soil fertility), such as addition of manures and bio fertilizers, green manuring, etc. can be used. Depending on the availability and suitability of crop, a variety of organic and bio fertilizers have been employed in organic farming (Indoria *et al.,* 2018). Organic manure encompasses various organic materials used to enrich soil. It includes animal-based materials like Farmyard manure (FYM) as well as plant-based materials like compost, leaf litter, crop residues, and green manures (Shakywal *et al*., 2023). FYM provides nutrients to plant, promotes soil aeration and organic matter, resulting in an increase in the number of soil microbes and accumulation of extra humus content (Dinesh *et al*., 2003; Sindhu *et al*., 2020). It also improves the chemical, biological, and physical properties of the soil (Singh *et al*., 2020).

Further, biofertilizer such as "*Azotobact*er" is a genus of free-living nitrogen-fixing bacteria which is commonly found in soil. These bacteria's are known for their capacity to convert environmental nitrogen gas (N_2) into ammonia (NH_3) through a biological process called nitrogen fixation (Bag *et al*., 2017; Macik *et al*., 2020). This ammonia can then be utilized by plants as a source of nitrogen for their growth and development. They colonise in the rhizosphere or interior of the plants, plantlet or seed surfaces, or soil, and encourage growth (Ahmed *et al*., 2017; Kumar *et al*., 2022a). *Azotobacter* sp. not only improves the quantity of nitrogen available to plants, but it also synthesizes chemicals that promote plant growth and development

(Gothandapani *et al*., 2017). Auxin, cytokinin, ethylene, and abscisic acid are some of the phytohormones secreted by biofertilizers (Egamberdieva *et al*., 2017). These phytohormones have a noticeable effect on plant metabolic activity and also indirectly contribute to the stimulation of defence as well as abiotic stress management (Ei-Lattief, 2016). In comparison to synthetic chemicals, biofertilizers are less expensive, more eco-friendly, and sustainable source of plant nutrients, hence they have developed worldwide attention and relevance in crop production (Swapna *et al*., 2016). Tejada *et al*., (2016) also reported that biological agents also improve the physical, chemical, and biological qualities of soil and ensure nutrient availability to plants. According to a study conducted by Schutz *et al*. (2018), biofertilizer inoculation boosted crop output by 16% on average when compared to controls (without-inoculation). Microbial biofertilizers acts an important role in sustaining soil fertility and enhancing soil structure by affecting soil particle aggregation (Rashid *et al*., 2016). They also improve crop-water relations (Xiang *et al*., 2012), increase drought tolerance, reduce plant susceptibility to some soil-borne diseases, particularly those caused by fungi that create mycotoxins (Simarmata *et al*., 2016), and reduce insect pest incidence (Macik *et al*., 2020).

Many significant vegetables belong to *Solanaceae, Brassicaceae, Cucurbitaceae, Alliaceae,* etc. family are grown in the trans-Himalayan region of Ladakh. Among them, vegetables belong to Brassicaceae family, popularly known as "cole crops" or the "mustard family" are in high demand at high altitude regions which is driven by their suitability for the challenging growing conditions, nutritional value, cultural significance, and adaptability to local preferences and dietary habits. Cabbage, cauliflower, knol-khol and radish are some of the members belonging to this family which are well known for their nutritional richness and bioactive compounds (Favela-Gonzalez *et al*., 2020). Cabbage (*B. oleracea* var. *capitata*) was introduced to cabbage multiple region of the world by Europeans (Dixon, 2007). Due to its head, cabbage has the variety name capitata. Additionally, to the fresh market, cabbage variants that are available include white headed, red headed, and savoy. Cabbage is now processed into cole slaws, kraut, and egg rolls. There may be additional specialist markets for these different varieties of *B. oleracea* var. *capitata* (Manchali *et al*., 2012). Each variety has its own unique characters, including variations in leaf color, texture, and flavor. There are many different culinary uses for *B. oleracea* var.

capitata. It can be eaten cooked in recipes like soups, stews, stir-fries, and sautés or eaten raw in salads, coleslaws, and sandwiches. It is common to prepare cabbage using the fermentation process, which produces foods like sauerkraut and kimchi, which have probiotic advantages (Valavanidis *et al*., 2004). Based on the conditions and time of the growing season, cabbage needs between 380 and 500 mm of even moisture to create healthy heads (Gelaye and Tadele, 2022).

Cauliflower (*Brassica oleracea* var. *botrytis* L.) is consumed for its white curd, but the stalk and surrounding thick, green leaves are discarded (Kowsalya and Sangeetha, 1999). It is believed that cauliflower originated on the Mediterranean's eastern coast. During the Roman period, genetic interchange occurred as a result of trade between numerous Mediterranean countries, and the variation of these cruciferous crops to different environments resulted in different types of *Brassica oleracea* var. *botrytis* (Branca, 2008). In the 15th century, a cauliflower plant with enormous sensitive flowering heads was produced. Botrytis refers to the cauliflower head's resemblance to a grape bunch. Cauliflower's mild flavor and distinct texture make it a popular cooking component. It can be eaten raw as a crunchy snack, mixed into salads, or cooked into a variety of recipes (Verkerk *et al*., 2009).

Knol-Khol (*Brassica oleracea* var. *gongylodes* L.), commonly referred as kohlrabi, is a vegetable grown for its delicious swelling stem that looks like a turnip or a cabbage (Manchali *et al.* 2012). Knol-Khol is grown mostly for its spherical, inflated stem, but its leaves are also delicious and can be used in a variety of culinary applications (Zhang *et al*., 2015). The term "Knol-Khol" is a combination of the German words "kohl" for cabbage and "rabi" meaning turnip, and it reflects the appearance and flavor of the vegetable. It is thought to have originated on Europe's northern shore, although it is now grown and consumed in many regions of the world (Bhandari *et al*., 2021; Mahdi *et al*., 2020). Its texture is crisp and crunchy; resembling that of a radish or a broccoli stem, and its flavor is distinctive, mild, sweet, and somewhat spicy. The knob stem is available in a variety of hues, such as light green, purple, and white (Fahey, 2015).

Radish (*Raphanus sativus*) is a root vegetable native to China that has been recorded in Egyptian, Roman, and Greek historical sources (Manchali *et al.,* 2012). Although it is uncommon in some societies, it is nevertheless regarded as a necessary component

of the human diet. *Raphanus sativus* are typically consumed as raw, a crunchy vegetable, mostly in salads, however they are also a common ingredient in many European recipes (Banihani, 2017). Some person, particularly in the Middle East, likes and prefers to drink its juice for health reasons. Radishes have different peel colors (which varies as red, purple, black, yellow, and white to pink), but their flesh is normally white. Moreover, the edible radish root also differs globally in terms of flavor, length, and size (Banihani, 2017; Oboh, 2005).

Furthermore, these cruciferous vegetables contains main nutritional components such as protein, carbohydrates, vitamin C, folic acid (Vitamin-B9), Vitamin E, and provitamin-A, calcium, phosphorous, magnesium, sodium, and potassium (as major macro elements), as well as iron, copper, manganese, zinc, and other micronutrients (Singh *et al*., 2001; Samec *et al*., 2011). The protein content of cruciferous vegetables varies between 1.0% to 3.3% (w/w) on fresh weight. It is a diet healthy for heart, due to its low fat content (less than 1.0%). They are also high in carbs, with carbohydrate content, ranging from 0.3% to 10% (w/w) on fresh weight basis (Manchali *et al*., 2012). The amount of free sugars in a plant has a significant impact on its flavor. The most common soluble sugars are fructose, glucose, and sucrose, with fructose accounting for the greatest percentage (Jahangir *et al*., 2009). Additionally, according to Singh et al. (2007), they have significant vitamin C content, with levels ranging from 15 to 50 mg/100g of fresh weight. According to Mangels *et al*., (1993), the maximum average betacarotene content of major cruciferous vegetables is in between 0.5-1.0 mg/100 g fresh weights. The contents of A-tocopherols in cruciferous vegetables ranged from 0.32 to 0.47 mg/100g fresh weight (Granado *et al.,* 2006). Additionally, cruciferous vegetables contain various phytochemicals as well, these phytochemicals are known as bioactive secondary metabolites. Polyphenols, phenolic acids, flavonoids, carotenoids (zeaxanthin, lutein, β-carotene), alkaloids, tannins, saponins, anthocyanins, phytosterols, chlorophyll, glucosinolates, phytosteroids, terpenoids, glycosides, and aromatic amines are among the bioactive phytochemical compounds that are typically present in the majority of cruciferous vegetables (Drozdowska *et al.*, 2020; Boivin *et al*., 2009; Zhang and Hamauzu, 2004). Due to the presence of these compounds, these plants exhibit biological effects against a wide range of diseases, including antimicrobial, antibacterial, antidiabetic, antimalarial, antiaging, antiulcer, anti-hyperglycemic, anti-hyperlipidemic, anti-proliferative,

neuroprotective, anti-genotoxic, and antioxidant activities (Moreno *et al.*, 2006; Nawaz *et al.*, 2018).

Thus, because of nutritional richness and presence of variety of phytochemicals in cruciferous vegetables, we have chosen cabbage, cauliflower, knol-khol, and radish for our present study. These are the most widely consumed and cultivated vegetables at high altitude region of Ladakh as well as at low altitude regions which can be consumed in its raw form or in its cooked form. The trans-Himalayan region of Ladakh remains cut-off for 4-5 months due to heavy snowfall, during this period there is no cultivation and harvesting of vegetables. Additionally, due to logistical difficulties, vegetables transported during this time period from low-altitude regions loses (drops) some of their nutritious contents. Therefore, both the local population and the soldiers stationed there are unable to access nutrient-rich food to easily thrive in the severe climate. Cabbage, cauliflower, knol-khol, and radish play a vital role in ensuring food security and dietary diversity in such regions. Therefore, it was intended that the local population and the soldiers stationed there would always have easy access to nutrient-rich food that would suit their dietary needs. In order to do this, the current investigation was planned, which compares the impact of organic manure (FYM) and biofertilizer (*Azotobacter*) on the morphology, biochemical, and phytochemical parameters of cruciferous vegetables grown at high-altitude region of Ladakh and low-altitude region of Chandigarh. To carry out the current study following objectives were formulated:

1. Comparative effect of FYM and *Azotobacter* on growth and yield parameters of cruciferous vegetables in high altitude (Leh) and in plain area (Chandigarh).

2. Comparative study of FYM and *Azotobacter* on nutritional parameters of edible portion of cruciferous vegetables in high altitude (Leh) and in plain area (Chandigarh).

3. Comparative study of FYM and *Azotobacter* on bioactive phytochemical marker based analysis of cruciferous vegetables in high altitude and in plain area.

Chapter -2

CHAPTER- 2
REVIEW OF LITERATURE

"Altitude" refers to a location elevated with respect to sea level, and it is universally categorized into three zones. The first is high altitude, spanning from 1,500 to 3,500 meters (4,900–11,500 feet), followed by very high altitude, ranging from 3,500 to 5,500 meters (11,500–18,000 feet), and finally, extreme altitude, exceeding 5,500 meters (18,000 feet) (Paralikar and Paralikar, 2010). Among the Himalayan high-altitude regions, Ladakh encompasses elevations from 1,500 to 5,500 meters, housing a diverse population, including armed forces stationed to safeguard international borders. However, the challenging climatic conditions in Ladakh lead to a substantial unmet demand for fresh vegetables. The harsh arid climate limits the cropping season to 4-5 months annually, resulting in restricted access to fresh and nutrient-rich food throughout the year. Historically, the need for fresh produce in Ladakh was fulfilled by transporting vegetables from lower altitudes, but logistical constraints and economic challenges often render this approach impractical. Importantly, vegetables transported from distant low-altitude locations may have reduced nutritional content due to prolonged transportation routes.

Current research predominantly focuses on advancement of agricultural practices, particularly through greenhouse technology to boost food crop production at high altitude (Stobdan *et al*., 2018). Despite its potential benefits, this method proves economically unviable for local farmers. Consequently, our present study emphasizes upon the significance of bio-organic fertilizer (FYM and Azotobacter) in influencing the growth, yield, nutritional, and phytochemical parameters of cruciferous vegetables cultivated in the high-altitude region of Ladakh. While the use of bio-organic fertilizers is well-documented in low-altitude areas, there is a scarcity of reports for high-altitude regions like Ladakh. Our research represents the first investigation into evaluating and comparing the influence of bio-organic fertilizers on cruciferous vegetables cultivated in the high-altitude regions of Ladakh and the low-altitude region of Chandigarh.

Cruciferous vegetables were selected for this study due to their global popularity, driven by nutritional value and antioxidant characteristics. These attributes are

particularly relevant for residents of high-altitude areas, aiding in coping with oxidative stress and high-altitude sickness. In this chapter, we endeavour to review all available literature on cruciferous vegetables at high and low altitudes. However, due to lack of sufficient published data, our investigation also encompasses research on other relevant crops, as outlined in the subsequent section.

2.1 Cruciferous family vegetables

Cruciferous ranks second in terms of vegetable production and consumption around the world, after Solanaceaeplant family, e.g., potatoes and tomatoes (Bennett *et al*., 2007). Cruciferae, also known as Brassicaceae, is a huge family of flowering plants that consists of more than 300 genera and almost 3,500 species; however, only a small percentage of these species are edible (Garg and Sharma, 2014). The term "cruciferae" derives from the cross-shaped petals that distinguish the plants of this family. Notable examples suitable for human consumption include rocket, mustard, kale, Brussels sprouts, broccoli, cauliflower, knol-khol, radish, and cabbage. Cruciferous plants may complete their biological cycle in one, two, or more years. They are mostly farmed in the Mediterranean area, as well as in a number of regions in North America, Europe, Southwest Asia, and Central Asia (Velasco *et al.* 2010).

Cruciferous vegetables boast essential nutritional components such as proteins, carbohydrates, vitamins (C, B-9, E, and provitamin-A), major macro elements (calcium, phosphorous, magnesium, sodium, and potassium), and micronutrients (Fe, Se, Cu, Mn, & Zn) (Singh *et al*. 2001; Samec *et al*. 2011). The flavor of Brassicaceae plants is significantly influenced by the quantity of free sugars, with fructose, glucose, and sucrose being the predominant soluble sugars, with fructose contributing the largest percentage (Jahangir *et al*., 2009).The Brassicaceae vegetables are abundant in phytochemicals that are known as bioactive secondary metabolites. These substances can operate on a wide range of molecular targets inside of cells and are produced naturally in these plants. Furthermore, Glucosinolates (GLSs) and their breakdown products, such as isothiocyanates and indoles, are prevalent in Brassicaceae plants, along with phenolic chemicals (Abbaoui *et al.,* 2018; Shankar *et al.,* 2019). Over 120 GSLs and isothiocyanates precursors were recently identified in plant, categorized as aliphatic, aromatic, and indolicglucosinolates based on their side chain structures (Hirai *et al*., 2007, Agerbirk and Olsen 2012). Sulforaphane, Indole-3-carbinol, and

kaempferol stand out as the most common glucosinolates in brassica species (Liang *et al*. 2006; Ahmed *et al*. 2014; Li *et al*. 2017). The presence of these compounds imparts cruciferous plants with various biological effects, encompassing antioxidant, neuroprotective, antimicrobial, antibacterial, antidiabetic, antimalarial, antiaging, and anti-proliferative properties against a broad spectrum of diseases (Moreno *et al.* 2006; Nawaz *et al.*, 2018).

2.2 Impact of Extreme Climatic Conditions on the Nutritional Quality of Cruciferous Vegetables

Extreme climatic conditions present a formidable challenge to agricultural practices while at the same time impacting various aspects of plant growth and development. The nutritional composition of cruciferous vegetables, comprising essential vitamins, minerals, and bioactive compounds crucial for human health, is susceptible to alteration under the influence of extreme climatic conditions marked by temperature fluctuations, erratic precipitation, and other environmental stressors. Recognizing these effects is crucial for addressing potential shifts in nutritional quality and ensuring food security in regions prone to extreme climate events.

From a wider perspective, global warming affects plant physiological responses, developmental processes, and molecular function (Myers *et al*. 2014). Furthermore, the influence on plants is closely tied to the genotype and the interplay of each nutrient synthesis pathway with environmental factors (Soares *et al*. 2019). For instance, the review by Soares *et al*. (2019) highlights that legume plants experience oxidative damage, impacting macronutrients, under conditions of drought and high temperatures. The level of risk that climate change poses to nutrition is closely linked to atmospheric CO_2 levels, as depicted in Figure 2.1. Elevated CO_2 levels, although contributing to increased yields, disturb the balance of the mineral content, nutrient-use efficiency, and carbon metabolism of plants (Nakandalange and Seneweera, 2018). The levels of several micronutrients in crops, such as P, K, Ca, S, Mg, Fe, Zn, Cu, and Mn, could potentially decrease by 6-10% under atmospheric CO_2 concentrations of 691 ppm, aligning with a 3.5°C warming scenario. This anticipated reduction, especially in zinc content, is forecasted to lead to an additional 160-210 million people facing Zn deficiency, further worsening existing deficiencies in over one billion individuals (Myers *et al.* 2017).

Moreover, there has been comparatively scant exploration of potential climate effects on malnutrition, particularly through mechanisms that could alter the nutrient composition of foods. Both major and minor minerals are fundamental elements of a balanced diet, essential for proper development, overall well-being, and disease prevention. Children in the age group of 6 months to 5 years, specifically, experience micronutrient deficiencies (WHO 2013), with Iron, iodine, and vitamin A are the most commonly occurring deficiency, posing significant public health concerns (Mirzabaev *et al.* 2023). In pregnant women, insufficient iron levels elevate the risks of maternal and child mortality, as well as low birth weight. Neurological development in children is adversely affected by iodine deficiency, and retinol deficiency heightens the likelihood of childhood blindness and mortality due to infectious diseases (WHO 2013). Thus, it is anticipated that agricultural crop production will play a major role in the ways that climate change affects human health.

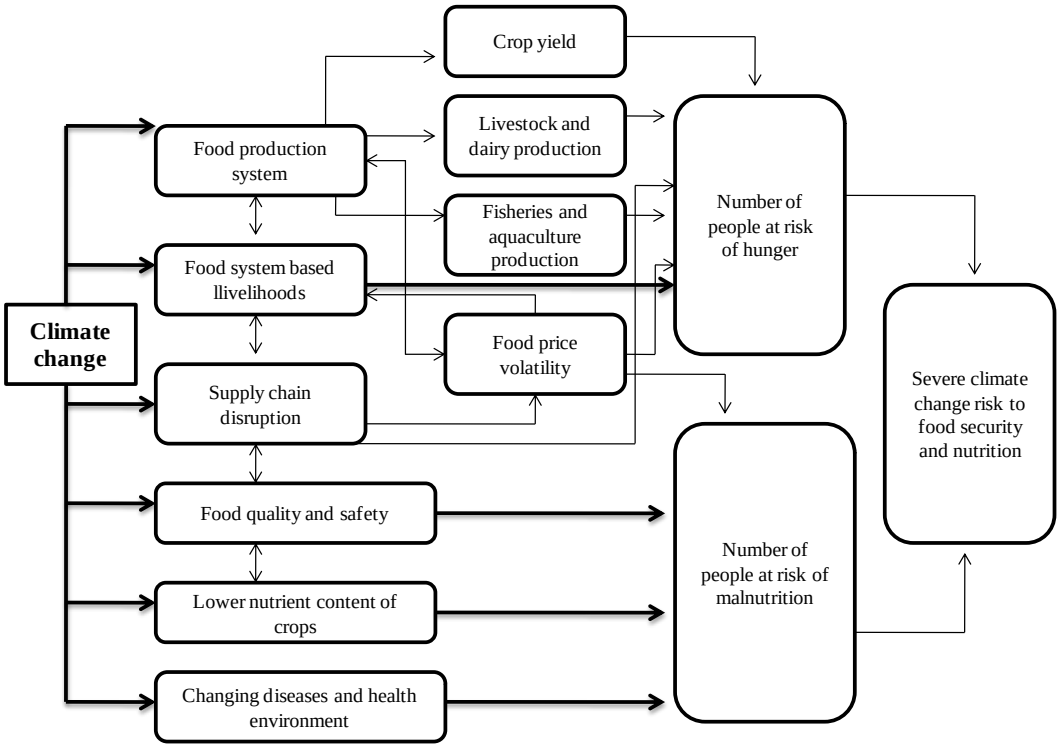

Figure 2.1: Climate change's effects on Malnutrition (Mirzabaev *et al*. 2023)

2.3 Organic farming

Organic farming is a system primarily characterized by the avoidance of artificial inputs, including fertilizers, pesticides, hormones, and feed additives, as defined by food safety regulatory bodies such as the United States Department of Agriculture

(USDA) (Gamage *et al*., 2023). Similarly, Organic farming' is defined by the International Federation of Organic Agriculture Movements (IFOAM) as an agricultural method that relies on naturally derived bio-fertilizers and pesticides, mostly derived from plant and animal waste and organic manure (Das *et al*., 2020).

Furthermore, organic farming enhances resilience within agro-ecosystems, helping them withstand adverse effects of climate change such as temperature fluctuations and drought, while also preventing soil erosion. The strategy advocates for sustainable and eco-friendly management, conservation practices, and restoration activities. The cost-effectiveness of organic farming makes it an attractive and viable option for cultivating crops in alignment with ecological principles, contributing to a more sustainable and resilient agricultural future. The following figure highlights the significance of organic farming, depicted in Figure 2.2.

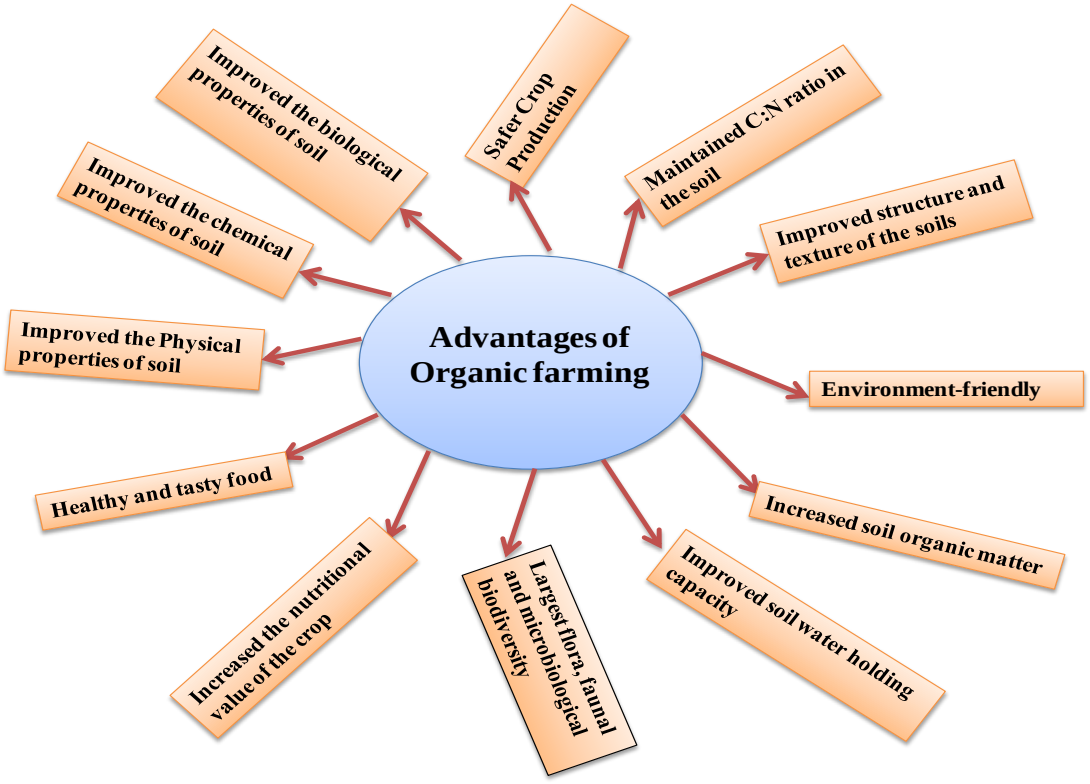

Figure 2.2: Advantages of organic farming

2.3.1 Methods of organic farming

Various forms of organic farming can be adopted by steering clear of chemical-based pesticides and fertilizers. The techniques utilized in organic farming seamlessly integrate current scientific knowledge and modern technology with longstanding traditional farming practices rooted in natural bioprocesses. The complexities of organic farming methods are the subject of several studies, with a focus on elements like crop rotation and intercropping, organic soil fertility, biological fertilizers, bio-pesticides, vermicomposting, integrated pest management, and waste management (Bhujel and Joshi, 2023). Figure 2.3 presents a visual depiction through a pictorial diagram, illustrating the essential methods employed in organic farming.

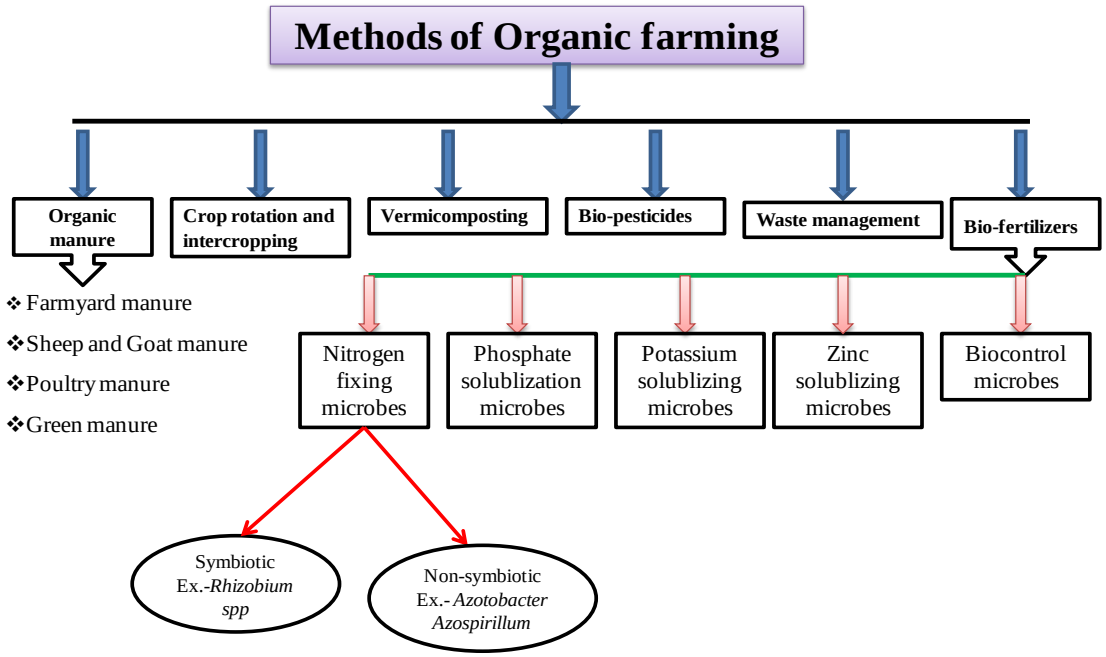

Figure 2.3: Methods of organic farming

2.3.2 Farmyard manure (FYM)

Farmyard manure (FYM) is a blended composition incorporating dung, urine, bedding, and straw. Dung is predominantly comprised of undigested material, while urine emanates from digested material. Over 50% of the organic matter in dung comprises complex products like lignin and protein, which resist rapid decomposition, resulting in a gradual release of nutrients. As indicated by Dey *et al.*, (2021), farmyard manure (FYM) typically encompasses approximately 5-6 kg of N, 1.2-2.0 kg P, and

5-6 kg K per ton. FYM plays a pivotal role in supplying essential plant nutrients, promoting soil aeration, and augmenting organic matter. As a result, this fosters a rise in soil microbes and the buildup of extra humus content (Dinesh *et al*., 2003; Sindhu *et al*. 2020). Moreover, farmyard manure (FYM) contributes to enhancing the physio-chemical and biological attributes of the soil (Singh *et al*. 2020).

2.3.3 Bio-fertilizers

Bio-fertilizers refer to biologically active inoculants or products, such as microbial inoculants containing beneficial bacterial or fungal strains. These inoculants perform an essential function in increasing plant morphology traits and soil structure by converting nutritionally important elements, such as phosphorus and nitrogen, from an unavailable to an available form. Upon application to the plant's surface, soil, rhizosphere, or plant interior, these strains colonize and facilitate this transformation (Zaidi *et al*., 2015). For example, certain bacteria transform atmospheric free nitrogen which is inaccessible for plants into usable forms of nitrogen like ammonia. Consequently, nitrogen becomes accessible for plant growth. The following are some of the reasons why biofertilizers are so important:

- The structure of the soil and the crop yield may both be improved using biofertilizers.
- Environmentally friendly and economical
- Prevent the growth and spread of pathogens.
- Eliminate a wide variety of hazardous compounds that may be present in the soil and are responsible for plant related diseases.
- Biofertilizers have been shown to be effective even in semi-arid environments.

2.3.4 Importance and mode of action of biofertilizers

In sustainable farming practices, biofertilizers serve as substitutes for chemical fertilizers, contributing to the improvement of plant growth, yield, and quality (Ajmal *et al.,* 2018). In comparison to synthetic chemicals, biofertilizers are a less expensive,

more ecologically friendly, and sustainable method for obtaining nutrient-rich plants, hence they have gathered worldwide attention and relevance in crop production (Swapna *et al*. 2016). Schutz *et al*. (2018), the inoculation of biofertilizers resulted in an average 16% increase in crop output compared to without-inoculated controls. Biological biofertilizers are essential to maintaining soil fertility and enhancing soil structure by influencing soil particle aggregation, as noted in a study by Rashid *et al*. (2016). These biofertilizers help to strengthen the relationship between crops and water (Xiang *et al*. 2012), give plants resistance to drought, decrease plant susceptibility to some soil-borne diseases, such as those brought on by fungi that produce mycotoxins (Simarmata *et al*. 2016), and reduce the amount of insect pests (Mącik *et al*. 2020). Tejada and colleagues (2016) further reported that biological agents have enhanced the physical, chemical, and biological qualities of soil, ensuring nutrient availability to plants. Therefore, much research has been devoted to find out the alternative source to chemical fertilizers. Bio-fertilizers are eco-friendly, non-toxic, low cost and live bacterial formulations that enhance soil fertility by solubilizing phosphorus, fixation of atmospheric nitrogen and other minerals, and promoting plant-growth *via* the synthesis of growth hormones. Importance and mode of action of biofertilizer was presented in Figure 2.4.

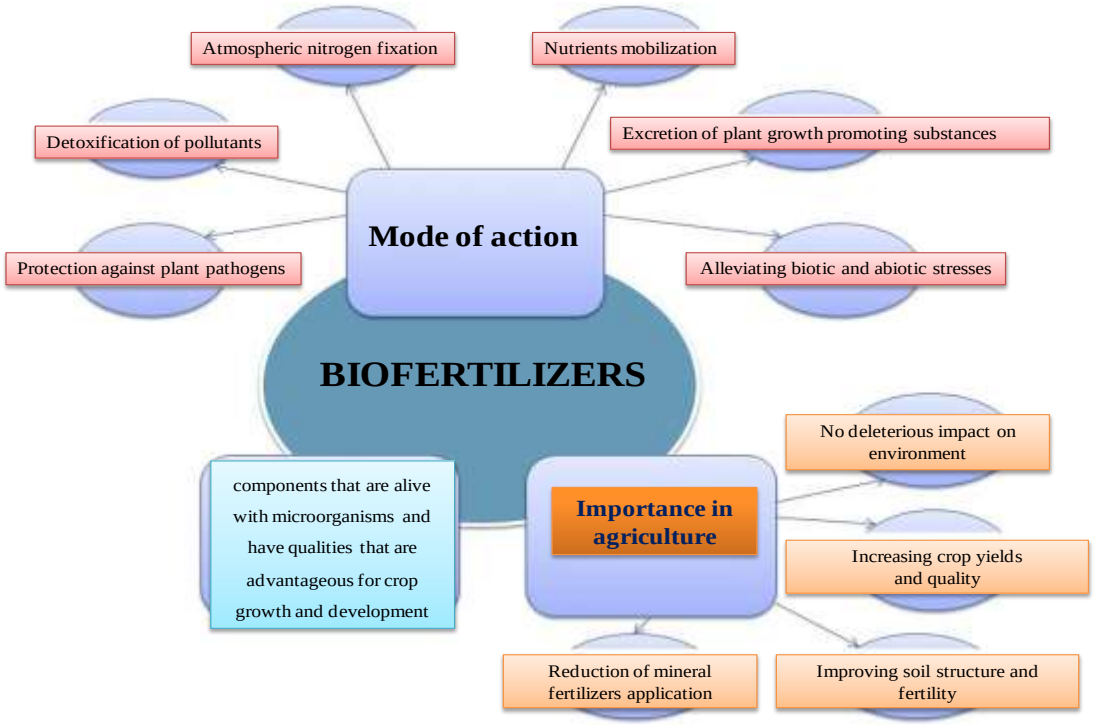

Figure 2.4: Importance and mode of action of biofertilizers (adopted from Macik *et al*. 2020)

2.3.5 Functional potential of microbes as biofertilizers

Across Asia, where conventional pesticides and fertilizers are extensively applied to boost crop yields, the substantial growth in their use over the past five decades has raised concerns (Park *et al*., 2005; Jin, 2012). The long-term application of these chemical inputs has been shown to have detrimental effects on soil health, human and animal populations, despite their short-term effectiveness in increasing agricultural productivity. Consequently, contemporary agricultural advancements are focused on alternative biotechnological approaches that can support plant growth and agricultural production while preserving soil fertility (Zhang*et al*., 2011). Therefore, the cultivation of pollutant-free crops is being actively promoted worldwide through the adoption of organic farming practices. This approach involves the utilization of bio pesticides and biofertilizers, employing green technologies to enhance crop nutritional content while effectively preventing various pests and diseases. Biofertilizers, consisting of beneficial microorganisms, play a pivotal role in enriching soil nutritional quality. Examples include bacteria, fungi, and algae, collectively contributing to the improvement of soil fertility. Among these, the subgroup known as "plant growth-promoting rhizobacteria" (PGPR), identified by Kloepper (1978) and Glick (1995), constitutes a specific set of rhizobacteria with positive effects on plants, commonly present in soil. The increased interest in PGPR as a potential biofertilizer has surged in recent years (Richardson, 2009), particularly as the costs associated with conventional pesticides and fertilizers continues to escalate, prompting farmers to seek viable alternatives such as biofertilizers.

2.3.6 Free-living non-symbiotic nitrogen fixing bacteria

Azotobacter, a group of free-living aerobic bacteria, primarily thrives in neutral and alkaline soils exhibits an average nitrogen-fixing capacity of around 20-22 kg N/ha/year (Bag *et al*., 2017; Mahanty *et al*., 2017). Six key species within the *Azotobacter* genus *i.e*. *A. armeniacus*, *A. beijerinckii*, *A. chroococcum*, *A. nigricans*, *A. paspali*, and *A. vinelandiihold* have a significant role in nitrogen fixation. Significant benefits result from using these species as biofertilizers, especially for non-legume crops like *Triticum aestivum*, *Hordeum vulgare*, *Oryza sativa*, *Helianthus annuus*, *Zea mays*, *Linum usitatissimum*, *Beta vulgaris*, Brassicaceae vegetables, *Camellia sinensis* and *Coffea arabica*. *Azotobacter*, is a free-living nitrogen-fixing

biofertilizer is applied to soil, they colonise in the rhizosphere or interior of the plants, plantlet or seed surfaces, or soil, and encourage growth (Ahmed *et al*. 2017; Kumar *et al*. 2022a). *Azotobacter sp.* produces compounds that promote plant growth and development in addition to increasing the amount of nitrogen that is available for plants as highlighted by Gothandapani *et al.* (2017). According to Egamberdieva et al. (2017), the biofertilizer secretes phytohormones including auxin, cytokinin, ethylene, and abscisic acid. These phytohormones significantly impact plant metabolic activity and, indirectly, contribute to the stimulation of defence mechanisms and the management of abiotic stress, as discussed by El-Lattief (2016).

2.4 Effect of bio-organic fertilizers on growth, yield and nutritional quality

Bahadur *et al*. (2006) investigated the influence of organic amendments and biofertilizers on the growth, production, and quality of Chinese cabbage. The research findings demonstrated that the combination of organic amendments with seedling inoculation of either PSM or VAM resulted in a head yield comparable to without treatment group, which underwent conventional fertilization. Significantly, the application of FYM or digested sludge independently, or in conjunction with phosphate-solubilizing microorganisms, notably increased the dry matter and TCC of the cabbage heads. In terms of nutritional value, compared to conventional fertilization, FYM (20 tonnes/ha) increased micronutrient contents e.g. vitamin C. Moreover, the total carotenoids levels were particularly higher when organic amendments were combined with seedling inoculation of either *Azotobacter* or PSM. The inclusion of FYM (200 q/ha) notably elevated the dietary fiber content. In a distinct study, **Upadhyay *et al.* (2012)** explored the impacts of three different biofertilizers, either combined with four diverse organic manures or alongside inorganic fertilizers, on dry matter partitioning, yield, and nutritive attributes of cabbage. The results revealed that the application of press mud or vermicompost, along with seedling inoculation in *Azospirillum* or PSM, resulted in head yields comparable to those achieved through conventional fertilization. The incorporation of organic manures and *Vesicular arbuscular mycorrhiza* or PSM significantly elevated the total carbohydrate content in the cabbage heads. Significantly, the application of organic manures and *Azospirillum* or PSM substantially enhanced the protein and fiber content in the cabbage heads. Additionally, the combination of farmyard manure

(FYM) with phosphate solubilizing microorganisms (PSM) resulted in the highest recorded total carotenoids and ascorbic acid content in the cabbage heads. **Verma *et al*. (2014)** explored the effects of incorporating Pseudomonas fluorescens and humic acid with three distinct fertilizer doses on growth, production and quality characteristics of *Brassica oleracea* L. The findings indicated that treatments incorporating 100 percent of the recommended fertilizer package, coupled with seedlings treated with Pseudomonas fluorescens and humic acid, resulted in notably higher PH, dry matter in leaves (head), an augmented number of outer leaf, and a head yield of 54.38 tonnes/ha. Moreover, the treatment resulted in elevated protein content (18.54%) and vitamin C levels (34.51 mg/100 g) compared to the chemical fertilizer alone.

Sharma and Sharma (2010) conducted a field trial in Himachal Pradesh (H.P.) to investigate the influence of different doses of bio-fertilizers, such as *Azotobacter* and PSB, in conjunction with four levels of NPK fertilizers, on the growth and production of the cauliflower hybrid 'Swati'. The utilization of bio-fertilizers, either independently or in combination, resulted in a significant improvement in several parameters, including PH, NL, CD, curd depth, gross weight per plant, marketable curd yield, and benefit-cost ratio. The combine utilization of *Azotobacter*+PSB, exhibited a notable rise in marketable curd yield when compared to the control group.

Batabyal *et al.* (2016) undertook an experiment on Nutrient Management (NM) technologies customized for cauliflower production, with a primary emphasis on yield, quality, profitability, energy balance, and ecology sustainability, with a specific focus on improving soil quality. The study examined fifteen NM technologies, incorporating three nutrient sources: organic FYM, vermicompost, and green manure. Additionally, chemical fertilizer was included, with the recommended NPK applied @ 198-45-80 kg/ha, along with selected combinations of these inputs. The findings illustrated that the Integrated Nutrient Management method was ecologically aware as well as economically viable. This approach not only led to the cultivation of superior-quality cauliflower but also resulted in the generation of enhanced value-added products, showcasing increased levels of crude protein, dietary fiber, and ascorbic acid. Moreover, the Integrated Nutrient Management (NM) technology played a simultaneous role in upholding soil quality by augmenting soil OC stock, microbial

biomass carbon, reducing bulk density, and enhancing the availability of extractable plant nutrients. In a similar vein, **Kachari and Korla (2009**) observed that integrated nutrient management, involving biofertilizer and FYM, significantly elevated the morphological attributes of cauliflower cv. PSB K-1. Additionally, **Prabhakar *et al.* (2015)** found that integrated nutrient management resulted in superior yield and quality parameters, including TAC, radical scavenging ability, TFC, and ascorbic acid, in comparison to the use of synthetic fertilizers alone.

Al-Gaadi *et al.* (2019) assessed the effects of organic fertilizers on lettuce, cauliflower, broccoli, and cabbage in a field experiment. The study's findings demonstrated that chicken dung outperformed cow manure in terms of the experimental crops' growth and nutritional factors. Among the crops under investigation, the plots treated with poultry manure displayed elevated chlorophyll content, larger curd or head sizes, and higher overall crop yields. Noteworthy is the substantial increase in crop yield observed when poultry manure was employed, surpassing the results obtained from plots treated with cow manure. Furthermore, according to **Maggio *et al.* (2013) and Tyagi *et al.* (2022)**, cauliflower exhibits enhanced quality and nutritional value when cultivated through organic farming in comparison to conventional farming methods. Similarly,

Zbar *et al.* (2021) explored the influence of different fertilizers on the mineral concentration in cauliflower. The fertilizers examined included a bio-fertilizer containing *Azotobacter chroococcum* (P_1), *Pseudomonas fluorescens* (P_2), and a combination of both (P_3). Additionally, two levels of organic fertilizers, O_1 and O_2, were studied, corresponding to 50 and 100 percent of the recommended dose of manual fertilizers. The findings indicated that all fertilizers resulted in an elevation N, P, K and Fe concentrations in the vegetative parts of cauliflower. Moreover, the combination of P_1 and P_2 (P_3) exhibited a notable impact. However, no significant differences were recorded when comparing the 50% and 100% of the recommended mineral fertilizer.

Yang *et al.* (2019) carried out field study in China, aimed to assess the impact of integrating biofertilizer with low doses of fertilizers on cauliflower physio-chemical, and soil characteristics. The findings revealed that employing fertilizer at 80% in conjunction with biofertilizer (80%) led to an increase in the vitamin C content of

cauliflower heads compared to the 100% fertilization treatment. Moreover, the 80% biofertilizer treatment resulted in minimum nitrate content and improved soluble sugar levels compared to the 100% fertilizer treatment. The conclusion drawn was that the combined use of biofertilizer along with a reduction in mineral N, P, K fertilizer can be effectively applied in cauliflower production. Similarly, **Shrestha *et al.* (2022)** revealed that combining a 75% nitrogen level with the *Azotobacter* significantly improved the morphology and yield attributes of cauliflower than to using the full dose of nitrogen alone.

Abd AL-Hseen and Manea (2020) explored the influence of bio-fertilization and organic extracts on the growth and yield of two cauliflower hybrids, revealing significant outcomes. Firstly, the findings indicated that the biological fertilization treatment excelled in various studied traits, encompassing PH, NL, LR, CD, K, Protein and N percent in the leaf and curds**.** Secondly, organic extracts showed significant superiority over the control treatment in most studied traits. Moreover, the study revealed significant synergies in the bio-interactions between bio-fertilization and organic extracts. The synergy between amino acids and bio-health demonstrated exceptional performance in key traits, including PH (0.576 m), LR (16.82 m^2) K (2.5 g/100g), N in curds (5.93 g/100g), N in leaves (5.39 g/100g), and protein (7.41 g/100g). These results emphasize the beneficial combined impact of bio-fertilization and organic extracts in augmenting cauliflower growth and yield

Abou-El-Hassan *et al.* (2020) implemented a field trial to enhance kohlrabi's organic production. The study incorporated diverse treatments, encompassing compost alone, compost combined with biofertilizer, and algae extract applied in combination. The findings resulted that the combined application of compost with biofertilizer and algae extract yielded the most significant improvements in growth, nutritional content, yield, and knob properties compared to other treatments.

Shah *et al.* (2019) assessed a study in Uttarakhand with the objective of assessing the effectiveness of a combined application of organic manure and fertilizer on knol-khol crops. Revealed that the collaborative use of organic manure+bio-fertilizer resulted a substantial enhancements in the growth, yield, and quality (assessed by TSS and Vitamin C content) of knol-khol plants, outperforming the results achieved with chemical fertilizer alone. Moreover, **Sahu *et al.* (2022)** illustrated that the concurrent

application of organic and mineral fertilizers higher the production of knol-khol. Notably, enhancements in nutritional quality were specifically observed in treatments combining organic fertilizers with biofertilizers.

Islam *et al*. (2020) examined the impact of various organic fertilizers and manures on the development and production of knol-khol. In this single-factor experiment, various kinds of fertilizers and manures were utilized, including 100% NPK, FYM, Vermicompost (VC), poultry manure (PM), 50% FYM+50% NPK, 50% VC+50% FYM, 50% VC+50% PM, and 25% FYM+25% VC+25% PM+25% NPK. Treatment 100% NPK demonstrated the highest plant height at 25, 35, and 45 DAT. Treatment VC, 50% Vermicompost+50% Poultry manure and PM resulted in the higher spread of canopy. Additionally, treatment T_1 yielded the highest economic yield, while T_7 produced the maximum biological yield. Moreover, Manhar *et al*. (2023) observed a significant improvement in the growth and production characteristics of knol-khol through the applied amount of organic manures and fertilizers. Similarly, **Antonova *et al.* (2014)** reveled that adopting an organic system with the utilization of biological fertilizer enhanced the morphology and yield attributes of kohlrabi.

Singh and Singh (2011) investigated in the Himachal Pradesh mid-hills to determine the impact of organic farming technology on *Raphanus sativus* L. productivity and quality. The experiment comprised nine treatments, including three sources of biofertilizer, namely B_1 (FYM+Dense organic manure), B_2 (Biofertilizer+Dense organic manure), and B_3 (synthetic fertilizer). Results indicated that treatments involving FYM combined with dense organic manure exhibited higher yield, increased protein content, and were also enriched in vitamin C content.

Jaiswal *et al*. (2020) examined the effect of organic manures and biofertilizers on growth and production of cabbage. The experiment involved ten distinct treatments, each with its specific composition of organic manure and biofertilizer. Among the various treatments, T_8, comprising Green ball + Poultry Manure @ 50 q/ha + *Azotobacter* at 2.5 kg per hectare, demonstrated the most favourable outcomes. This treatment showcased the maximum PH, SL, the highest NNL, extensive PS, maximum head size, HD, gross head weight, HC, head yield per hectare, and the highest net return. The study highlighted that cabbage exhibited exceptional growth,

yield, and quality under the influence of T_8, underscoring the synergistic effects of Green ball, Manure, and *Azotobacter*.

Sarkar *et al*. (2021) conducted an experiment to evaluate the joined effects of seedling bio-priming and fertilizers on the mineral content, bioactive compounds, and production of red cabbage. The application of 75% fertilizer along with *T. harzianum* and *P. fluorescens* showed in maximum concentrations of head N, Cu, and protein, as well as an increased head yield. Incorporating two bacterial inoculations (*P. fluorescens* and *B. subtilis*) in the integrated system led to the maximum levels of head P, K, Fe, Zn, and total carbohydrate content. The inclusion of a microbial consortium consisting of *T. harzianum* and *B. subtilis* enhanced the head Mn and vitamin C content. Overall, the bio-priming treatments exhibited superior crop quality and yield compared to the control and use of synthetic fertilizers only.

Khede *et al.* (2019) investigate to examine the effects of organic manures, fertilizers, and their mixtures on the development, production, and quality of Japanese White Radish. The results indicated that treatment T_8 (50% RDF + 25% VC + 25% PM) demonstrated the highest values for various parameters, including PH, LL, fresh weight of root, dry weight of root, RL, RD, average weight of root, yield of root, TSS (5.09 $^{\circ}$Brix), and fiber content (749.87 mg). Similarly, Shani *et al*. (2016) recorded that usages of a combination comprising 25% PSB, 25% *Azospirillum*, 25% recommended dose of fertilizer (RDF), and 25% *Azotobacter* (T_{12}) resulted in increased PH, NL, LL, length of root, RD, FW leaves, DW leaves, root weight, yield, as well as enhanced levels of vitamin C, reducing sugar, non-reducing sugar, total sugar, and TSS.

Kopta and Pokluda (2013) investigated the production, quality, and mineral attributes of organically grown radish in the Czech Republic. An open field was used for the organic cultivation of three cultivars: Jarola, Miyashige, and Red Meat. There were adequate yields for every radish cultivar, ranging from 320 to 420 q/ha of marketable yield. In terms of nutritional properties, cv. Red Meat exhibited significantly maximum ascorbic acid (270 mg/kg) content. Cultivar Miyashige displayed the maximum fiber content (1.70%). While the potassium content was reasonably high, there were notable differences between the cultivars, with cv. Jarola standing out for the highest calcium content (157 mg/kg). Furthermore, Gyewali *et al*.

(2020) field trial was conducted in Nepal on organically grown radish, revealing that the combination of FYM with PSB significantly enhanced the growth and production attributes.

Gelaye and Tadele (2022) investigate the two locations within the East Gojjam zone of north western Ethiopia in which the impact of bud numbers and organic manure (FYM) doses on cabbage's morphological parameters was assessed. The findings revealed significant interaction effects, with the most favourable outcomes observed when 2 buds of cabbage were combined with 50 quintal of FYM. This combination emerged as a recommended strategy for economically optimizing cabbage production in north western Ethiopia and similar environments.

Kaur *et al.* (2023) explored the impact of organic manures and bio-fertilizers on radish growth and yield. Their study indicated that the application of PSB @ 4 kg/ha combined with vermicompost at 50 q/ha resulted in increased parameters such as NL, LL, LR, RL, RD, PH, root weight, FW and DW of the plant, root yield per plot, TSS, vitamin C, and the benefit-cost ratio. Similar findings were reported by Teeraj *et al.* (2019), Upadhyay and Prasad (2021), and Pathak *et al.* (2018), emphasizing the positive impact of organic manures with biofertilizers, specifically *Azotobacter*, on radish yield and nutritional parameters.

Goswami *et al.* (2017) study, water hyacinth drum compost (DC) and traditional VC significantly improved soil health and plant growth in an agro-ecosystem dedicated to intensive cultivation of tomato and cabbage. The application of these composts enhanced soil nutrient availability, physical stability, and microbial diversity, contributing to increased yield and improved product quality for both *Solanum lycopersicum* and *Brassica oleracea* var. *capitata* crops.

Choudhary *et al.* (2017) investigated the impact of biofertilizers (*Azospirillum*, *Azotobacter*, and PSB) and different fertility levels of NPK on Knol-khol (*Brassica caulorapa* L.). The study revealed that PSB inoculation resulted in the maximum values for various growth, yield, and quality parameters, surpassing other biofertilizers and comparable to *Azospirillum*. The inoculation of *Azotobacter* also demonstrated notable performance, positively influencing growth, yield, and quality parameters than control group. Similarly,Mishra *et al.* (2014)found that the

application of biofertilizer at 2000 g/ha for *Azotobacter*, *Azospirillum* &PSB resulted in maximum crop dry weight, production, chlorophyll content, TSS, vitamin C and protein content.

Saffeullah *et al.* (2021) conducted an assessment of the influence of *Azotobacter* bacterization on two cabbage genotypes Pusa Early Golden Acre and Pusa Drum Head under field conditions. The plants underwent various treatments involving nitrogen (N) alone and in conjunction with seedling inoculation with *Azotobacter*. The findings revealed a notable elevation in chlorophyll content, nitrate reductase (NR) activity, protein content, sugar content, and phenol content in plants subjected to a combined application of nitrogen and *Azotobacter*. This underscores the advantageous impact of integrating *Azotobacter* into nutrient management systems, emphasizing its potential as a beneficial biofertilizer. Such practices not only reduce the reliance on synthetic fertilizers but also contribute to sustainable agriculture goals.

Hasan *et al.* (2018) investigated the morphology responses of cabbage cultivars under the influence of both organic and mineral fertilizers. The trail incorporated three cabbage varieties Atlas 70 (V_1), Keifu 65 (V_2), and Autumn 60 (V_3) and 4 multiple fertilizers labelled as control (F_0), Cow dung (F_1), Poultry manure (F_2), and Inorganic fertilizer (F_3). Using a RBD Design with three replications, the findings revealed that among the varieties, Atlas 70 (V_1) exhibited superior results in various parameters, including PH, LL, SL, HD, whole plant weight, entire production, marketable production, and economic yield at harvest. Furthermore, considering the interaction impact between cultivar and fertilizer, the combination of Atlas 70 with PU (V_1F_2) demonstrated the enhanced whole plant weight, gross yield, and marketable yield. Consequently, the study suggests that the pairing of Atlas 70+PU proves possess the greatest favourable combination for achieving highest cabbage production. In addition, Chaudhary *et al*. (2018) documented that incorporating farmyard manure (FYM) at a rate ranging from 150 to 200 quintals per hectare played a significant role in enhancing the sustainability of cabbage production. Similarly, **Kumar *et al*. (2017)** found that a combination of 50% FYM, 50% *Azospirillum*, and 100% *Azotobacter* at optimal levels proved to incredibly efficient at fostering cabbage growth, yield, and quality. This treatment led to increased plant height, leaf count, head size, spread, and

yield per hectare, while also increasing the attribute of cabbage heads *i.e*. TSS, acidity, and vitamin c content.

Rajwade and Bahadur (2018) explored the effect of organic manures and chemical fertilizer on the growth attributes of Radish. Their findings highlighted that the usage of 50% of the recommended nutrient dose combined with 50% primary manure resulted in significantly higher values for PH, NL, LL, and shoot weight, particularly observed at 45 days after sowing. Similarly, Subedi *et al.* (2018) revealed that the administration of FYM, alone and in conjunctionwith inorganic fertilizer, led to an enhancement in the growth and production parameters of radish.

Helaly *et al.* (2020) a research experiment was carried out on collard (*Brassica oleracea* var. *acephala*) cv Georgia from the Brassicaceae family, the objective was to explore the impact of biofertilization using four bacterial strains of PGPB in comparison to control plants. The findings showed that the application of AP-303 resulted in the most significant improvements in PH, NL, and LR across both seasons. The highest yields were observed in the AP-303 treatment group, with 0.1178 Kg and 0.1174 Kg per plant, respectively. Following closely were the AP-19 treatments, yielding 0.1114 Kg and 0.1098 Kg per plant, respectively. In contrast, both AP-4 and control treatments exhibited the minimum yields in winter period**.**

Liao *et al.* (2019) conducted an experiment on pakchoi (*Brassica campestris* sp. *chinensis* L.) under greenhouse conditions to investigate the impact of rhizobacteria under various fertilization treatments. The study's findings indicated that the use of bioorganic fertilizer resulted in significant enhancements in soil properties. Moreover, it led to increased productivity of pakchoi, elevated levels of antioxidants, flavonoids, and phenolic content when compared to the effects of chemical fertilizer.

Nurhidayati *et al.* (2016)A factorial block randomized structure with two components was used to study the impact of three types of vermicompost materials and the population of *P. corethrurus* on cabbage production and attribute in organic growing media, than chemical fertilizer. The first component is vermicompost treatment with (V_1: a mixture of mushroom media waste+ FYM + vegetable wastes; V_2: mushroom media waste + FYM + leaf litter; V_3: mushroom media waste + FYM + vegetable wastes + leaf litter). The second component involved the biomass of

P.corethrurus with 5 levels (0, 25, 50, 75, and 100 indiv./m^2), along with one control treatment (chemical fertilizer). The findings indicated that the application of treatment V_1 and V_2 resulted in high yields with populations of 0-25 and 50 indiv./m^2, systematically. Treatment V_3 demonstrated a maximum production even without the inoculation of the earthworm *P. corethrurus*. *Brassica oleracea* var. *capitata* treated with these 3 types of treatments exhibited highest sugar and ascorbic acid contents, with an average increment of 12% and 57%, respectively. These results suggest that the utilization of organic manure has the potential to enhance both the production and quality of cabbage.

Meena *et al.* (2017) examine the effects of biological fertilizer and organic compost on broccoli cv. KTS-1 growth, yield, and quality. The treatment involving 50% vermicompost and 50% *Azotobacter* exhibited the highest values for various parameters, including PH, NL, LL, LW, SL, days to curd, days to fifty percent head initiation and fifty percent head maturity, CD, curd weight, overall yield, and quality attributes such as maximum ascorbic acid content (0.90%), T.S.S content (8.80 $^{\circ}$B), and levels of reducing sugar (3.25 g/100g), non-reducing sugar (790 mg/100g), and total sugars (3970 mg/100g). Similarly, findings by **Demir *et al.* (2023) and Doklega and El-Hady (2017)** also support the concept that biofertilizers contribute to the enhanced growth, yield, and mineral concentration of lettuce and broccoli.

Srichandan *et al.* (2015) revealed that the application of a combination consisting of 75% nitrogen fertilizer, 100% potassium, bioinoculants, and vermicompost (50 q/ha) significantly boosted growth factors, including plant height, leaf area, and curd diameter. Furthermore **Shankar *et al.* (2019)** found that applying a combination of 50% NPK and 50% farmyard manure (FYM) resulted in increased yield and improved nutritional quality of broccoli.

Singh *et al.* (2014) assess the impact of biofertilizers on both the production and mineral properties of broccoli. Various biofertilizers such as *Azospirillum*, PSB, *Azotobacter*, and VAM were used individually and in different conjunction, with chemical fertilizer and no manuring as control. The results revealed that the application of the inoculants *Azospirillum* 50%+*Azotobacter* 50% consequently improved curd size, curd yield, protein and lipid profile, as well as elevated phosphate and sulphate content in the broccoli curd. Additionally, experiments by **Mohamed *et***

***al*. (2021) and Ollio *et al.* (2023)** reveled that plants treated with phosphoric acid and inoculated with mycorrhiza exhibited enhanced values in terms of head length, total carbohydrate, and TSS content.

Altuntas *et al*. (2018) investigated the impact of different origin of fertilizers on the yield of broccoli and selected characteristics. Various fertilizers were examined, including Farm Manure (2-4% N), mineral fertilizer (46:46:51 NPK), Humic Acid, Amino Acid, Humic and Fulvic Acid, Microalgae, *Arthrobacter* sp., and *Bacillus subtilis* strain. The findings indicated that organic and biofertilizers significantly improved the production and different crop morphology traits, nutrient uptake and the vitamin c content of broccoli heads.

Aisha *et al*. (2014)conducted an field trial in Egypt, the effects of organic compost fertilizer and humic acid on the growth, physical, and chemical qualities of turnip plants (c.v. Balady) were investigated. The turnip plants with the highest levels of compost and humic acid also had the highest values for growth and root characteristics; the turnip root tissues also had the highest percentages of protein, nitrogen, phosphorus, potassium, carbohydrates, and iron.

Tamchos *et al*. (2018) carried out an investigation in the trans-Himalaya region of Ladakh, assessing the influence of different ratios of FYM and inorganic fertilizer on the production and superiority of radish. The results indicated that higher applications of FYM (150-200 q/ha) combined with inorganic fertilizer significantly improved parameters such as LL, NL, root length, root weight, root yield, TSS, and vitamin C content in radish.

Kapoulas *et al.* (2017) investigated the influence of production systems (organic) and growing seasons (autumn or spring) on the production and property attributes of lettuce and green onion when cultivated as companion plants (the same piece of land is used to cultivate each crop). Across both production systems, lettuce plants exhibited higher levels of potassium (K), boron (B), zinc (Zn), and iron (Fe) compared to onions. Organically cultivated green onions displayed elevated levels of all major and microelements (other than copper) encountered to traditionally cultivated onions. The total phenolic content (TPC) in green onions surpassed that of lettuce, a trend similarly measured in DPPH content. Notably, lettuce exhibited

significantly superior nitrate concentrations than onions. In conclusion, companion planting demonstrated the potential to increase vegetable productivity each unit of land and enhance net revenues.

Das *et al*. (2016) carried out an experiment focusing on the standardized pungency and hotness of *C. chinense* in geographically distant locations through integrated nutrient management schemes. Assam soil cultivation showed an elevated bitterness and capsaicin level compared to West Bengal. Vermicompost alone in Assam increased fruit production, TSS, protein, fiber, and lycopene. In West Bengal, the combination of NPK and vermicompost showed optimal results. **Abu-Zahra (2016)** found that organic treatments consistently yielded fruits with higher anthocyanin, TSS percentage, dry matter, vitamin c, TPC, and dietary fiber content.

Sepat *et al*. (2012) conducted a field experiment at the DIHAR, Partapur, Ladakh aiming to evaluate the effects of biofertilizer, fertility levels & FYM on the growth, production, and attribute of tomato. The findings revealed that treatments involving either 100% NPK or FYM 100 q/ha, either independently or combined with *Azotobacter*, significantly impacted plant growth and production attributes compared to the control group. Notably, the application of 50% NPK + FYM + *Azotobacter* resulted in superior PH, branches, fruit clusters, fruit size, weight of fruit, and overall fruit yield.

Helaly *et al*. (2022) explored the influence of bacterial bio-fertilization employing three distinct strains AP-28 (*Pseudomonas koreensis*), AP-29 (*Ralstonia pickettii*), and AP-51 (*Bacillus cereus*) on kale crop. The results unveiled that the application of AP-51 demonstrated the most substantial positive impact on morphological traits, including PH, NL, and LR, when compared to the control group. Additionally, the AP-51 treatment led to significantly elevated levels of ascorbic acid, phenolic contents, macronutrients (Nitrogen, Phosphorus, potassium, calcium, and magnesium), as well as essential microelements (iron, copper, and zinc) in contrast to the control group. These findings imply that utilizing the *Bacillus cereus* strain AP-51 as a biofertilizer holds promise in enhancing the overall growth and nutritional quality of kale plants, presenting it as a viable option for sustainable and eco-friendly agricultural practices.

Ranjan *et al.* (2010) investigate to assess how garlic responds to the application of nitrogen-fixing and phosphate-solubilizing biofertilizers in various combinations with a reduced dose of fertilizer (RDF) in the high altitude of the northwest Himalayas. The study revealed that the growth characteristics, including plant height, neck thickness, bulb yield, and bulb weight, were maximized in plants treated with a combination of *Azotobacter* sp., *Microphos* sp., *Azospirillum* sp., half dose NP, and full K.

Kumar *et al.* (2012) investigate an exploring the effects of integrated nutrient management on both soil fertility and potato yield in the elevated regions of the Eastern Himalayas. Employing a split-plot design, the experiment incorporated eight nutrient management practices in the main plots, comprising combinations of organic manures such as FYM, PM, VC, and chemical fertilizers. Additionally, the subplots involved seed tuber treatment with three biofertilizers *i.e*., *Azotobacter*, PSB, and *Azotobacter* + PSB. The findings revealed that the application of fifty percent NPK by chemical fertilizers, combined with fifty percent nitrogen by organic manures significantly influenced tuber production, nutrient uptake, and soil fertility compared to other treatments. Similarly, **Kumar *et al.* (2013)** studied the combined application of fifty percent NPK by chemical fertilizers and 50% Nitrogen by *Azotobacter* + PSB significant increase in shoot number, PH, leaf area index (LAI), dry matter accumulation, and tuber production.

Dinu *et al.* (2015) a field trial was conducted at Romania, involving several tomato cultivars, namely Antalya, Cemil, and Lorely, to assess the effects of foliar treatment with three distinct biofertilizers. The study revealed that the biochemical alterations and resulting production exhibited variations based on both the type of fertilizer applied and the specific cultivar under consideration. The higher content of chlorophyll, total carotene was observed in the combination of biofertilizers, humic acids, extract from *Vitis vinifera* seeds, and Boron. Furthermore, **Kannahi *et al.* (2015)** investigate the application of biofertilizers led to a significant improvement in both the production and quality of tomatoes.

Shilpa *et al.* (2024) carried out research to evaluate the synergistic effects of organic manure, PGPR, and inorganic fertilizers on bell pepper (Capsicum cv. Solan Bharpur). Employing a three replications with 15 treatment combinations, the

research investigated their impact on growth, production, soil fertility, nutritional value and field income. The results indicated that the treatment comprising 75% nitrogen-phosphorus (NP) in combination with vermicompost (VC) and enriched compost (EC) at 250 q/ha, along with PGPR, led to the highest levels of available nitrogen, phosphorus, potassium, OC, and electrical conductivity in the soil. Additionally, this treatment combination exhibited the highest values for yield and its nutritive value parameters, including fruit number, pericarp thickness, length and diameter of fruit. Notably, it resulted in a remarkable 60.89% improvement in yield and elevated levels of TSS (5.94 $^{\circ}$B) and vitamin c content (0.182%).

Dawa*, et al.* (2012) investigated the impact of two organic fertilizer sources (PM & FYM) and biofertilizers (*A. chroococcum, B. circulans* bacteria, and *Mycorrhiza* fungi) on capsicum. The study revealed distinct effects on various aspects of plant growth and chemical composition. Notably, plants receiving chicken manure demonstrated superior vegetative parameters, including PH, leaf count, branch number, LR, fresh and dry weights. Furthermore, these plants exhibited higher concentrations of essential leaf constituents such as chlorophylls nitrogen, phosphorus, and potassium percent compared to those supplied with compost. Additionally, the incorporation of biofertilizers contributed to improved growth performance and chemical composition in leaves.

2.5 Effect of bio-organic fertilizers on bioactive phytochemical compound

Naguib *et al*. (2012) evaluated the influence of organic and bio-organic fertilizers on the growth, production, and nutritive value of 2 cultivars, Calabrese and Southern star of broccoli. The results indicated that application of organic fertilizers resulted in higher levels of TPC, TFC, and total glucosinolates content in the Calabrese cultivar. Assessment of antioxidant activities revealed that the Calabrese cultivar displayed greater DPPH scavenging activity, with an IC_{50} of 16.42µg/mg, than Southern star (18.38µg/mg). Furthermore, when organic fertilizer was utilized, the Calabrese cultivar exhibited maximum chelating power (74.29µg/mg) compared to Southern star (73.39 µg/mg) at a concentration of 30 µg/mg. These findings suggest the potential for enhancing antioxidant compounds in broccoli through organic fertilization, highlighting its viability as a valuable natural source of antioxidants for nutraceutical purposes and emphasizing its economic production potential.

Velasco *et al*. (2021) an experiment focused on *Trichoderma hamatum*, the research aimed to explore its impact on productivity, glucosinolate content, and antioxidant potential in various leafy brassica crops such as *Brassica oleracea* var. *sabellica*, *Brassica oleracea* var. *capitata*, *Brassica napus*, and *Brassica rapa* sp. rapa. The ground breaking results revealed a substantial increase in the productivity of *Brassica oleracea* var. *sabellica* by 55%, *Brassica oleracea* var. *capitata* by 36%, and *Brassica rapa* sp. rapa by 46% through root inoculation with *T. hamatum*. Additionally, the fungal inoculation demonstrated a noteworthy rise in the total glucosinolate (GSL) content in *Brassica oleracea* var. *capitata* and *Brassica rapa* sp. rapa. This was accompanied by an enhancement in the antioxidant capacity of these crops. The findings suggest that *T. hamatum* root inoculation can positively influence both yield and nutritional quality, particularly by elevating glucosinolate levels and antioxidant potential in Brassica crops.

Dos Reis *et al*. (2015) carried out an experiment on impact of various processing conditions, including boiling, steaming, microwaving, and sous vide, on the stability of Total Soluble Solids (TSS), chlorophyll content, flavonoids, antioxidants, carotenoids, quercetin, kaempferol, and vitamin A in broccoli and cauliflower inflorescences cultivated within an organic system. The findings indicated that each processing method contributed to the enhancement of antioxidant compound content. Notably, sous vide processing demonstrated superior antioxidant capacity for both vegetables under the tested conditions. Additionally, post-processing, inflorescences of broccoli and cauliflower from an organic system exhibited increased levels of bioactive substances.

Dutta and Neog, (2016) investigated the potential medicinal uses of a methanol raw extract made from the rhizome of turmeric that was co-inoculated with orginated arbuscular mycorrhizal fungi, phosphate-solubilizing *B. megaterium*, and diazotrophic microbes (A. *amazonense* and *Azotobacter* sp.). Various parameters, including free-radical scavenging capacity (DPPH, ABTS), TPC, TFC, and curcumin content, were evaluated. The findings revealed that the inoculated plants had significantly higher levels of these secondary metabolites than the control plants did. This suggests that turmeric has strong antioxidant properties against DPPH and ABTS radicals, with levels ranging from 80% to 97%. Moreover, the heightened levels of flavonoids

(ranging from 179.07 to 492.88 mg RE/g) and phenolic contents (ranging from 82.97 to 151.54 mg GAE/g) in all methanolic extracts of dried rhizomes suggest a potential correlation with the observed antioxidant activity.

Khalid *et al.* (2017) explored the influence on soil fertility and phytochemical concentrations in spinach of biofertilizers containing mycorrhizal fungus, either separately or in conjunction with N-fixer (*A. chroococcum*), K solubilizer (*B. mucilaginous*), and P solubilizer (*B. megaterium*). In treatments where mycorrhizal fungi were combined with bacterial inoculation, there was an increase in root colonization compared to alone inoculation treatments. The biofertilizer containing both mycorrhizal fungi and bacterial species significantly heightened the levels of TPC, TFC, and phenolic acids in spinach. HPLC analysis revealed a notable augmentation in antioxidant activity in spinach, and this enhancement correlated with increased levels of quercetin and chlorogenic acid. Overall, the combined use of mycorrhizal fungi and bacterial species in bio fertilizers demonstrated positive effects to enhanced phytochemical levels, indicating potential benefits for spinach growth and nutritional quality.

Asghari *et al*. (2020) conducted a greenhouse study employing a factorial experiment in a completely randomized design to examine the impact of PGPR in protecting *Mentha pulegium*, an important industrial and functional plant, from drought-induced damages. *Azotobacter chroococcum* (Ac), *Azospirillum brasilense* (Ab), Ac+Ab, and a control group lacking PGPR were all included in the first factor, which entailed PGPR inoculation. Field capacity (FC), 0.7 & 0.4 FC were the three irrigation regimes that made up the second factor. Under water deficit conditions, the plant exhibited increased levels of secondary metabolites such as TPC, TFC and along with enhanced DPPH activity of menthe extract. The study uncovered variations in the efficacy of bacteria in enhancing plant characteristics, with the co-inoculation of Ac and Ab proving more effective in improving physio-chemical attributes of *Mentha pulegium*. Under extreme drought stress, plants treated with Ac+Ab showed the highest concentrations of TPC, TFC, and oxygenated monoterpenes as well as the greatest capacity to scavenge free radicals (DPPH).

Couto *et al.* (2011) studied on soybean inoculated with *Bradyrhizobium japonicum*, investigating its impact on the metabolite profile and antioxidant potential of the

plant's aerial parts. Utilizing HPLC–DAD for phenolic compounds and HPLC–UV for organic acids, the researchers analyzed extracts. Acidic and methanolic extracts were tested for their antioxidant capacity adverse DPPH. Results indicated that nodolous induced by japonicum had to increased concentrations of both phenolic compounds and organic acids. Phenolic extracts exhibited superior antioxidant capacity compared to acid extracts. Crops nodulated with B. japonicum showed substantially more antioxidant activity in extracts than in control samples. These outcomes demonstrate that inoculation with nodulating B. japonicum has the potential to effectively notable metabolite content in the parts of soyabean, influencing its antioxidant properties.

Sousa *et al.* (2005) conducted a comprehensive phytochemical study on cabbage grown under both conventional and organic farming methods, with samples collected at various time points. Qualitative and quantitative differences were observed between the internal and external leaves of the cabbage. HPLC was employed to analyze the phenolic composition of the internal leaves, revealing a unique phenolic profile compared to the external leaves.

Picchi *et al*. (2012) Investigate to compare the phytochemical content of two cauliflower genotypes, Emeraude and Magnifico, cultivated under both conventional and organic management practices. Notably, under organic management, employing higher fertilization levels had a pronounced effect on the phytochemical production of Magnifico, specifically leading to a significant increase in polyphenols. Conversely, the same fertigation treatments resulted in a decrease in the phytochemical production of Emeraude, particularly affecting glucosinolates and ascorbic acid. These findings underscore the genotype-specific and differential responses to organic cultivation practices, particularly in terms of phytochemical composition influenced by fertilization levels.

Lombardi-Boccia *et al*. (2004) studies on *Prunus domestica* cultivated both conventionally and organically within the same farm. The research aimed to examine the influence of different agronomic convention on the concentrations of antioxidants, vitamins, and phenolics. Three organic cultivation methods were utilized: tilled soil, mulching with trifolium, and mulching with natural meadow. Plums planted on mulching with trifolium exhibited the maximum total polyphenols content, whereas conventionally grown plums showed higher concentrations. Regarding flavonols,

quercetin concentration was greater in conventional plums, while myrecitin and kaempferol were more abundant in organic plums. These findings underscore the influence of different agronomic practices on the specific antioxidant compounds present in yellow plums, emphasizing the variability in phenolic composition based on cultivation methods.

Hashemi *et al.* (2022) found that the utilization of *Azotobacter chroococcum*, a plant growth-promoting agent, in conjunction with mild drought stress, significantly ($p \leq 0.05$) augmented the biosynthesis and bioactivity of *Trachyspermum ammi* seed essential oil. This combined treatment led to increased levels of TPC and TFC, accompanied by heightened antioxidant activity. These results suggest a positive influence on the production of bioactive compounds in *Trachyspermum ammi* seeds in response to the synergistic effects of *Azotobacter chroococcum* and mild drought stress.

Jacob *et al.* (2012) utilized extraction protocols to evaluate the antioxidant characters of green and red cabbage, emphasizing their total antioxidant capacities through ABTS radical scavenging method. The study established a robust correlation between the total antioxidant capacity of both green and red cabbage and the content of TPC and TFC present in the extracts. Remarkably, the ABTS radical scavenging capacity of red cabbage extract exceeded that of green cabbage. Through time-resolved absorption kinetic spectro-photometry, free radical reactions with extracts from both cabbage types were investigated. Further examination using pulse radiolysis revealed that the extracts could effectively scavenge radicals, demonstrating antioxidant properties and suggesting potential in repairing free-radical damage to biologically significant guanosine radicals. Additionally, Rokayya *et al*. (2013) identified that among various cabbage varieties, red heads exhibited the maximum total antioxidant contents, followed by Savoy, Chinese, and green heads. The green variety demonstrated the maximum DPPH, reaching 90.19 micromoles per gram fresh weight. The red cabbage exhibited the maximum FRAP, recording at 79.88 μmol/g FW. TPC ranged from 16.18 to 31.58 mM Trolox equivalent while TFC ranged from 40.11 to 73.19 mg/g of quercetin.

Agrawal and Verma (2014) conducted a study on the radical scavenging ability and biochemical analysis of leaf and root extracts of radish. The phytochemical analysis

of the leaf extract revealed a phenol content (0.0270 mg/g) and root extract (0.0375 mg/g) fresh weight, along with the presence of alkaloids, flavonoids, triterpenoids, and steroids. Notably, the study concluded that the leaf extract exhibited higher antioxidant potency compared to the root.

Singh *et al.* (2006) conducted in a plain area, antioxidant phytochemicals in cabbage were investigated across eighteen different cultivars. The variability in antioxidant phytonutrients among the cultivars was assessed, revealing a lutein content ranging from 0.022 to 0.26 mg/100 g fresh weight. Additionally, TPC was measured in 14 cultivars, showing values ranging from 11.98 to 33.65 mg/100 g fresh weight. Red cabbage exhibited higher levels of TFC, FRAP, and DPPH content. The study concludes that cabbage has the potential to make a significant contribution to overall phytonutrients intake, and the observed substantial variability within the cultivars underscores the importance of considering specific cabbage types for maximizing antioxidant benefits.

Chorol *et al.* (2019) investigate the field trial on antioxidant content in different parts of radish from Ladakh region of Trans- Himalaya. It was observed that the combined methanolic and acetone extract of radish sprouts exhibited the highest Total Phenolic Content (TPC) across all three radish cultivars- Gya Labuk, Tsentay Labuk, and Pusa Himani. Notably, the peel showed the minimum TPC values for all three cultivars significant ($p \leq 0.05$). Furthermore, the FRAP analysis revealed that the leaf of Gya Labuk, Tsentay Labuk, and Pusa Himani had the maximum FRAP values, while the peel had the minimum values. These differences were statistically significant at $p \leq 0.05$. Similarly, in the case of DPPH, the maximum values were measured in the leaf of all three cultivars, while the peel exhibited the minimum values at $p \leq 0.05$. The findings from this study align with epidemiological evidence suggesting that the consumption of vegetables, such as radishes, can play a preventive role against degenerative diseases associated with oxidative stress. Another finding by **Chorol *et al.* (2018)** results indicated that growing radish plants at higher altitudes had increased in the concentration of glucosinolate contents in radish seeds.

Kusznierewicz *et al.* (2008) conducted an investigation into glucosinolates, antioxidative compounds, and total radical scavenging activities in white cabbage samples collected from various European regions during both spring and autumn. The

lyophilized vegetables demonstrated a total glucosinolates content ranging from 3.41 to 7.80 mm/g DW. Electron transfer-based assays (TPC, ABTS, and DPPH) were utilized to evaluate total radical scavenging activities and major bioactive compounds in cabbage. Total polyphenols ranged from 2.31 to 4.89 GAE/g DW, and total radical scavenging activities varied from 2.69 to 8.18 mmol TE/g DW in the ABTS test and from 2.38 to 5.36 mmol TE/g DW in the DPPH assay. Cabbages harvested in autumn in Belgium exhibited the highest recorded amounts of polyphenol compounds, antioxidant activity, and total glucosinolates content, while the lowest values were found for Poland 2 cabbage harvested in spring. Statistically significant correlations were calculated between antioxidative properties and the abundance of bioactive compounds. This suggests that total radical scavenging activities could serve as a standardization method for natural mixtures, particularly in cabbage, facilitating the comparison of results from biological studies conducted on vegetable-derived samples.

Samec *et al*. (2011) observed the evolution of total phenols, TFC, and antioxidant capacity (evaluated through FRAP, DPPH, and ABTS assays) in juices derived from Croatian white cabbage cultivars Vara zdinski and Ogulinski, as well as Chinese cabbage at different growth stages. Over the initial 8–12 weeks, a notable increase in TPC and TFC contents occurred, with antioxidant capacity doubling in most analyzed juice samples. Variability in TPC, TFC, and antioxidant capacity was observed at the fully mature stage, distinguishing between white and Chinese cabbage juices and among cultivars Ogulinski and Vara zdinski.

Seong *et al*. (2016) investigate the antioxidant capacities and polyphenolic of Chinese cabbage leaves. The research revealed that the outer leaf demonstrated the highest antioxidant activity, accompanied by maximum levels of TPC, TFC, and ascorbic acid. HPLC and liquid chromatography/mass spectrometry (LC/MS), antioxidant-related components such as TPC and TFC were isolated and identified. Notably, the outer leaves exhibited superior antioxidant activity compared to the mid and inner leaves, suggesting a gradient of antioxidant contents within different layers of Chinese cabbage leaves.

Ahmed *et al*. (2014) utilized the HPLC method to analyze Phenolic Compounds in *Brassica Oleracea* L. var. *capitata*. The mobile phase, composed of a mixture of

Acetonitrile and Phosphate buffer (pH-5.8) in a 55:45 ratio, facilitated the rapid and straightforward quantitative determination of Rutin, Quercetin, and Kaempferol in cabbage. The study's findings underscored the efficiency and accuracy of this HPLC method for quantifying these phenolic compounds in the specified variety of *Brassica oleracea*.

Liang *et al.* (2006) employed high-performance liquid chromatography (HPLC) to quantify sulforaphane in *Brassica oleracea* var. *italica* and *Brassica oleracea* var. *capitata*. The method involved methylene chloride extraction and reversed-phase HPLC (RP-HPLC) with gradient of acetonitrile in water. The efficacy of this process was assessed by measuring sulforaphane levels in the edible portion broccoli and cabbage, as well as various parts of *Brassica oleracea* var. *italica*. Results revealed that the mean sulforaphane content in broccoli was almost five times maximum than that in *Brassica oleracea* var. *capitata*. Within different parts of broccoli, the florets exhibited the highest sulforaphane content, while the leaves showed the lowest. This study offers valuable insights into sulforaphane distribution in broccoli and cabbage, highlighting the potential benefits of this analytical method for sulforaphane analysis in cruciferous vegetables.

Liang *et al.* (2009) investigated the phytochemicals and antioxidant activity in four commonly consumed varieties of head cabbages in China. The findings revealed that red head cabbage exhibited the greatest levels of TPC and TFC, measuring 152.89 mg gallic acid equivalents/100g and 50.29 mg rutin equivalents/100g, respectively. In contrast, *Brassica oleracea* var. *capitata* showed the lowest levels. The antioxidant activity, assessed through DPPH and ABTS, as well as FRAP assays, was notably higher in red head cabbage compared to the other varieties. This study suggests that different head cabbage varieties offer distinct nutritional advantages, providing valuable information for recommending specific varieties to consumers.

Lee *et al*. (2014) conducted an experiment assessing 62 varieties of Chinese cabbage (*Brassica rapa* L. ssp. *pekinensis*), determining glucosinolate (GSL) and antioxidant activity through HPLC, DPPH, HRSA, and FRAP assays. The study identified five aliphatic glucosinolates and one aromatic GSL (gluconasturtiin). Total GSL contents varied widely among the 62 varieties, ranging from 2.79 to 47.49 mM/g DW, with significant variations in both total and individual GSL contents observed among

different varieties. Although indoles and aromatic GSLs collectively constituted 25.80% of the total GSLs, differences among varieties in this regard were minimal. Most Chinese cabbage varieties exhibited noteworthy antioxidant activities compared to the positive control.

Kumar *et al.* (2022) compared the phytochemical composition and antioxidant benefits of *Eruca sativa* (Arugula) crop cultivated at Ladakh versus Chandigarh. The phytocompounds of *E. sativa* exhibited favourable physicochemical characteristics for oral bioavailability. The extract from High altitude cultivated crop demonstrated more valuable for Total Phenolic Content (TPC) at 31.90±1.09 µg GAE/ml, Ferric Reducing Antioxidant Power (FRAP) at 216.46±3.53 µM TE/ml, Total Flavonoid Content (TFC) at 33.54±0.92 µM RE/ml, as well as lower IC50 values for DPPH (3.60±0.22 mg/g). Polyphenols and flavonoids exhibited a positive correlation with antioxidant activities. RP-HPLC–DAD with MS-analysis evident significantly higher levels of kaempferol (7.01±0.11 µg/ml) and quercetin (0.33±0.00 µg/ml) in Leh samples compared to Chandigarh samples. In conclusion, this study suggests that arugula cultivated at higher altitudes tends to accumulate higher levels of health-promoting bioactive phyto-compounds compared to sample grown at low altitudes.

Li *et al.* (2017) implemented a sensitive and rapid HPLC method to evaluate indole-3-carbinol in cabbage and broccoli. The method showcased qualities of sensitivity, selectivity, rapidity, and reproducibility, with a robust recovery rate of 99.25%. Noteworthy features included a quick retention time (4.80 min) and excellent linearity (R^2=0.9991). The study revealed significant variations in indole-3-carbinol contents among all the materials ($p \leq 0.05$). Additionally, the research established a simple extraction method for indole-3-carbinol, affirming the accuracy, reliability, and stability of the determination method.

Ares *et al.* (2014) determined sulforaphane levels in *Brassica oleracea* var. *italica* florets, stems, and leaves using liquid chromatography coupled to diode array detection. The technique used isocratic elution to achieve fast isolation on a C_{18} column with a liquid phase of ammonium formate (20 mM) in water and acetonitrile (55:45 v/v). The validation process demonstrated selectivity, linearity within the range of 2.48 to 800 mg/kg, and precision. Additionally, limits of detection and quantification were established at 0.8 and 2.5 mg/kg, respectively. The proposed

method successfully analyzed sulforaphane in *Brassica oleracea* var. *italica* cultivar Parthenon and Marathon.

Jaakola *et al*. (2010) reviled that the impact of light intensity, photoperiod, and temperature on the gene-environment interaction influencing flavonoid biosynthesis. Higher plants' secondary metabolite composition is influenced by altitude as well. Apart from introducing various climatic variations, altitude also affects the nature of radiation. Specifically, alpine sites exhibit elevated levels of UV-B radiation compared to lower habitats. The conclusion drawn from the investigation suggests that increased light irradiation leads to elevated contents of flavonols, particularly quercetin derivatives, and enhances antioxidant properties in plants.

2.6 Research Gap

Based on the research papers discussed above, it is evident that the majority of the investigations into the impact of biofertilizers and organic manure on the growth, yield, nutritional content, and phytochemical compounds of vegetables have predominantly taken place in low-altitude regions, with no available dataset elucidating how these agricultural inputs might influence the physiochemical and phytochemical parameters of cruciferous vegetables cultivated under extreme climatic conditions of high altitude regions. Therefore, the current investigation was planned, which compares the efficacy of organic manure (FYM) and biofertilizer (*Azotobacter*) on the morphology, biochemical, and phytochemical parameters of cruciferous vegetables grown at high-altitude region of Ladakh and low-altitude region of Chandigarh.

Chapter -3

CHAPTER- 3
MATERIALS AND METHODS

The current research on **"**Comparative Study of FYM and *Azotobacter* on the Growth, Yield, Qualitative Traits and Phytochemical Aspects of Cruciferous Vegetables at Cold Desert Region and Plain Area" was conducted during the years 2020-2021 and 2021-22. The research was carried out at two locations: the high-altitude setting of Defence Institute of High Altitude Research (DIHAR) in Leh-Ladakh, and the low-altitude location at DIHAR's base lab in Chandigarh, both affiliated with Defence Research and Development Organization (DRDO). This chapter provides a detailed account of the materials used and methods employed throughout the investigation, outlining the specifics of the procedures and materials implemented in the study.

3.1 Experimental site: The experiment was conducted in 2020-2021 and 2021-22 at high altitude location (HA) *vs.* low altitude (LA). HA- Agriculture Research Unit (ARU), Defence Institute of High Altitude Research (DIHAR)-Defence Research and Development Organization (DRDO), HQ, Leh-Ladakh, India which is situated at 34°08′2″ North Latitude and 77°34′3″ East Latitude, with the average elevation and a mean sea level of 3340 m.

LA- Defence Institute of High Altitude Research (DIHAR), Defence Research and Development Organization (DRDO), Base lab Chandigarh, India which is situated at 30º41′31″ North Latitude and 76º47′10″ East Latitude, with the average elevation and a mean sea level of 321 m (Figure 3.1).

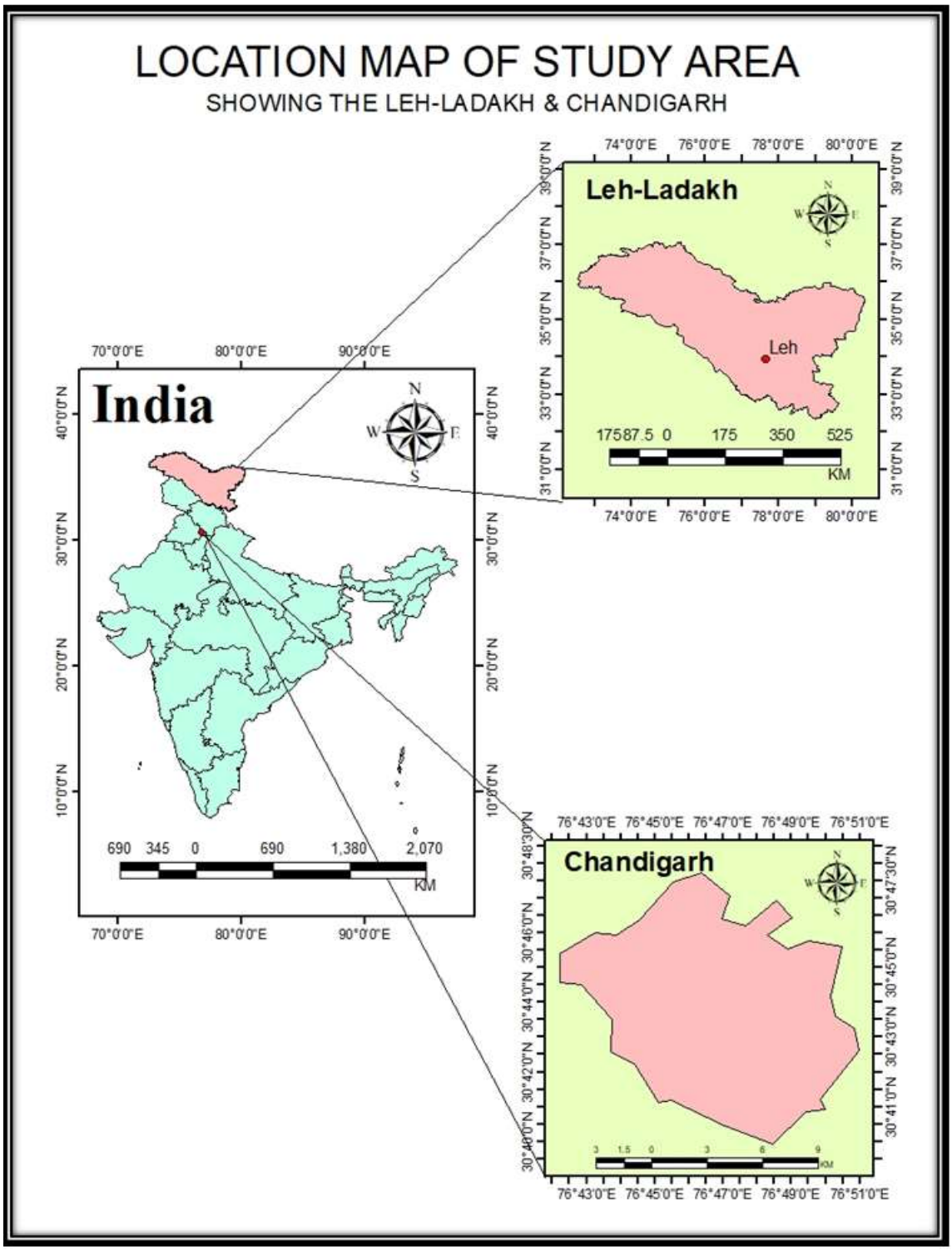

Figure 3.1 Experimental site of the study area

3.2 Climatic condition: The high-altitude region of Leh-Ladakh experiences challenging climatic conditions characterized by drastic temperature fluctuations, minimal precipitation (<100 mm annually) primarily in the form of snow, elevated wind speeds, limited plant density, a thin atmosphere exposing it to high levels of UV

radiation, and a delicate ecosystem. The extended and severe winter limits the cropping season to a mere 4-5 months (Stobdan *et al.*, 2018).

The climate in low altitude region (Chandigarh) is humid subtropical with moderate winters and extremely hot summers, unpredictable precipitation, and wide temperature ranges (10ºC to 45 °C). The average yearly rainfall is approximately 1100.70mm.

The meteorological data during course of investigation has been obtained from the Hygro-thermometer to assess the impact of meteorological observation in term of temperature and relative humidity in support of experimental findings. The meteorological data are exhibited in Figure 3.2.

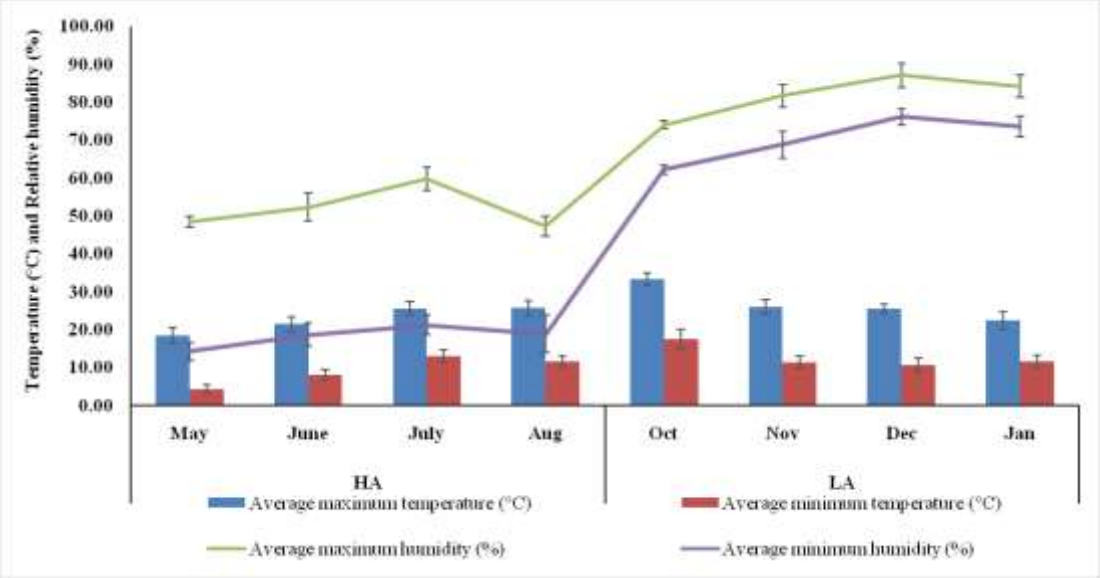

Figure 3.2 Average means minimum and maximum temperatures, as well as relative humidity, at high and low altitudes during growth period. HA: High altitude and LA: low altitude.

3.3 Soil of the experiment site:

The comprehensive details of soil characteristics before the cultivation of crops are presented in Table 3.1**.** The analysis of the soil's chemical properties was conducted using the method outlined by Page et al. (1982). Soil samples were gathered from a depth of 0 to 30 centimetres, both before cultivation and after crop harvest, at both study locations. Soil samples from each replicate treatment were collected aggregated and shade-dried. Then, any visible noticeable organic debris such as roots, leaves, and

twigs were removed. Mechanically field samples were sieved with a 2 mm mesh sieve. After mixing these replicates, a composite sample for each treatment was prepared. The soil's pH was measured using a pH meter (Hanna HI 8424 pH meter, Europe) in a 1:2.5 soil suspension, while the electrical conductivity was assessed with a conductivity meter (Sn X24560 thermo scientific, Indonesia).Organic matter was determined by wet digestion according to Walkley and Black (1934). The Kjeldahl method [(K-355, Buchi Labortechnik, Switzerland) was used to determine available soil N (Kjeldahl, 1883)]. Available soil P was evaluated by $NaHCO_3$ extraction (Olsen and Sommers, 1982) and computed via colorimetric estimation at 880 nm. Flame photometry (Jenway PFP7, Bibby scientific Ltd, UK) was used to determine the available soil K. Metals such as Mg, Zn, Cu, Fe, and Mn in soil were outlined by DTPA extractable with technique of Lindsay and Norvell (1978).

Table 3.1 Comparative chemical properties of soil before cultivation at high and low altitude

Soil parameters	HA	LA
pH	7.34±0.08***	8.19±0.02
EC (ms/cm)	0.39±0.02	0.65±0.03***
OC (%)	0.65±0.03***	0.35±0.06
N (Kg/ha)	37.21±0.97	34.02±1.66
P (Kg/ha)	13.18±0.56***	9.04±0.09
K (Kg/ha)	189.06±2.38**	175.78±2.99
S (mg/kg)	138.03±2.55***	104.72±3.22
Zn (mg/kg)	2.17±0.01	3.42±0.02***
Fe (mg/kg)	3.03±0.18	4.56±0.29***
Mg (mg/kg)	1.59±0.04	4.16±0.11***
Cu (mg/kg)	3.86±0.26	4.91±0.11**
Mn (mg/kg)	3.05±0.16***	1.78±0.16

HA- high altitude and LA- low altitude, Values presented as means ± SD.
*Values in columns were significantly different at *** p≤0.001, ** p≤0.01 and * p≤0.05, via., Independent T-test analysis between HA and LA.*

3.4 Experimental material

3.4.1 Organic input:

(A) Farm Yard Manure (FYM): It is produced from the dairy wastage. Farmyard manure provides vital plant nutrients, promotes soil aeration and organic matter, leads to in a rise in number of soil microbes and an accumulation of extra humus content (Dinesh *et al*., 2003; Sindhu *et al*., 2020) and improves enhances the physio-chemical and biological properties of the soil (Singh *et al*., 2020). It contains 0.50-1.2% nitrogen, 0.25-0.40% phosphorus and 0.45-1.00% potassium.

(B) Biofertilizer (*Azotobacter*): *Azotobacter*, is a free-living nitrogen-fixing biofertilizer is applied to soil, they colonise in the rhizosphere or interior of the plants, plantlet or seed surfaces, or soil, and encourage growth (Ahmed *et al*., 2017; Kumar *et al*., 2022a). Auxin, cytokinin, ethylene, and abscisic acid are some of the phytohormones secreted by biofertilizer (Egamberdieva *et al*., 2017). These phytohormones have a noticeable effect salient impact on plant metabolic activity and have also indirectly contributed obliquely furnished to the stimulation of defence as well as abiotic stress management (Ei-Lattief, 2016).

3.4.2 Crops and cultivar: In cruciferous vegetable, Cabbage (Videshi), cauliflower (W.S.909), knol-khol (White Vienna) and radish (Pusa Himani) waere used for field experiment at both high and low altitude locations.

3.4.3 Selection of cruciferous vegetable: To the best of our knowledge, cruciferous vegetables are more in demand by the local person as well as army soldiers due to their nutritional value and potential health benefits. Moreover, very limited information is available on cruciferous vegetable wither aspect to the study that looks at how biofertilizers and organic manures can influence the morphology, nutritional value, and bioactive chemical profiling of cruciferous vegetables. Furthermore, No studies on the production of cruciferous vegetables have been conducted or a comparison of their phytochemical composition when grown at high and low altitudes.

3.4.4 Characteristics of cultivar

3.4.4.1 Cabbage cultivar (Videshi): This hybrid cultivar, developed by Beejo Sheetal Seeds Pvt. Ltd. in Jalna, Maharashtra, is characterized by robust plant growth with upright outer leaves. The heads exhibit a dark green, smooth, and highly compact appearance, becoming ready for harvest within 80 to 95 days after transplanting. With an average head weight ranging from 1.0 to 2.5 kg, it yields an average of 400 to 750 quintals per hectare.

3.4.4.2 Cauliflower cultivar (W.S. 909): This synthetic cauliflower variety features a longer stem and sparsely semi-erect leaves. The curds are hemispherical, exhibiting a creamy to yellow-white color, medium compactness, non-ricey texture, and reaching maturity within 80-90 days after transplanting. With an average curd weight ranging from 0.75 to 1.5 kg, this variety has a yield potential of 200 to 350 quintals per hectare.

3.4.4.3 Knol-khol cultivar (White Vienna): This early variety boasts globular, light green knobs with a smooth and tender texture, featuring creamy-white flesh with a delicate flavor. The plants are dwarf in stature, and both leaves and stems display a medium-green hue. With a yield potential ranging from 450 to 600 quintals per hectare, it matures within 55-65 days after transplanting. In summary, it is an early type characterized by smooth, medium-sized, globular knobs, light green in color, offering creamy-white, tender flesh with a subtle flavor. The dwarf plants with medium-green leaves and stems have a yield potential of 450 to 600 quintals per hectare, reaching maturity 55-65 days after transplantation.

3.4.4.4 Radish cultivar (Pusa Himani): It is European or temperate type variety of radish. The radish has a pure white skin and crisp, sweet-flavored flesh with a mild pungency. The roots measure 30-35 cm in length and 40-50 mm in diameter, presenting as pure white with a green stem end. These roots are semi-stump to tapering in shape, accompanied by short tops. It mature 50to60 days after sowing. It is European or temperate type variety of radish. The flesh is crisp, sweet, and mildly pungent, while the skin is a flawless white colour. The roots have a diameter of 40 to 50 mm and a length of 30-35 cm. With a green stem end, roots are completely white.

They have short tops and a semi-stump to tapering shape. It matures fifty to sixty days after seeding.

3.5 Experimental details

3.5.1 Detail of treatments: Four different treatment of organic manure (FYM), biofertilizer (*Azotobacter*) alone and combination of FYM+*Azotobacter* along with one control were imposed on cruciferous vegetable at both high and low altitude locations (Table 3.2).

Table 3.2 Experimental details of treatments plots at both high and low altitude

Sr.No.	Treatments	Application
1	T_1	FYM @ 150 q/ha
2	T_2	*Azotobacter* @ 8.6 kg/ha
3	T_3	FYM @ 150 q/ha+ *Azotobacte*r @ 8.6 kg/ha
4	T_4	Control (without any treatment)

3.5.2 Experimental design and layout: The present experiment was laid out in open field condition at both HA and LA with two factorial Randomized Blok Design (Factor A- 2 Locations and Factor B- 4 treatments). Total eight treatments were taken and replicated thrice.

Year of experiment	:	2020-21 and 2021-2022
Location	:	HA and LA
Treatment	:	4
Total number of treatment	:	2×4 = 8 (Factor A- 2 Locations and Factor B- 4 treatments)
Number of replication	:	3
Design	:	2FRDB (Two factorial Randomized Blok Design)
Net plot size	:	1.35 x 1.20 m^2
Total number of plot	:	12
Spacing	:	Cabbage and Cauliflower - 60×45 cm Knol-khol - 30×20 cm Radish - 30×10 cm

3.6 Agronomic operation

3.6.1 Field preparation: The experimental plot was prepared with cultivator followed by planking to bring the field good tilth condition. After that, a scientific layout was executed in field with the help of rope, measuring tape, tags etc. The ridge and furrow were made 50 cm apart on each plot and irrigation channels were provided proper irrigation.

3.6.2 Application of FYM and *Azotobacter*: After the completion of layout, FYM and *Azotobacter* were applied as per the treatment combinations in randomized manner. The organic manure (FYM) was incorporated in experimental field as per the treatments suggested prior to 15^{th} days of transplanting of seedlings or seed sowing. However, the biofertilizer (*Azotobacter*) was applied in the soil at the time of seedling transplanting or seed sowing.

3.6.3 Nursery sowing: Quality seeds were used for sowing purpose. Cabbage, cauliflower and knol-khol seeds were shown in trench in month of April at high altitude (Leh) whereas, open field condition in month of September at low altitude (Chandigarh). After sowing of seeds, light watering was done with the help of watering cane.

3.6.4 Seedling transplanting: The transplantation of seedling was done at 12 to 15 cm height (Figure 3.3). Seedling transplanting was done in month of May at HA whereas; month of October at LA. After seedling transplanting, surface irrigation was done at both the locations.

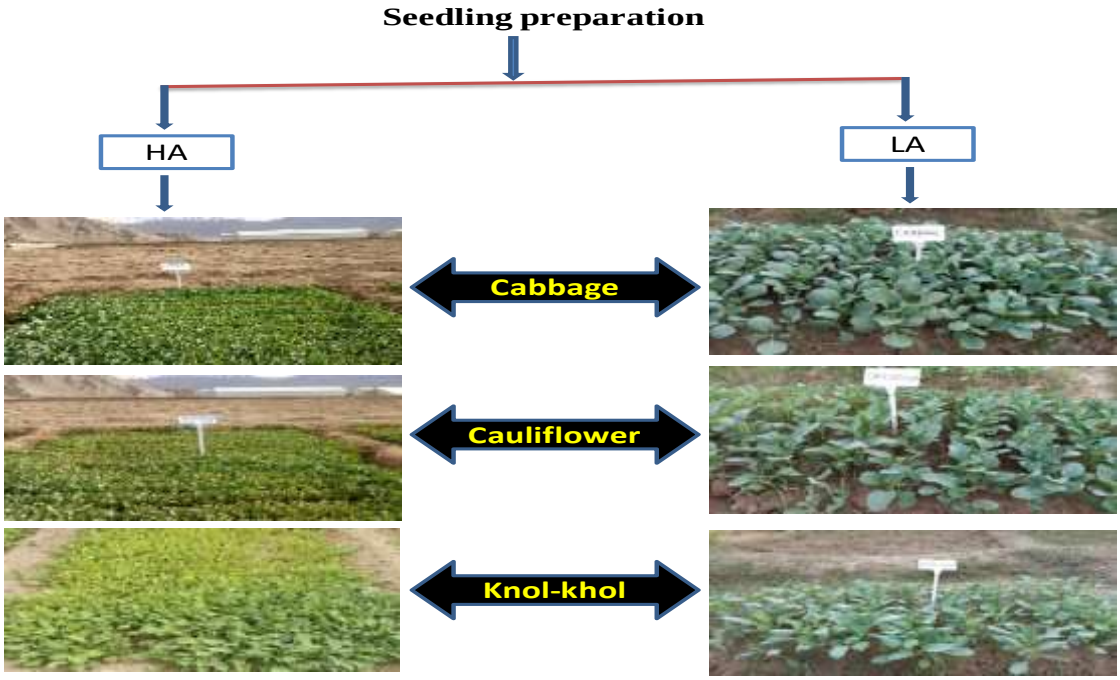

Figure 3.3 Seedling preparations of Brassicaceae vegetables at high altitude and low altitude

3.6.5 Seed sowing: In radish crop, seeds were sown directly to the experimental plots at a spacing of 30 x 10 cm. Two-three seeds were sown in each hill. The seeds were germinated after 7-8 days of sowing. After sowing of seeds, watering was done at both the locations.

3.6.6 Irrigation: The field was irrigated by flooding at an interval of three days at high altitude and 6-7 days interval at low altitude during early stage of plant establishment pursued by one week interval (HA) and two weeks interval (LA) at later stages.

3.6.7 Weed management: In order to check the weed growth and to make the soil friable, manual weeding were done frequently as per the requirement of crops in experimental plot at both HA and LA. To maintain weed free experimental plots, two to three times weeding were done at the interval of 30 days after transplanting (DAT) or sowing of seed at both high and low altitude locations, respectively.

3.6.8 Plant protection measures: There was no usage of artificial pesticides or fertilizers at any site. To manage the various insect pests and diseases, organic pesticide was utilized. In order to prevent this, a preventive spray of *Azadirachtin*

indica oil was applied @ 5 mL/Liter of water every 25 days following seed sowing or transplanting.

3.6.9 Sampling: Five representative plants were chosen at random from each plot, and the plants were tagged for further measurement.

3.7 Observations recorded

The data collected for various traits during the experimental period was categorized into three groups for analysis.

3.7.1 Morphological parameters

The morphological parameters of cabbage and cauliflower were carried out at various successive stage of growth *i.e.*,30, 45, 60, 75 and 90 days after transplanting (DAT) Whereas, 30, 45 and 60 DAT in knol-khol and 30, 45, and 60 days after sowing (DAS) in radish at both HA and LA location (Figure 3.4).

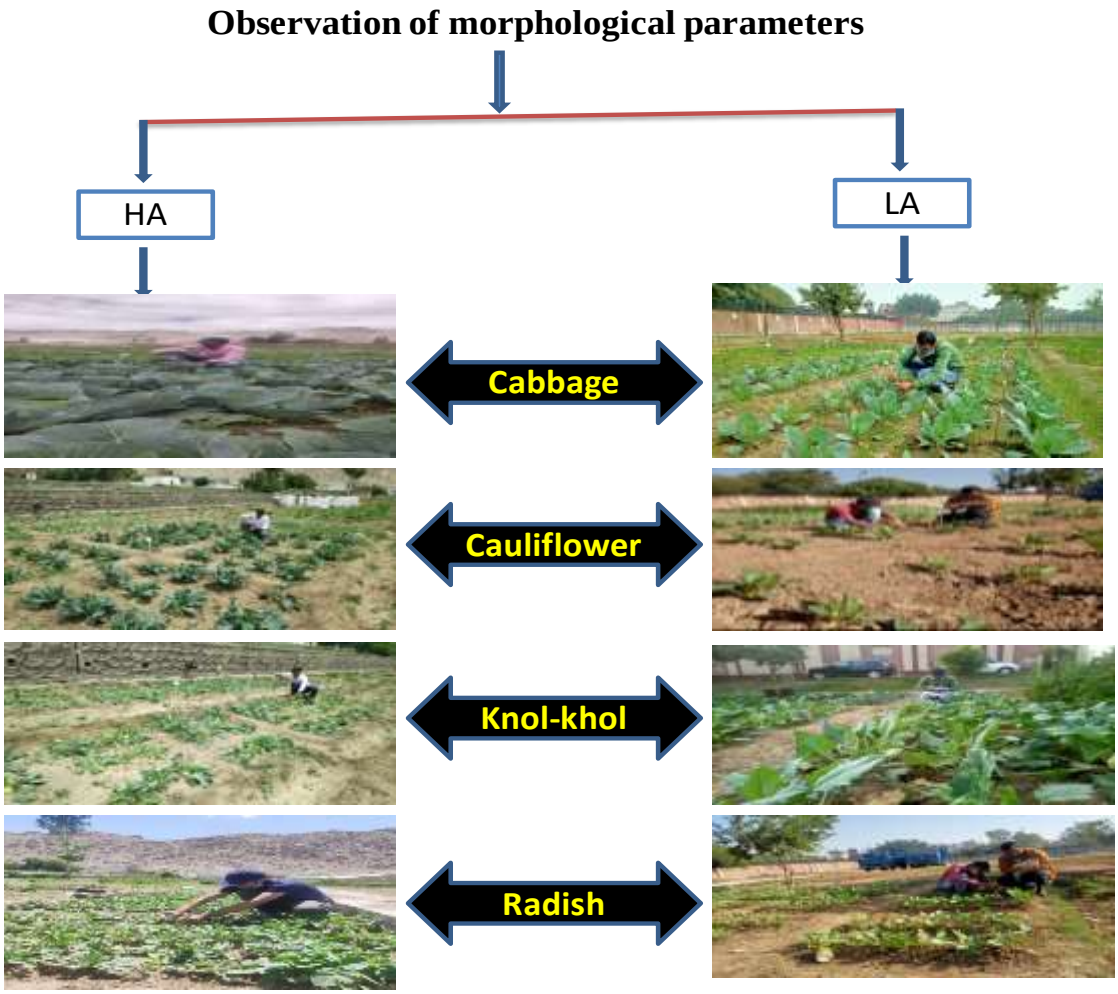

Figure 3.4 Observation of morphological parameters of Brassicaceae vegetables grown at HA and LA

3.7.1.1 Plant Height (cm): A measuring scale was used to determine the plant's height from the base to the top of the longest leaf. The recorded vine length presented as the mean value.

3.7.1.2 Number of leaves per plant: The leaf count per plant was determined by counting the leaves on randomly tagged plants at different successive stages of growth.

3.7.1.3 Leaf length with petiole (cm): Leaf length with petiole was recorded from the leaf petiole up to the leaf apex at different growth stages with the help of measuring scale.

3.7.1.4 Leaf width (cm): The broadest portion of the leaves was measured by measuring scale at different growth stages

3.7.1.5 Leaf area (cm^2): Leaf area (cm^2) was calculated by multiplying the length of the leaf without petiole and width of the leaf.

3.7.1.6 Plant spread (cm): Plant spreading was recorded from middle portion to outer leaf of the head/curd/knob with the help of measuring scale.

3.7.1.7 Stem diameter (mm): Stem diameter was recorded from the first secondary root level to the position of first leaves in cabbage and cauliflower at different growth stages with the help of vernier callipers.

3.7.1.8 Leaf chlorophyll content: A portable chlorophyll meter was used to measure the chlorophyll content of the leaf (CCM-200 plus, ADC Bioscientific, UK). The results were expressed chlorophyll content index (cci).

3.7.1.9 Leaf anthocyanin content: A portable anthocyanin meter was used to measure the anthocyanin content (ACI) of the leaf (ACM-200 plus, ADC Bioscientific, UK). The results were expressed anthocyanin content index (aci).

3.7.1.10 Polar diameter (mm): Polar diameter was measured after cutting the curd/head into two equal halves longitudinally with help of scale.

3.7.1.11 Equatorial diameter (mm): It was measured by vernier calliper at widest part of head/curd/knob.

3.7.1.12 Compactness: Cabbage and cauliflower compactness rate was recorded by formula given by Raid *et al.* (2009). A compactness value of 1 indicates that the head is exceedingly compact and does not contain any air. The lower the rate of compaction, the more compact the cranium, and vice versa.

$$\text{Compactness} = \frac{\text{Head volume } \left(\frac{3}{4}\pi \text{radius}^3\right)}{\text{Head weight (g)}}$$

3.7.1.13 Root length (cm): In radish, length of root was measured with the help of measuring scale.

3.7.1.14 Root diameter (mm): In case of radish, the root diameter was measured at three distinct locations namely, the stalk end, middle section, and floral end utilizing a vernier calliper. The average diameter was then calculated from these measurements.

3.7.1.15 Yield per plant (g): The weight of five randomly chosen heads/curds/knobs/roots per plant was recorded using a weighing balance. The weight of the average result was then reported as the yield per plant.

3.7.1.16 Yield (q/ha): The weight of head/curd/knob/roots per plot (kg) was recorded and converted per ha. The result was expressed as total yield (q/ha).

3.7.2Economic of treatments

At the end of the study, the cost of cultivation, gross return, net return, and benefit cost ratio were computed. The average treatment yield and the market rates/prices for inputs and products were utilized to calculate economics. The cost of cultivation for each treatment was deducted from the gross returns obtained from the economic yield in order to compute the net returns. The benefit-cost (B:C) ratio was calculated by dividing gross returns by cultivation costs for each treatment.

3.7.3 Biochemical parameters: The fresh and dried samples were stored at -20^{0}C after harvesting for future analysis.

3.7.3.1 Chemicals and Reagent:

HPLC-grade methanol, acetonitrile, acetone, sodium nitrite, sodium hydroxide, and gallic acid were obtained from Merck (India). Hydrogen sulphate, DPPH (2,2-

diphenyl-1-picrilhydrazyl), potassium persulfate (PPS), Folin–Ciocalteu (FC) reagent, aluminum chloride, trolox, quercitin, kaempferol, indole-3-carbinol, sulforaphane, and anion multi-element standard were procured from Sigma Aldrich Pvt. Ltd (Switzerland). Sodium bicarbonate, sodium chloride, boric acid, rutin trihydrate, and sodium carbonate were sourced from Himedia (India). Water from the water purification instrument [Merck Millipore Academic, United States of America (USA)] was utilized for various analyses. Additionally, all other solvents of analytical grade were purchased from Rankem, Loba Chemie, and Qualigens Fisher Scientific.

3.7.3.2 Estimation of Total soluble solids (TSS) and Titratable acidity (TA):

Approximately 10 g of fresh sample was blended, and juice was extracted to estimate TSS using a hand refractometer (ATAGO, Tokyo). Titratable acidity (TA) was calculated evaluated as a percentage of malic acid by titrating fresh sample juice with 0.1 N NaOH up to pH 8.2 (Upadhyay *et al*., 2012).

3.7.3.3 Determination of Anions (Nitrate, Phosphate and Sulphate):

Ion exchange chromatography was used to determine the anions (Nitrate, Phosphate, and Sulphate) in fresh cruciferous samples (Cataldi *et al*., 2003) (Figure 3.5). Fresh vegetable samples (1000 mg) were homogenized using a homogenizer at 12000 rpm in deionized water for two minutes, followed by 35 minutes of sonication in an ultrasonic bath (Ultrasonic cleaner YJ5120-1, India). The resulting supernatant was diluted in distilled water and filtered through a 0.22 µM syringe filter. For the chromatographic analysis, an injection volume of 20 µL with a flow rate of 0.6 mL/min was applied to a column (Metrosep A Supp 5- 250/4.0), using a mobile phase containing 3.2 mM sodium carbonate and 1 mM sodium bicarbonate (930 Compact IC flex Metrom; Switzerland). The conductivity detector was used for detection, and the outcomes were given in mg/Kg of fresh weight (FW).

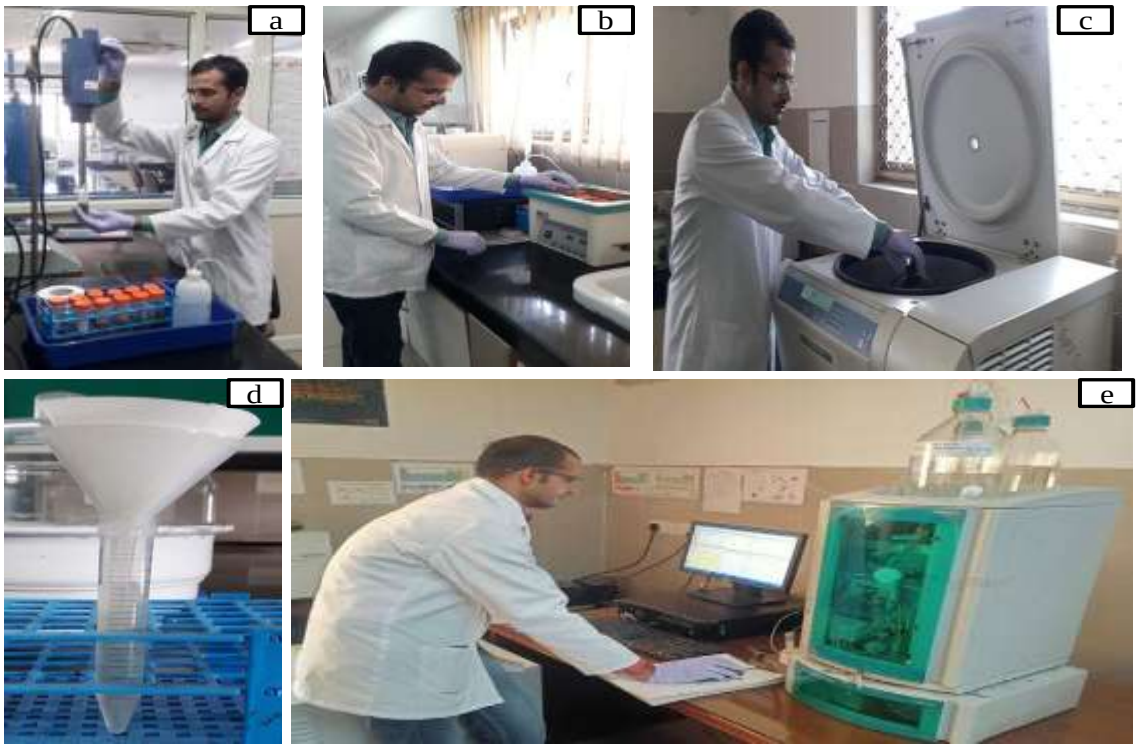

Figure 3.5 (a-e): (a) Homogenization (b) sonication (c) centrifugation (d) filtration (e) ion-chromatography analysis

3.7.3.4 Estimation of crude fat:

The Soxhlet system was used to calculate the crude fat content of dried samples (Horwitz and Latimer, 2005). The dried cabbage powder (3000 mg) was extracted in three soxhlet extractors using continuous petroleum ether at a flow rate of 2-3 drops per second, followed by sample drying at 95±4°C. The percentages of crude fat were calculated using the formula:

$$\text{Crude fat (\%)} = \frac{\text{Flask weight with fat} - \text{Flask weight without fat}}{\text{Sample weight}} \times 100$$

3.7.3.5 Estimation of Dietary fiber:

The Dietary fiber of vegetable samples was estimated according to the method no. 978-10 (AOAC, 2006) with some modification. Moisture free and defatted sample (2000 mg) was digested with 0.128 M (200 mL) of boiling H_2SO_4 for 35 minutes. The digested sample was filtered after the acid was discarded, and then it was washed with boiling distilled water to remove any remaining acid. To eliminate all base solubilized fractions, the sample was next subjected to a 35-minute treatment with 200 mL of

boiling NaOH (0.313 M) solution. Once more filtering and washing with hot distilled water. The residual remains were dried at 180°C for 95 minutes, weighed, and then ignited in a muffle furnace (Scientech laboratory equipment, India) at 560±10°C for 2 hours. The following subsequent equation was used to calculate the percentage of dietary fiber:

$$\text{Dietary fiber (\%)} = \frac{(B - C)}{A} \times 100$$

Where: A= crude sample weight, B= sample weight before ignition, C= sample weight after ignition.

3.7.3.6 Estimation of Ash content:

Method No. 942-05 was used to determinate the ash content in cabbage samples (AOAC, 2006). The 5000 mg sample was put in a crucible, heated to 560 ± 10 °C in a muffle furnace for six hours, until whitish grey residues were formed. The sample was cooled before being weighed. Percentage of ash content was computed by the following subsequent formula:

$$\text{Ash(\%)} = \left(\frac{A_2 - A_1}{A_s}\right) \times 100$$

Where: A_1= weight of crucible, A_2= weight of crucible with ash, A_s= weight of sample.

3.7.3.7 Estimation of Total Kjeldahl Nitrogen and crude protein:

The total kjeldahl nitrogen and crude protein in cruciferous vegetable samples was examined in accordance with modified method of Kjeldahl instrument (K-355, Buchi Labortechnik, Switzerland) (AOAC, 2006). For this, 0.2 g of oven dried sample was digested through concentrated H_2SO_4 and digestion tablets until light greenish color was attained which was obtained after two to three hours. Distillation was done with 32% NaOH after digestion. The released ammonium gas was captured in 4% boric acid solution consisting of methyl red and bromo cresol green (indicator), generating ammonium borate that indicates nitrogen content. At last, the distillate was titrated with 0.25 M H_2SO_4 till light pinkish color and the volume consumed was noted and outcomes were demonstrated in mg/100 g of DW (Figure 3.6). To calculate crude

protein in dried sample, nitrogen was multiplied by correction factor (*i.e*. 6.25) (Wang *et al*., 2016).

Figure 3.6 (a-d): Kjeldahl analysis (a-b) digestion (c) titration (d) distillation

The amount of total nitrogen present in each sample was computed by using the following subsequent formula:

$$W_{(N)} = \frac{(Vol_{samp} - Vol_{blank}) \times z \times c \times f \times Mn}{m_{sample} \times 1000}$$

Whereas

$W_{(N)}$: Nitrogen weight fraction

Vol_{blank}: mean titrant volume for the blank (mL)

Vol_{samp}: volume of titrant for sample (mL)

f: titrant factor

c: titrant concentration (mol L^{-1})

z: molar valence factor (2 for H2SO4)

Mn: nitrogen molecular weight (14.007gm mol^{-1})

M_{sample}: sample volume (gm)

1000: conversion factor (mL/L)

3.7.3.8 Determination of macro and micro elements:

The total contents of potassium and sodium were measured using a flame photometer (Jenway PFP7, Bibby Scientific Ltd, UK) (Lee *et al*., 2010), whereas Zn, Cu, Fe, Mn, and Mg were measured using an atomic absorption spectrophotometer (AAS ZEEnit 700 plus, analytik Jena AG, Germany) (AOAC, 2006; Bhargava, and Raghupathi, 2005). Dry sample powder (200 mg) was digested with a micro digester (Analytik Jena AG, Germany) in a 3:1 ratio of nitric acid and hydrochloric acid. The supernatant was diluted in distilled water to a volume of 50 mL and filtered through a Whatman filter paper grade 1 filter. The results out comes were indicated in mg/100 g of DW (Figure 3.7).

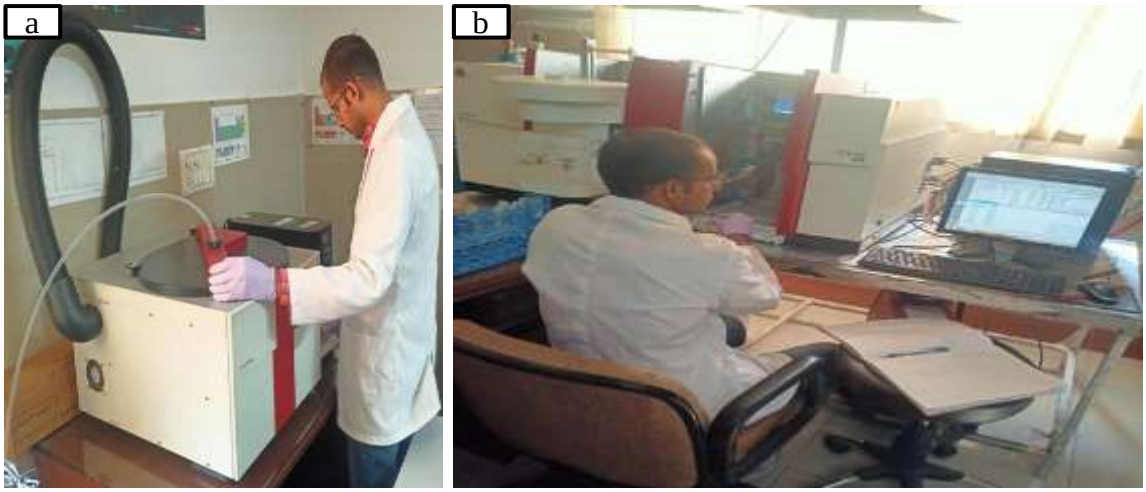

Figure 3.7 (a-b): (a) Sample digestion with micro digester (b) sample analysis using atomic absorption spectrophotometer

3.7.4 Phytochemical Parameters

3.7.4.1 Sample extraction:

There are numerous extraction techniques available today, from basic ones as maceration to more advanced ones that use microwave and ultrasonic technology (Azwanida, 2015). The goal of the procedures for extracting bioactive components from plants depend on five different functioning techniques: (1) extract particular complicated compounds; (2) make the methods more specific; (3) concentrate compounds; (4) evolution compounds into simpler forms; and (5) develop an effective and consistent method (Azmir *et al*., 2013). A suitable solvent choice must be made

based on the polar nature of the target solute for extracting bioactive chemicals from plants. Therefore, maceration was used in this study (Figure 3.8). A pulverized sample weighing thirty grams underwent three successive extractions over a 24-hour period, each time using 100 mL of solvent (composed of 80% methanol and 20% distilled water). The extraction process was conducted in darkness and at room temperature. Subsequently, all these extracts were filtered through Whatman filter paper grade 1. The filtered extract was concentrated using a Rotavapor (Buchi R-215, Switzerland) at a temperature of 40°C, followed by lyophilization (Esquire biotech Freeze dryer 18N, India) at -80°C and 0.050 mbar pressures. The resulting product was then stored in an airtight plastic container at -20°C for future analysis.

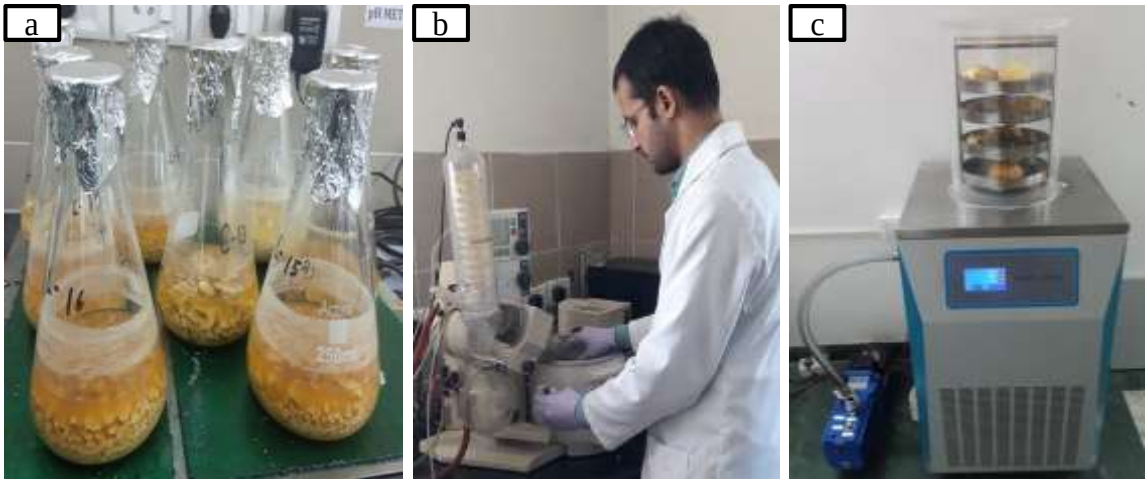

Figure 3.8 (a-c): (a) maceration (b) rotary evaporator (c) lyophilization

3.7.4.2 Determination of total carbohydrate content:

The total carbohydrate content of the extracts was determined using the anthrone method with slight modifications as per Arguello *et al*. (2006). Anthrone (200 mg) was dissolved in 100 mL of concentrated sulfuric acid and cooled using ice cooling. A mixture of 400 µL of various concentrations of standard solution (Glucose; 3.9-1,000 µg/mL)/extracts was combined with 2,000 µL of anthrone reagent, followed by placement in a water bath at 95 °C for 17 minutes and subsequent cooling to room temperature. The absorbance of both the standard and samples was measured at 620 nm using a spectrophotometer (Molecular Devices UV-Visible SpectraMax i3x Spectrophotometer, USA). The outcomes were presented in micrograms of glucose equivalent per gram of Dry Powder Extract (DPE).

3.7.4.2 Evaluation of total phenolic content:

The assessment of Total Phenolic Content (TPC) in sample extracts was carried out using the Folin–Ciocalteu reagent with slight modifications, as described by Jacob *et al*. (2011) and Kumar et al. (2022b). Seventy microliters of various concentrations of the standard solution (Gallic acid; 2.000–0.332 µg/mL)/extracts were combined with 630 µL of deionized water, followed by the addition of 70 µL of FC reagent. The mixture was incubated at room temperature for 5 minutes. Subsequently, in each reaction mixture, 140 µL of sodium carbonate solution (20%) was added, and the mixture was further incubated in the dark for 60 minutes at ambient temperature. After the incubation process, the absorbance of both the samples and standard was spectrophotometrically measured at 750 nm. The results were expressed in micrograms of Gallic Acid Equivalent (GAE) per gram of DPE. The calibrated equation for gallic acid was $y = 0.0087x - 0.0012$ ($R^2 = 0.998$), where x represents concentration and y is the absorbance at 750 nm.

3.7.4.3 Evaluation of total flavonoids content (TFC):

Total Flavonoid Content (TFC) was determined using the aluminum chloride method with slight modifications, following the procedures outlined by Samec *et al*. (2011) and Bhardwaj *et al*. (2019). One hundred seventy microliters of various concentrations of the standard solution (Rutin trihydrate; 1.46-3.000 µg/mL)/two extracts were mixed with 680 µL of deionized water, followed by the addition of 51 µL of sodium nitrite solution (0.724 M) and incubation for 5 minutes. Subsequently, in each reaction mixture, 51 µL of aluminum chloride (0.75 M) was added and incubated for 6 minutes. Furthermore, 340 µL of sodium hydroxide (1.0 M) was added to each reaction mixture. The total reaction volume was adjusted to 1700 µL by adding 408 µL of deionized water. Finally, the absorbance was recorded at 510 nm using a spectrophotometer. The outcomes were presented in micrograms of rutin trihydrate equivalent (RE) per gram of DPE.

3.7.4.4 Evaluation of ferric reducing antioxidant power (FRAP):

The FRAP assay was conducted following the method suggested by Alam *et al.* (2021) and Kumar *et al.* (2022b) with slight modifications. A FRAP solution was prepared by combining acetate buffer (pH 3.6) at 300 mM, TPTZ solution (20 mM in 40 mM HCl), and 20 mM FeCl3 (dissolved in water) in a ratio of 10:1:1. This FRAP solution was then reacted with the methanol extract of the sample (10.000 mg/mL) in a ratio of 1:30, followed by incubation in the dark for 30 minutes at 37°C. The resulting blue-colored product, known as the ferrous tripyridyltriazine complex, was obtained, and its absorbance was spectrophotometrically recorded at 593 nm. Trolox (0.976-250.00 µg/mL) served as the assay standard, and the outcomes were expressed in micrograms of trolox equivalent (TE) per gram of DPE.

3.7.4.5 Evaluation of Antioxidant capacity (DPPH radical scavenging activity):

The DPPH radical scavenging activity of the extracts was assessed following the method outlined by Liang *et al.* (2019) and Bhardwaj *et al*. (2019) with minor adjustments. A DPPH solution (0.135 milli molar) in methanol was prepared, and the methanolic extract of cruciferous vegetables (30.000 mg/mL) or standard (0.480-1.500 µg/mL) was mixed in a 1:15 ratio using vortex, then left for 30 minutes in the dark at room temperature. After the incubation period, the absorbance was measured using a spectrophotometer at 517 nm. Quercetin (QR) served as the reference standard. The ability to scavenge radicals was determined using the formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Rsam} - \text{Rsas}}{\text{Rsam}} \times 100$$

R_{sam} = DPPH radical absorbance in methanol; R_{sas} = DPPH radical absorbance in sample/standard.

3.7.4.6 Separation of key phytocompound by Reverse phase high-performance liquid chromatography (RP-HPLC) analysis

The determination of key phytocompound with kaempferol, Indole-3-carbinol and sulforaphane were measured by using RP-HPLC technique (Agilent, Infinity 1200 Series) (Figure 3.9) with photodiode array detector (DAD) method explained by Ahmed*et al*., (2014); Kumar *et al.* (2022b) – Kaempferol, Li *et al*., (2017)- Indole-3-carbinol and Liang *et al*., (2006)- sulforaphane with some modifications, respectively. Sample peaks were separated on a Phenomenex C18 column (5µm

100A, 250 × 4.6 mm), temperature was maintained at 25 °C with flow rate (0.6 mL/min) and injection volume was 10 µL. Prior to being employed for analysis, all HPLC quality grade solvents were filtered using a 0.45-micron filter disc. For kaempferol determination, an isocratic solvent system was deputed using 50% formic acid (0.1%, v/v) and 50% acetonitrile for 18 minutes with absorbance at 254 nm. For indole-3-carbinol estimation, gradient elution: mobile phase A: acetonitrile; mobile phase B: water-formic acid (99.9: 0.1, v/v) with absorbance at 280 nm. The gradient method was as follows: from 0 to 4 min, 30% A; from 4 to 10 min, 50% A; from 10 to 12 min, 30% A; from 12 to 16 min, 30% A;. The determination of sulforaphane, for best separation, the following mobile phase gradient was used: mobile phase A: acetonitrile; mobile phase B: water-formic acid (99.9: 0.1, v/v) with absorbance at 254 nm. The gradient method was as follows: from 0 to 4 min, 40% A; from 4 to 10 min, 70% A; from 10 to 12 min, 70% A; from 12 to 20 min, 40% A;. Kaempferol, Indole-3-carinol and sulforaphane standards were used for identification and quantification by making a comparison between RT (retention times) of unspecified peaks with specified standard, and outcomes were presented as µg per mg of DPE.

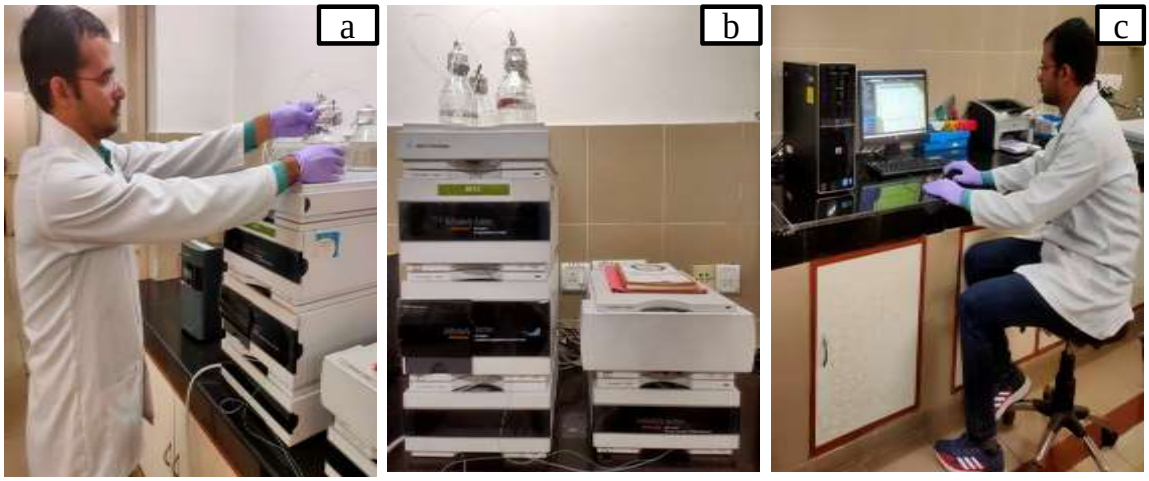

Figure 3.9 (a-c): RP-HPLC-DAD analysis (Agilent, Infinity 1200 Series)

3.8 Statistical analysis

All experimental data were conducted in triplicate and presented as mean ± standard deviation (SD). The data collected over both years of the study were combined. To determine the significance of the results for various morphological, nutritional, and phytochemical parameters of vegetables samples from high and low altitudes, statistical analyses such as independent t-tests, one-way ANOVA, and post hoc

analysis with Duncan's multiple range tests ($p \leq 0.05$) were carried out using SPSS 16.0 (SPSS Corporation, Chicago, IL). Additionally, a Two-way ANOVA test was applied to analyze soil fertility, morphology traits, biochemical, and phytochemical attributes to identify the interaction between altitude and treatments. Correlation analysis was performed using non-linear regression analysis.

Chapter -4

CHAPTER-4
EXPERIMENTAL RESULTS AND DISSCUSSION

The present investigation on **"Comparative Study of FYM and *Azotobacter* on the Growth, Yield, Qualitative Traits and Phytochemical Aspects of Cruciferous Vegetables at Cold Desert Region and Plain Area**" was carried out in 2020-2021 and 2021-22 at high altitude location, Defence Institute of High Altitude Research (DIHAR) - Defence Research and Development Organization (DRDO), HQ, Leh-Ladakh and at low altitude location, Defence Institute of High Altitude Research (DIHAR), Defence Research and Development Organization (DRDO), Base lab Chandigarh. The statistical data in regards to different observations were assembled, tabulated and analyzed statistically in order to draw the valid conclusions and presented with corresponding tables and figures under the following heads and sub heads:

4.1 Comparative impact of FYM and *Azotobacter* on growth and yield parameters of cruciferous vegetables grown at HA *vs.*, LA

The various growth parameters of cruciferous vegetable (*i.e.,* cabbage, cauliflower, knol-khol, and radish) like plant height, number of leaves, leaf length with petiole, leaf width, leaf area, plant spread, stem diameter, leaf chlorophyll content, leaf anthocyanin content, and yield parameters (*i.e*., polar and equatorial diameter, total yield) were significantly influenced by FYM, *Azotobacter* and their combination as compared to control at both HA and LA locations followed by comparing HA and LA grown cruciferous vegetable during the course of investigation. The detailed experimental findings are given below:-

4.1.1 Plant height (cm) of cruciferous vegetable at different days after transplanting

4.1.1.1 Cabbage cultivar Videshi

According to the current investigation, the plant height of cabbage was found to be considerably impacted by each of the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) both at HA and LA locations. The data are present in Table 4.1.

At HA, maximum plant height (18.33±0.42 cm, 29.05±0.23 cm, 31.97±0.71 cm, 33.93±0.42 cm, and 35.96±0.61 cm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (17.18±0.70 cm, 27.41±0.88 cm, 29.05±0.21 cm, 30.46±0.88 cm, and 32.39±0.88 cm) and T_2 (17.17±0.85 cm, 27.24±0.57 cm, 28.77±0.38 cm, 30.63±0.63 cm, and 32.79±0.99 cm) which included FYM and *Azotobacter* respectively. The lowest plant height (13.53±0.28 cm, 20.58±0.47 cm, 25.55±0.68 cm, 27.19±0.50 cm and 28.46±0.68 cm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum plant height (26.11±0.48 cm, 29.38±0.32 cm, 30.69±0.40 cm, 31.29±0.54 cm, and 32.27±0.60 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (23.92±1.08 cm, 26.90±1.01 cm, 28.39±0.84 cm, 29.05±0.60 cm, and 29.93±0.51 cm) and T_2 (23.97±0.98 cm, 26.65±0.90 cm, 28.29±0.99 cm, 28.92±0.91 cm, and 29.99±1.04 cm) which included FYM and *Azotobacter* respectively. Lowest plant height (21.88±0.56 cm, 24.07±0.67 cm, 25.33±0.40 cm, 25.73±0.37 cm and 26.69±0.40 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on plant height at low and high-altitude regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum plant height at both sites. Furthermore, at 30 DAT, the height of plants cultivated in the LA region was 42.44% greater than HA region. However, no significant change was observed in plant height during 45 and 60 DAT at both the locations. However, after 75 and 90 days of transplanting, the height of plants grown in the HA region was found to be increased by 8.44% and 11.43%, respectively, as compared to the plants grown at the LA region. The altitudes and treatments significantly affected the plant height at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was not significant except at 45 DAT (Table 4.1).

Table 4.1 Comparative effect of location and treatments on plant height of *Brassica oleracea* var. capitata cultivar Videshi

High altitude (HA)										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	17.40 ± 0.79^{b}	16.96±0.68^{b}	27.62 ± 1.05^{bc}	27.19±0.83^{b}	28.84 ± 0.20^{b}	29.26±0.29^{b}	30.51 ± 0.91^{b}	30.40±0.84^{b}	32.56 ± 0.85^{b}	32.22±0.92^{b}
T_2	17.37 ± 0.84^{b}	16.98±0.87^{b}	27.40 ± 1.00^{b}	27.08±0.14^{b}	28.49 ± 0.44^{b}	29.05±0.33^{b}	30.77 ± 0.67^{b}	30.50±0.6^{b}	32.93 ± 0.95^{b}	32.64±1.03^{b}
T_3	18.52 ± 0.50^{b}	18.14±0.34^{c}	29.11 ± 0.19^{c}	28.99±0.27^{c}	31.76 ± 0.70^{c}	32.18±0.73^{c}	34.03 ± 0.45^{c}	33.83±0.43^{c}	36.19 ± 0.57^{c}	35.72±0.64^{c}
T_4	13.86 ± 0.36^{a}	13.21±0.20^{a}	20.69 ± 0.89^{a}	20.48±0.14^{a}	25.36 ± 0.82^{a}	25.74±0.56^{a}	27.41 ± 0.67^{a}	26.97±0.32^{a}	28.59 ± 0.80^{a}	28.32±0.61^{a}
Low altitude (LA)										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	24.82 ± 1.15^{ab}	23.01±1.20^{b}	27.53 ± 0.77^{b}	26.27±1.26^{b}	28.66 ± 0.52^{b}	28.12±1.21^{b}	29.21 ± 0.50^{b}	28.90±0.71^{b}	30.14 ± 0.42^{b}	29.71±0.59^{b}
T_2	24.83 ± 1.15^{ab}	23.11±0.84^{b}	27.28 ± 1.34^{b}	26.01±0.51^{b}	28.53 ± 1.33^{b}	28.04±0.84^{b}	29.11 ± 1.28^{b}	28.72±0.64^{b}	30.36 ± 1.39^{b}	29.64±0.76^{b}
T_3	26.61 ± 0.73^{b}	25.61±0.22^{c}	29.72 ± 0.80^{c}	29.03±0.44^{c}	31.06 ± 0.70^{c}	30.33±0.17^{c}	31.54 ± 0.76^{c}	31.03±0.38^{c}	32.70 ± 0.87^{c}	31.84±0.34^{c}
T_4	23.12 ± 0.65^{a}	20.63±0.51^{a}	25.03 ± 0.63^{a}	23.10±0.75^{a}	25.93 ± 0.68^{a}	24.73±0.19^{a}	26.30 ± 0.73^{a}	25.17±0.20^{a}	27.38 ± 0.73^{a}	26.01±0.20^{a}
Pooled										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	17.18±0.70^{b}	23.92±1.08^{b***}	27.41±0.88^{b}	26.90±1.01^{b}	29.05±0.21^{b}	28.39±0.84^{b}	30.46±0.88^{b}	29.05±0.60^{b}	32.39±0.88^{b*}	29.93±0.51^{b}
T_2	17.17±0.85^{b}	23.97±0.98^{b***}	27.24±0.57^{b}	26.65±0.90^{b}	28.77±0.38^{b}	28.29±0.99^{b}	30.63±0.63^{b}	28.92±0.91^{b}	32.79±0.99^{b*}	29.99±1.04^{b}
T_3	18.33±0.42^{c}	26.11±0.48^{c***}	29.05±0.23^{c}	29.38±0.32^{c}	31.97±0.71^{c}	30.69±0.40^{c}	33.93±0.42^{c**}	31.29±0.54^{c}	35.96±0.61^{c**}	32.27±0.60^{c}
T_4	13.53±0.28^{a}	21.88±0.56^{a***}	20.58±0.47^{a}	24.07±0.67^{a**}	25.55±0.68^{a}	25.33±0.40^{a}	27.19±0.50^{a*}	25.73±0.37^{a}	28.46±0.68^{a*}	26.69±0.40^{a}
ALT	***		*		*		***		***	
TRE	***		***		***		***		***	
ALT×TRE	NS		***		NS		NS		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels:

****p≤0.001; **p≤0.01; *p≤0.05.*

Table 4.2 Comparative effect of location and treatments on plant height (cm) of *Brassica oleracea* var. botrytis cultivar WS 909

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	12.17 ± 0.46^{bc}	12.10±0.29^{b}	18.66 ± 0.81^{c}	18.18±0.49^{c}	28.68 ± 1.48^{b}	29.51±1.06^{b}	35.68 ± 0.81^{b}	34.63±0.27^{b}	43.76 ± 0.93^{c}	44.93±0.76^{c}
T_2	11.58 ± 0.64^{b}	11.60±0.44^{b}	17.01 ± 0.88^{b}	17.04±0.57^{b}	28.32 ± 0.13^{b}	29.14±0.63^{b}	34.23 ± 0.55^{b}	33.77±0.60^{b}	41.57 ± 0.49^{b}	42.51±0.77^{b}
T_3	12.84 ± 0.02^{c}	12.80±0.20^{c}	21.09 ± 0.61^{d}	20.95±0.14^{d}	32.39 ± 0.39^{c}	32.96±0.51^{c}	39.26 ± 1.08^{c}	38.21±1.15^{c}	47.69 ± 0.63^{d}	48.60±0.80^{d}
T_4	9.79 ± 0.34^{a}	9.58±0.37^{a}	14.58 ± 0.27^{a}	14.12±0.37^{a}	26.53 ± 0.59^{a}	25.91±0.80^{a}	29.13 ± 0.82^{a}	28.66±0.82^{a}	34.46 ± 0.91^{a}	32.48±1.85^{a}
	Low altitude (LA)									
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	28.42 ± 1.52b^{c}	28.26±0.98^{b}	30.69 ± 1.77b^{c}	30.67±1.49^{b}	33.28 ± 1.58^{b}	34.21±0.38^{b}	36.54 ± 1.66^{b}	36.36±0.81^{b}	40.72 ± 1.55^{b}	38.91±1.34^{b}
T_2	26.92 ± 1.70^{b}	26.70±1.00^{b}	29.36 ± 1.96^{b}	29.23±1.41^{b}	31.90 ± 1.62^{b}	33.53±1.50^{b}	35.03 ± 1.75^{b}	35.75±0.69^{b}	39.08 ± 0.97^{b}	38.99±0.60^{b}
T_3	30.52 ± 0.79^{c}	30.96±0.12^{c}	33.17 ± 0.44^{c}	34.79±1.21^{c}	35.84 ± 0.77^{c}	36.89±0.42^{c}	40.63 ± 0.44^{c}	39.06±0.56^{c}	45.26 ± 0.70^{c}	42.52±1.83^{c}
T_4	22.47 ± 1.22^{a}	21.04±0.87^{a}	24.76 ± 0.64^{a}	24.82±0.52^{a}	26.64 ± 0.65^{a}	26.86±0.38^{a}	29.76 ± 0.66^{a}	28.69±0.36^{a}	32.58 ± 0.22^{a}	31.00±1.94^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	12.13±0.37^{b}	28.34±1.21^{b***}	18.42±0.64^{c}	30.68±1.51^{b***}	29.10±1.27^{b}	33.75±0.95^{b**}	35.16±0.54^{b}	36.45±0.86^{b}	44.34±0.76^{c**}	39.82±0.79^{b}
T_2	11.59±0.54^{b}	26.83±0.95^{b***}	17.03±0.72^{b}	29.29±1.61^{b***}	28.73±0.35^{b}	32.72±1.52^{b*}	34.00±0.55^{b}	35.39±1.19^{b}	42.04±0.61^{b***}	39.03±0.20^{b}
T_3	12.82±0.10^{c}	30.74±0.34^{c***}	21.02±0.28^{d}	33.98±0.40^{c***}	32.67±0.42^{c}	36.37±0.28^{c***}	38.74±1.11^{c}	39.84±0.28^{c}	48.14±0.69^{d**}	43.89±1.26^{c}
T_4	9.68±0.35^{a}	21.76±1.04^{a***}	14.35±0.26^{a}	24.79±0.55^{a***}	26.22±0.69^{a}	26.76±0.28^{a}	28.89±0.80^{a}	29.22±0.32^{a}	33.47±1.34^{a}	31.79±1.06^{a}
ALT	***		***		***		**		***	
TRE	***		***		***		***		***	
ALT×TRE	***		NS		**		NS		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.1.1.2 Cauliflower cultivar WS 909

The plant height of cauliflower plants was significantly influenced by all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control) at both high altitude (HA) and low altitude (LA) locations, as indicated by the data provided in Table 4.2.

At HA, maximum plant height (12.82±0.10 cm, 21.02±0.28 cm, 32.67±0.42cm, 38.74±1.11 cm, and 48.14±0.69 cm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (12.13±0.37 cm, 18.42±0.64 cm, 29.10±1.27 cm, 35.16±0.54 cm, and 44.34±0.76 cm) and T_2 (11.59±0.54 cm, 17.03±0.72 cm, 28.73±0.35 cm, 34.00±0.55 cm, and 42.04±0.61 cm) which included FYM and *Azotobacter* respectively. The lowest plant height (9.68±0.35cm, 14.35±0.26 cm, 26.22±0.69 cm, 28.89±0.80 cm and 33.47±1.34 cm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum plant height (30.74±0.34 cm, 33.98±0.40 cm, 36.37±0.28 cm, 39.84±0.28 cm, and 43.89±1.26 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (28.34±1.21cm, 30.68±1.51 cm, 33.75±0.95 cm, 36.45±0.86 cm, and 39.82±0.79 cm) and T_2 (26.83±0.95 cm, 29.29±1.61 cm, 32.72±1.52 cm, 35.39±1.19 cm, and 39.03±0.20 cm) which included FYM and *Azotobacter* respectively. Lowest plant height (21.76±1.04 cm, 24.79±0.55 cm, 26.76±0.28 cm, 29.22±0.32 cm and 31.79±1.06 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on plant height at low and high-altitude regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum plant height at both locations. Furthermore, at 30, 45 and 60 DAT, the height of plants cultivated in the LA region was 139.78%, 61.66% and 11.33% greater than HA region. However, not significant change was measured in plant height during 75 DAT at both the locations. However, after 90 days of transplanting, the height of plants grown in the HA region was found to be increased by 9.68%, as compared to the plants grown at the LA region. The altitudes and treatments significantly affected the plant height at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was found to be significant at 30 and 60 DAT.

4.1.1.3 Knol-khol cultivar White Vienna

The plant height of knol-khol plants was notably influenced by all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control) at both high altitude (HA) and low altitude (LA) locations, as evidenced by the data presented in Table 4.3.

Table 4.3 Comparative effect of location and treatments on plant height (cm) of Brassica oleracea var. gongylodes cultivar White Vienna

High altitude (HA)						
Treatments	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	16.09 ± 0.51^{b}	16.00±0.55^{b}	24.07 ± 0.95^{b}	24.01±0.32^{b}	28.80 ± 0.92^{b}	29.42±1.00^{b}
T_2	17.17 ± 0.84^{b}	16.99±0.37^{b}	23.21 ± 1.00^{b}	23.26±0.42^{b}	27.33 ± 0.86^{b}	28.49±0.39^{b}
T_3	20.54 ± 1.00^{c}	20.53±0.88^{c}	26.31 ± 0.87^{c}	26.29±0.56^{c}	32.74 ± 1.52^{c}	33.26±1.24^{c}
T_4	14.12 ± 0.42^{a}	13.98±0.44^{a}	18.49 ± 0.85^{a}	18.44±0.65^{a}	22.40 ± 0.82^{a}	22.63±1.04^{a}
Low altitude (LA)						
Treatments	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	29.38 ± 1.08^{b}	28.4±1.13^{b}	31.53 ± 0.96^{b}	32.33±0.83^{b}	32.97 ± 1.10^{b}	32.06±1.35^{b}
T_2	28.56 ± 2.01^{b}	28.67±0.99^{b}	29.84 ± 1.58^{b}	30.93±1.51^{b}	31.44 ± 1.11^{b}	31.72±1.29^{b}
T_3	32.77 ± 2.07^{c}	31.11±0.92^{c}	34.18 ± 1.87^{c}	34.89±1.06^{c}	35.42 ± 1.43^{c}	36.68±1.89^{c}
T_4	24.64 ± 0.77^{a}	22.08±0.45^{a}	26.43 ± 0.55^{a}	25.38±1.14^{a}	27.51 ± 0.66^{a}	27.51±0.75^{a}
Pooled						
Treatments	**30 DAT**		**45 DAT**		**60 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	16.04±0.53^{b}	28.89±0.75^{b***}	24.04±0.64^{b}	31.93±0.39^{c***}	29.11±0.95^{b}	32.51±1.08^{b*}
T_2	17.08±0.60^{b}	28.61±1.31^{b***}	23.23±0.70^{b}	30.39±0.97^{b***}	27.91±0.60^{b}	31.58±0.96^{b**}
T_3	20.54±0.94^{c}	31.94±0.78^{c***}	26.30±0.71^{c}	34.53±1.09^{d***}	33.00±1.22^{c}	36.05±0.91^{c*}
T_4	14.05±0.43^{a}	23.36±0.60^{a***}	18.47±0.74^{a}	25.90±0.64^{a***}	22.52±0.91^{a}	27.51±0.10^{a***}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	*		NS		NS	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum plant height (20.54±0.94 cm, 26.30±0.71 cm and 33.00±1.22 cm) was recorded at different days after transplanting (30, 45 and 60 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (16.04±0.53 cm, 24.04±0.64 cm, and 29.11±0.95 cm) and T_2 (17.08±0.60 cm, 23.23±0.70 cm, and 27.91±0.60 cm) which included FYM and *Azotobacter* respectively. The lowest plant height (14.05±0.43 cm, 18.47±0.74 cm, and 22.52±0.91 cm at 30, 45 and 60 DAT respectively) was observed in control. Similarly, at LA maximum plant height (31.94±0.78 cm, 34.53±1.09 cm, and 36.05±0.91 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, and 60 days after transplanting followed by the treatment T_1 (28.89±0.75 cm, 31.93±0.39 cm and 32.51±1.08 cm) and T_2 (28.61±1.31 cm, 30.39±0.97 cm and 31.58±0.96 cm) which included FYM and *Azotobacter* respectively. Lowest plant height (23.36±0.60 cm, 25.90±0.64 cm and 27.51±0.10 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on plant height at low and high-altitude regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum plant height at both locations. Furthermore, at 30, 45 and 60 DAT, the height of plants cultivated in the LA region was 55.50%, 31.29% and 9.24% higher than in the HA region. The altitudes and treatments significantly affected the plant height at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was significant at 30 DAT.

4.1.1.4 Radish cultivar Pusa Himani

The study revealed that at both the HA and LA locations, the plant height of radish was considerably impacted by all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control). Table 4.4 contains the information.

Table 4.4 Comparative effect of location and treatments on plant height (cm) of Raphanus sativus cultivar Pusa Himani

High altitude (HA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T1	11.11 ± 0.44^{bc}	11.1±0.39^{b}	17.10 ± 0.70^{b}	17.16±0.90^{b}	21.78 ± 0.94^{b}	21.76±0.79^{b}
T2	10.50 ± 0.65^{b}	10.57±0.59^{b}	16.91 ± 0.88^{b}	16.96±0.42^{b}	21.50 ± 0.09^{b}	21.67±0.38^{b}
T3	12.01 ± 0.43^{c}	12.07±0.38^{c}	21.22 ± 0.44^{c}	21.26±0.27^{c}	24.39 ± 0.39^{c}	24.01±0.88^{c}
T4	9.43 ± 0.46^{a}	9.40±0.37^{a}	13.58 ± 0.08^{a}	13.43±0.29^{a}	19.01 ± 0.49^{a}	18.94±0.16^{a}
Low altitude (LA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T1	18.92 ± 1.01^{bc}	18.47±0.92^{b}	25.87 ± 0.96^{b}	26.37±0.38^{b}	27.90 ± 0.76^{b}	29.33±1.05^{b}
T2	17.30 ± 1.30^{b}	18.28±0.95^{b}	24.04 ± 1.71^{b}	25.94±0.89^{b}	27.32 ± 1.59^{b}	29.94±0.53^{b}
T3	19.94 ± 0.56^{c}	21.77±1.19^{c}	28.62 ± 1.32^{c}	29.48±0.73^{c}	30.88 ± 0.34^{c}	32.54±0.70^{c}
T4	15.03 ± 1.13^{a}	15.96±0.96^{a}	20.54 ± 1.33^{a}	21.12±0.54^{a}	21.92 ± 1.79^{a}	23.70±0.52^{a}
Pooled						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T1	11.11±0.41^{b}	18.73±0.75^{b***}	17.13±0.80^{b}	26.12±0.61^{b***}	21.77±0.87^{b}	28.62±0.73^{b***}
T2	10.53±0.62^{b}	17.79±1.11^{b***}	16.93±0.65^{b}	24.99±1.29^{b***}	21.58±0.24^{b}	28.63±0.61^{b***}
T3	12.04±0.40^{c}	21.29±0.79^{c***}	21.19±0.35^{c}	29.05±0.95^{c***}	24.20±0.54^{c}	31.71±0.47^{c***}
T4	9.42±0.41^{a}	15.50±0.87^{a***}	13.51±0.16^{a}	20.83±0.54^{a***}	18.98±0.31^{a}	22.81±0.70^{a***}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	*		NS		***	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, the tallest plants (12.04±0.40 cm, 21.19±0.35 cm, and 21.19±0.35 cm) were observed in the T_3 treatment (FYM+*Azotobacter*) at 30, 45, and 60 days after sowing (DAS), followed by T_1 (11.11±0.41 cm, 17.13±0.80 cm, and 21.77±0.87 cm) and T_2 (10.53±0.62 cm, 16.93±0.65 cm, and 21.58±0.24 cm) which included FYM and *Azotobacter* respectively. The control exhibited the lowest height (9.42±0.41 cm, 13.51±0.16 cm, and 18.98±0.31 cm) at 30, 45, and 60 DAS respectively. Similarly, at LA maximum plant height (21.29±0.79 cm, 29.05±0.95 cm, and 31.71±0.47 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, and 60 days after sowing followed by the treatment T_1 (18.73±0.75 cm, 26.12±0.61 cm and 28.62±0.73 cm) and T_2 (17.79±1.11 cm, 24.99±1.29 cm and 28.63±0.61 cm) which included FYM and *Azotobacter* respectively. Lowest plant height (15.50±0.87 cm, 20.83±0.54 cm and 22.81±0.70 cm) was observed in control.

Comparing treatments (T_1, T_2, T_3, and T_4) between low and high-altitude regions revealed that treatment T_3 (FYM+*Azotobacter*) consistently produced the tallest plants at both locations. Additionally, at 30, 45, and 60 DAS, plants in the LA region were 76.83%, 37.09%, and 31.03% taller than those in the HA region. Altitude and treatments significantly influenced plant height at various days after transplanting, with the interaction between altitude and treatment (ALT×TRE) being significant at 30 and 60 DAS.

In the current investigation, it was measured that treatment T_3 (FYM+*Azotobacter*) significantly impact the plant height of cruciferous vegetables (cabbage, cauliflower, knol-khol, and radish) at both high altitude (HA) and low altitude (LA). This effect is likely attributed to the presence of free-living nitrogen-fixing bacteria *i.e. Azotobacter*, which not only fix atmospheric nitrogen but also release phytohormones like GA3, IAA, and cytokinin. These phytohormones stimulate plant growth and enhance nutrient availability to the roots by promoting nutrient dissolution (Mahato & Kafle, 2018; Abou El-Magd *et al.,* 2014). These findings are consistent with previous studies on plant height conducted by other researchers (Upadhyay *et al.*, 2012; Bahadur *et al.,* 2006 on cabbage; Meena *et al*., 2017 on broccoli; Sepat *et al*., 2012 on tomato).

Furthermore, during the early stage *i.e.* 30 DAT/DAS, cabbage, cauliflower, knol-khol and radish plants grew taller at LA than at HA. It is being proposed that this could be an effect of abiotic stressors including cold, frost, drought, low oxygen, high wind velocity and intense UV radiations *etc*. at high altitude (Kumar, 2020). At 60 DAS/DAT, radish and knol-khol also showed a larger plant height at LA than HA location. It might have appeared due to the difference in species and environmental factors. Our results is similarly consisting with (Singh *et al*., 2011a); (Kumar, 2020). However, later stage *i.e.* 90 DAT, the higher plant height in cabbage (35.96±0.61 cm) and cauliflower (48.14±0.69 cm) was observed at HA than LA grown cabbage (32.27±0.60 cm) and cauliflower (43.89±1.26 cm) respectively. It might be because of cumulative effect of bio-organic treatment along with extended exposure to sunlight at high altitude which generally increases photosynthesis rate and enhances the growth of plant. Saapilin *et al*., (2022) also reported that plants cultivated in high light intensity had a higher growth rate or biomass than plants grown in low light intensity.

Table 4.5 Comparative effect of location and treatments on number of leaves per plant of *Brassica oleracea* var. capitata cultivar Videshi

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	9.11 ± 0.38^{b}	9.00±0.33^{b}	13.44 ± 0.19^{b}	13.11±0.19^{b}	13.56 ± 0.19^{b}	14.11±0.77^{b}	14.56 ± 0.51^{b}	14.33±0.34^{b}	15.00 ± 0.33^{b}	14.45±0.39^{b}
T_2	8.78 ± 0.38^{b}	8.89±0.51^{b}	13.11 ± 0.19^{b}	13±0.33^{b}	13.44 ± 0.19^{b}	14.00±0.33^{b}	14.33 ± 0.58^{b}	14.11±0.19^{b}	15.00 ± 0.33^{b}	14.22±0.19^{b}
T_3	10.11 ± 0.19^{c}	10.22±0.19^{c}	14.33 ± 0.33^{c}	14.22±0.19^{c}	14.89 ± 0.38^{c}	15.44±0.20^{c}	15.78 ± 0.19^{c}	15.67±0.34^{c}	16.44 ± 0.19^{c}	15.78±0.51^{c}
T_4	7.44 ± 0.38^{a}	7.33±0.34^{a}	9.56 ± 0.38^{a}	9.44±0.20^{a}	10.67 ± 0.00^{a}	10.78±0.19^{a}	11.11 ± 0.19^{a}	10.89±0.19^{a}	11.44 ± 0.38^{a}	11.33±0.34^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	13.56 ± 0.19^{b}	11.11±0.38^{b}	14.33 ± 0.33^{bc}	13.22±0.69^{b}	14.33 ± 0.33^{b}	14±0.33^{b}	15.11 ± 0.19^{b}	14.67±0.34^{b}	16.11 ± 0.19^{b}	15.78±0.19^{b}
T_2	13.67 ± 0.00^{b}	11.11±0.19^{b}	14.89 ± 0.19^{b}	13.33±0.58^{b}	14.89 ± 0.19^{c}	14.11±0.19^{b}	15.33 ± 0.33^{b}	14.89±0.51^{b}	16.00 ± 0.33^{b}	15.78±0.39^{b}
T_3	14.33 ± 0.58^{c}	13.22±0.19^{c}	15.44 ± 0.38^{c}	15.11±0.19^{c}	15.44 ± 0.38^{d}	15.33±0.34^{c}	16.56 ± 0.51^{c}	16.22±0.51^{c}	17.89 ± 0.51^{c}	17.22±0.51^{c}
T_4	12.44 ± 0.19^{a}	10.11±0.19^{a}	13.44 ± 0.19^{a}	12.11±0.19^{a}	13.44 ± 0.19^{a}	12.89±0.19^{a}	13.78 ± 0.19^{a}	13.11±0.19^{a}	14.44 ± 0.19^{a}	13.89±0.19^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	9.06±0.34^{b}	12.33±0.17^{b***}	13.28±0.19^{b}	13.78±0.48^{b}	13.83±0.44^{b}	14.17±0.34^{b}	14.44±0.42^{b}	14.89±0.10^{b}	14.72±0.25^{b}	15.94±0.10^{b***}
T_2	8.83±0.44^{b}	12.39±0.1^{b***}	13.06±0.2^{b}	14.11±0.19^{b**}	13.72±0.25^{b}	14.5±0.17^{b*}	14.22±0.39^{b}	15.11±0.38^{b*}	14.61±0.26^{b}	15.89±0.35^{b**}
T_3	10.17±0.17^{c}	13.78±0.35^{c***}	14.28±0.25^{c}	15.28±0.25^{c**}	15.17±0.29^{c}	15.39±0.26^{c}	15.72±0.09^{c}	16.39±0.10^{c***}	16.11±0.19^{c}	17.56±0.10^{c***}
T_4	7.39±0.35^{a}	11.28±0.09^{a***}	9.50±0.29^{a}	12.67±0.17^{a***}	10.72±0.09^{a}	13.17±0.00^{a***}	11.00±0.00^{a}	13.44±0.10^{a***}	11.39±0.26^{a}	14.17±0.00^{a***}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	NS		***		***		***		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.1.2 Number of leaves of cruciferous vegetable at different days after transplanting

4.1.2.1 Cabbage cultivar Videshi

The numbers of leaves of cabbage at various days after transplanting were found to be significantly affected by all the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) both at HA and LA locations. The data are present in Table 4.5.

At HA, maximum number of leaves (10.17±0.17, 14.28±0.25, 15.17±0.29, 15.72±0.09 and 16.11±0.19) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (9.06±0.34, 13.28±0.19, 13.83±0.44, 14.44±0.42, and 14.72±0.25) and T_2 (8.83±0.44, 13.06±0.20, 13.72±0.25, 14.22±0.39, and 14.61±0.26) which included FYM and *Azotobacter* respectively. The lowest plant height (7.39±0.35, 9.50±0.29, 10.72±0.09, 11.00±0.00 and 11.39±0.26 at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum number of leaves (13.78±0.35, 15.28±0.25, 15.39±0.26, 16.39±0.10, and 17.56±0.10 were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90days after transplanting followed by the treatment T_2 (12.39±0.10, 14.11±0.19, 14.50±0.17, 15.11±0.38, and 15.89±0.35 cm) and T_1 (12.33±0.17, 13.78±0.48, 14.17±0.34, 14.89±0.10, and 15.94±0.10) which included FYM and *Azotobacter* respectively. Lowest number of leaves (11.28±0.09, 12.67±0.17, 13.17±0.00, 13.44±0.10 and 14.17±0.00) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on number of leaves at HA and LA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum number of leaves at both sites. Furthermore, at 30, 45, 75 and 90 DAT, the number of leaves in the LA region was 35.50%, 7.00%, 4.26% and 9.00% respectively, as compared to the plants grown at the HA region. However, no significant change was observed in number of leaves during 60 DAT at both the locations. The altitudes and treatments significantly affected the number of leaves at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was found significant ($p \leq 0.05$) except at 30 DAT.

4.1.2.2 Cauliflower cultivar WS 909

The leaf count of cauliflower was significantly impacted by all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control-no treatment) at both high altitude (HA) and low altitude (LA) locations. The data are present in Table 4.6.

Table 4.6 Comparative effect of location and treatments on number of leaves per plant of *Brassica oleracea* var. botrytis cultivar WS 909

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	6.53 ± 0.38^{b}	6.56±0.2^{b}	11.78 ± 0.69^{b}	11.56±0.51^{b}	13.00 ± 0.58^{b}	13.22±0.39^{b}	16.00 ± 0.33^{b}	15.78±0.39^{b}	17.56 ± 0.19^{b}	17.44±0.51^{b}
T_2	6.67 ± 0.00^{b}	6.78±0.19^{b}	11.33 ± 0.58^{b}	11.44±0.2^{b}	12.78 ± 0.19^{b}	13.11±0.19^{b}	15.78 ± 0.19^{b}	15.67±0.34^{b}	17.22 ± 0.38^{b}	17.33±0.00^{b}
T_3	7.33 ± 0.00^{c}	7.58±0.16^{c}	13.22 ± 0.51^{c}	13.45±0.39^{c}	14.56 ± 0.19^{c}	14.89±0.19^{c}	18.44 ± 0.19^{c}	18.22±0.39^{c}	20.33 ± 0.33^{c}	20.56±0.51^{c}
T_4	5.44 ± 0.19^{a}	5.44±0.2^{a}	8.33 ± 0.33^{a}	8.44±0.20^{a}	10.78 ± 0.19^{a}	10.89±0.19^{a}	12.89 ± 0.69^{a}	12.55±0.69^{a}	14.00 ± 0.33^{a}	14.11±0.19^{a}
	Low altitude (LA)									
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	12.22 ± 0.19^{b}	10.67±0.34^{b}	13.89 ± 0.19^{b}	13.11±0.19^{b}	15.00 ± 0.58^{b}	14.11±0.51^{b}	17.78 ± 0.69^{b}	16.44±0.20^{b}	19.22 ± 0.69^{b}	18.22±0.19^{b}
T_2	12.33 ± 0.33^{b}	10.33±0.34^{b}	13.56 ± 0.51^{b}	13.00±0.67^{b}	14.44 ± 0.51^{b}	14.33±0.34^{b}	17.44 ± 0.19^{b}	16.22±0.51^{b}	19.00 ± 0.33^{b}	18.11±0.38^{b}
T_3	13.22 ± 0.19^{c}	12.44±0.84^{c}	14.78 ± 0.19^{c}	14.67±0.34^{c}	16.33 ± 0.33^{c}	15.55±0.39^{c}	19.78 ± 0.38^{c}	18.22±0.39^{c}	21.67 ±± 0.33^{c}	20.11±0.51^{c}
T_4	10.89 ± 0.38^{a}	8.33±0.67^{a}	12.22 ± 0.38^{a}	10.78±0.51^{a}	13.11 ± 0.51^{a}	12.33±0.34^{a}	15.78 ± 0.51^{a}	14.55±0.39^{a}	17.22 ± 0.51^{a}	16.22±0.19^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	6.55±0.25^{b}	11.45±0.25^{b***}	11.67±0.60^{b}	13.50±0.00^{b**}	13.11±0.48^{b}	14.56±0.42^{b*}	15.89±0.35^{b}	17.11±0.38^{b*}	17.5±0.33^{b}	18.72±0.25^{b**}
T_2	6.72±0.09^{b}	11.33±0.17^{b***}	11.39±0.38^{b}	13.28±0.58^{b**}	12.94±0.20^{b}	14.39±0.35^{b**}	15.72±0.25^{b}	16.83±0.29^{b**}	17.28±0.19^{b}	18.56±0.34**
T_3	7.46±0.08^{c}	12.83±0.44^{c***}	13.33±0.44^{c}	14.72±0.09^{c**}	14.72±0.09^{c}	15.94±0.10^{c***}	18.34±0.29^{c}	19.00±0.33^{c}	20.44±0.42^{c}	20.89±0.26^{c}
T_4	5.44±0.20^{a}	9.61±0.51^{a***}	8.39±0.10^{a}	11.50±0.44^{a} ***	10.83±0.17^{a}	12.72±0.42^{a} **	12.72±0.69^{a}	15.17±0.44^{a**}	14.05±0.25^{a}	16.72±0.35^{a***}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	*		**		NS		**		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum number of leaves (7.46±0.08, 13.33±0.44, 14.72±0.09, 18.34±0.29 and 20.44±0.42) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (6.55±0.25, 11.67±0.60, 13.11±0.48, 15.89±0.35, and 17.50±0.33) and T_2 (6.72±0.09, 11.39±0.38, 12.94±0.20, 15.72±0.25, and 17.28±0.19) which included FYM and *Azotobacter* respectively. The lowest plant height (5.44±0.20, 8.39±0.10, 10.83±0.17, 12.72±0.69 and 14.05±0.25at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum number of leaves (12.83±0.44, 14.72±0.09, 15.94±0.10, 19.00±0.33, and 20.89±0.26) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (11.45±0.25, 13.50±0.00, 14.56±0.42, 17.11±0.38, and 18.72±0.25) and T_2 (11.33±0.17, 13.28±0.58, 14.39±0.35, 16.83±0.29, and 18.56±0.34) which included FYM and *Azotobacter*, respectively. Lowest number of leaves (9.61±0.51, 11.50±0.44, 12.72±0.42, 15.17±0.44and 16.72±0.35) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on number of leaves at HA and LA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum number of leaves at both sites. Furthermore, at 30, 45 and 90 DAT, the number of leaves in the LA region was 72.06%, 10.40% and 8.26% respectively, as compared to the plants grown at the HA region. The altitudes and treatments significantly affected the number of leaves at different days after transplanting.

4.1.2.3 Knol-khol cultivar White Vienna

At both HA and LA locations, it was found that all four treatments (T_1, T_2, T_3 and T_4) had a significant impact on the number of leaves of knol-khol. The information is available in Table 4.7.

Table 4.7 Comparative effect of location and treatments on number of leaves per plant of *Brassica oleracea* var. gongylodes cultivar White Vienna

High altitude (HA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	8.00 ± 0.67^{b}	7.78±0.39^{b}	11.78 ± 0.51^{b}	11.78±0.19^{c}	15.44 ± 0.69^{b}	15.78±0.69^{b}
T_2	7.44 ± 0.51^{b}	7.56±0.51^{b}	11.00 ± 0.58^{b}	11.22±0.39^{b}	14.78 ± 0.69^{b}	15.89±0.51^{b}
T_3	8.44 ± 0.38^{b}	8.33±0.34^{c}	13.22 ± 0.19^{c}	13.11±0.19^{d}	18.78 ± 0.51^{c}	19.11±0.38^{c}
T_4	6.22 ± 0.51^{a}	6.11±0.19^{a}	9.22 ± 0.51^{a}	9.11±0.19^{a}	12.44 ± 0.19^{a}	12.78±0.19^{a}
Low altitude (LA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	12.67 ± 0.88^{b}	11.11±0.38^{b}	15.56 ± 0.51^{bc}	14.33±0.58^{b}	16.44 ± 0.19^{b}	16.00±0.33^{b}
T_2	12.44 ± 1.68^{b}	11.11±0.51^{b}	14.78 ± 1.35^{b}	13.89±0.51^{b}	15.89 ± 1.39^{b}	15.89±0.19^{b}
T_3	14.33 ± 0.33^{b}	13.33±0.58^{c}	16.89 ± 0.69^{c}	15.78±0.19^{c}	18.67 ± 0.33^{c}	18.11±0.19^{c}
T_4	10.11 ± 0.19^{a}	9.22±0.51^{a}	12.67 ± 0.33^{a}	11.55±0.39^{a}	13.67 ± 0.58^{a}	12.78±0.51^{a}
Pooled						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	7.89±0.51^{b}	11.89±0.26^{b***}	11.78±0.19^{c}	14.94±0.10^{b***}	15.61±0.70^{b}	16.22±0.19^{b}
T_2	7.50±0.44^{b}	11.78±1.08^{b**}	11.11±0.48^{b}	14.33±0.88^{b**}	15.33±0.60^{b}	15.89±0.79^{b}
T_3	8.39±0.35^{c}	13.83±0.44^{c***}	13.17±0.00^{d}	16.33±0.44^{c***}	18.94±0.42^{c}	18.39±0.10^{c}
T_4	6.17±0.34^{a}	9.67±0.17^{a***}	9.17±0.34^{a}	12.11±0.10^{a***}	12.61±0.19^{a}	13.22±0.35^{a}
ALT	***		***		NS	
TRE	***		***		***	
ALT×TRE	*		NS		NS	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum number of leaves (8.39±0.35, 13.17±0.00, and 18.94±0.42) was recorded at different days after transplanting (30, 45, and 60 DAT) in T3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (7.89±0.51, 11.78±0.19and 15.61±0.70) and T_2 (7.50±0.44, 11.11±0.48and 15.33±0.60) which included FYM and *Azotobacter* respectively. The lowest plant height (6.17±0.34, 9.17±0.34, and 12.61±0.19at 30, 45, and 60 DAT respectively) was observed in

control. Similarly, at LA maximum number of leaves (13.83±0.44, 16.33±0.44, and 18.39±0.10) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, and 60 days after transplanting followed by the treatment T_1 (11.89±0.26, 14.94±0.10, and 16.22±0.19) and T_2 (11.78±1.08, 14.33±0.88, and 15.89±0.79) which included FYM and *Azotobacter,* respectively. Lowest number of leaves (9.67±0.17, 12.11±0.10, and 13.22±0.35) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on number of leaves at HA and LA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum number of leaves at both sites. Furthermore, at 30 and 45 DAT, the number of leaves in the LA region was 64.84% and 23.99% respectively, as compared to the plants grown at the HA region. However, no significant change was observed in number of leaves during 60 DAT at both the locations. The altitudes significantly affected the number of leaves at different days after transplanting except 60 DAT. The interaction between altitude and treatment (ALT×TRE) was found significant at 30 DAT.

4.1.2.4 Radishcultivar Pusa Himani

The number of leaves per plant of radish was found to be significantly affected by all the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) at both HA and LA locations. The data are present in Table 4.8.

Table 4.8 Comparative effect of location and treatments on number of leaves per plant of *Raphanus sativus* cultivar Pusa Himani

High altitude (HA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	7.33 ± 0.33^{b}	7.33±0.34^{b}	14.89 ± 0.69^{b}	14.78±0.51^{b}	15.11 ± 0.51^{b}	15±0.58^{b}
T_2	7.67 ± 0.00^{b}	7.56±0.20^{b}	14.78 ± 0.84^{b}	14.67±0.34^{b}	15.78 ± 0.96^{b}	15.56±0.84^{b}
T_3	9.44 ± 0.51^{c}	9.33±0.34^{c}	16.78 ± 0.77^{c}	16.66±0.58^{c}	17.89 ± 0.19^{c}	17.78±0.39^{c}
T_4	6.11 ± 0.38^{a}	6.22±0.19^{a}	10.89 ± 0.69^{a}	10.89±0.38^{a}	12.11 ± 0.69^{a}	12.22±0.51^{a}
Low altitude (LA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	8.22 ±0.69^{b}	8.00±0.33^{b}	11.56 ± 0.38^{b}	11.56±0.20^{b}	12.44 ± 0.51^{b}	12.67±0.34^{b}
T_2	8.00 ± 1.45^{b}	7.78±0.19^{b}	11.22 ± 0.84^{b}	11.45±0.39^{b}	12.56 ± 0.96^{b}	12.45±0.69^{b}
T_3	10.11 ± 0.19^{c}	9.56±0.20^{c}	13.00 ± 0.33^{c}	13.44±0.51^{c}	15.33 ± 0.88	14.55±0.39^{c}
T_4	6.22 ± 0.19^{a}	6.22±0.51^{a}	9.22 ± 0.77^{a}	9.56±0.20^{a}	10.33 ± 0.58^{a}	10.56±0.20^{a}
Pooled						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	7.33±0.34^{b}	8.11±0.51^{b}	14.83±0.6^{b***}	11.55±0.25^{b}	15.06±0.54^{b**}	12.56±0.34^{b}
T_2	7.61±0.10^{b}	7.89±0.42^{b}	14.72±0.58^{b***}	11.33±0.44^{b}	15.67±0.88^{b**}	12.50±0.67^{b}
T_3	9.39±0.42^{c}	9.83±0.17^{c}	16.72±0.68^{c***}	13.22±0.25^{c}	17.83±0.29^{c**}	14.94±0.59^{c}
T_4	6.16±0.29^{a}	6.22±0.35^{a}	10.89±0.54^{a*}	9.39±0.35^{a}	12.17±0.60^{a*}	10.45±0.39^{a}
ALT	*		***		***	
TRE	***		***		***	
ALT×TRE	NS		**		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.
*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At the HA, the treatments T_3 (FYM+*Azotobacter*) consistently exhibited the greatest number of leaves (9.39±0.42, 16.72±0.68, and 17.83±0.29) at 30, 45, and 60 DAS, respectively. This was followed by T_1 (7.33±0.34, 14.83±0.6, and 15.06±0.54) and T_2 (7.61±0.10, 14.72±0.58, and 15.67±0.88), which included FYM and *Azotobacter* individually. Conversely, the control displayed the lowest leaf count (6.16±0.29, 10.89±0.54, and 12.17±0.60) at these respective time points. Similarly, at LA, T_3 treatment (FYM+*Azotobacter*) also demonstrated the maximum number of leaves (9.83±0.17, 13.22±0.25, and 14.94±0.59) at 30, 45, and 60 DAS. This was trailed by T_1 (8.11±0.51, 11.55±0.25, and 12.56±0.34) and T_2 (7.89±0.42, 11.33±0.44, and 12.50±0.67), incorporating FYM and *Azotobacter* separately. The control exhibited the lowest plant height (6.22±0.35, 9.39±0.35, and 10.45±0.39) at these respective time points.

However, upon comparing the effects of treatments (T_1, T_2, T_3, and T_4) on leaf count at both HA and LA, it was evident that treatment T_3 (FYM+*Azotobacter*) consistently yielded the highest number of leaves at both locations. Furthermore, at 45 and 60 days after sowing, the number of leaves in the HA region was 26.48% and 19.34% higher, respectively, compared to those in the LA region. However, no significant changes were noted in leaf count during 30 DAS at both locations. Altitude significantly influenced the number of leaves at various time points after sowing. The interaction between altitude and treatment (ALT×TRE) was found to be significant ($P < 0.05$) at 45 DAS.

In current study treatment T_3, involving the application of FYM and *Azotobacter*, exhibited the highest number of leaves per plant across different days after transplanting (30-90) and sowing (30-60) for both high altitude (HA) and low altitude (LA) Brassicaceae vegetables. The observed increase in leaf count could be attributed to the synergistic effect of FYM and *Azotobacter*. These findings are consistent with prior research by Bahadur *et al*. (2006), Hasan *et al*. (2018) on cabbage, and Kumar *et al*. (2017) on radish. Moreover, at 90 days after transplanting (DAT), LA-grown cruciferous vegetable had a higher maximum leaf count per plant compared to HA-grown crops. This difference in leaf count between altitudes could potentially be attributed to the differential environmental influences, with low altitudes exhibiting a more pronounced effect compared to high altitudes. Conversely, in radish, at 60 DAS, the highest leaf count (17.83±0.29) was recorded at HA, as compared to LA (14.94±0.59). It might be attributed to the combined influence of bio-organic fertilizer which increased the nitrogen content and higher light intensity, which notably boosted leaf production. These finding are consistent with prior research highlighting the elevated light intensity and ultraviolet radiation in the high alpine region of Leh Ladakh (Stobdan *et al*., 2018; Allen, 2016).

4.1.3 Leaf length with petiole (cm) of cruciferous vegetable at different days after transplanting

4.1.3.1 Cabbage cultivar Videshi

According to the present study, the leaf length with petiole of cabbage was found to be significantly affected by all the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) both at HA and LA locations. The data are present in Table 4.9.

Table 4.9 Comparative effect of location and treatments on leaf length with petiole (cm) of *Brassica oleracea* var. capitata cultivar Videshi

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	15.77 ± 0.67^{b}	15.67±0.40^{b}	25.11 ± 0.57^{b}	24.92±0.52^{b}	26.60 ± 0.24^{b}	26.90±0.25^{b}	28.29 ± 0.33^{b}	27.92±0.40^{b}	29.58 ± 0.67^{b}	29.22±0.48^{b}
T_2	15.72 ± 0.74^{b}	15.65±0.52^{b}	25.02 ± 0.44^{b}	24.95±0.25^{b}	26.51 ± 0.69^{b}	26.82±0.65^{b}	28.38 ± 0.32^{b}	28.24±0.32^{b}	30.63 ± 0.87^{b}	30.02±0.75^{b}
T_3	17.16 ± 0.55^{c}	17.10±0.29^{c}	27.07 ± 0.52^{c}	26.90±0.24^{c}	29.94 ± 0.53^{c}	30.09±0.54^{c}	31.18 ± 1.09^{c}	30.91±1.29^{c}	33.37 ± 0.89^{c}	33.04±0.90^{c}
T_4	12.78 ± 0.26^{a}	12.70±0.32^{a}	18.79 ± 0.62^{a}	18.71±0.57^{a}	22.41 ± 0.34^{a}	22.35±0.33^{a}	25.69 ± 0.65^{a}	24.21±0.63^{a}	26.04 ± 0.30^{a}	26.17±0.36^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	23.53 ± 1.10^{b}	20.89±0.98^{b}	25.59 ± 0.96^{b}	24.32±0.25^{b}	26.76 ± 0.66^{b}	25.23±0.34^{b}	27.22 ± 0.63^{b}	26.02±0.19^{b}	28.39 ± 0.56^{b}	27.07±0.17^{b}
T_2	23.27 ± 1.30^{ab}	20.87±0.98^{b}	25.59 ± 1.25^{b}	24.13±0.74^{b}	26.61 ± 1.28^{b}	25.77±0.92^{b}	27.12 ± 1.26^{b}	26.38±0.90^{b}	28.32 ± 1.33^{b}	27.21±0.80^{b}
T_3	25.64 ± 0.64^{c}	23.61±0.53^{c}	28.06 ± 1.23^{c}	26.72±0.31^{c}	29.43 ± 0.78^{c}	28.10±0.20^{c}	29.90 ± 0.79^{c}	28.82±0.30^{c}	30.99 ± 0.75^{c}	29.59±0.29^{c}
T_4	21.56 ± 0.68^{a}	18.83±0.15^{a}	23.06 ± 0.71^{a}	21.50±0.41^{a}	23.97 ± 0.64^{a}	22.28±0.29^{a}	24.50 ± 0.62^{a}	23.03±0.17^{a}	25.61 ± 0.49^{a}	24.08±0.08^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	15.72±0.52^{b}	22.21±0.82^{b***}	25.02±0.54^{b}	24.95±0.60^{b}	26.75±0.23^{b}	25.99±0.50^{b}	28.1±0.37^{**b}	26.62±0.37^{b}	29.4±0.58^{**b}	27.73±0.26^{b}
T_2	15.69±0.63^{b}	22.07±1.11^{b***}	24.99±0.34^{b}	24.86±0.91^{b}	26.67±0.67^{b}	26.19±1.06^{b}	28.31±0.31^{b}	26.75±0.95^{b}	30.33±0.81^{*b}	27.77±0.93^{b}
T_3	17.13±0.42^{c}	24.63±0.58^{c***}	26.98±0.38^{c}	27.39±0.63^{c}	30.02±0.54^{*c}	28.77±0.47^{c}	31.05±1.15^{c}	29.36±0.48^{c}	33.21±0.89^{**c}	30.29±0.44^{c}
T_4	12.74±0.19^{a}	20.19±0.37^{a***}	18.75±0.60^{a}	22.28±0.50^{a***}	22.38±0.34^{a}	23.12±0.46^{a}	24.95±0.56^{**a}	23.79±0.38^{a}	26.11±0.33^{**a}	24.85±0.21^{a}
ALT	***		***		NS		***		***	
TRE	***		***		***		***		***	
ALT×TRE	NS		***		NS		NS		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf length with petiole (17.13±0.42 cm, 26.98±0.38 cm, 30.02±0.54 cm, 31.05±1.15 cm, and 33.21±0.89 cm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (15.72±0.52 cm, 25.02±0.54 cm, 26.75±0.23 cm, 28.10±0.37 cm, and 29.40±0.58 cm) and T_2 (15.69±0.63 cm, 24.99±0.91 cm, 26.67±0.67 cm, 28.31±0.31 cm, and 30.33±0.81 cm) which included FYM and *Azotobacter* respectively. The lowest leaf length with petiole (12.74±0.19 cm, 18.75±0.60cm, 22.38±0.34 cm, 24.95±0.56 cm and 26.11±0.33 cm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf length with petiole (24.63±0.38 cm, 27.39±0.63 cm, 28.77±0.47 cm, 29.36±0.48 cm, and 30.29±0.44 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (22.21±0.82 cm, 24.95±0.60 cm, 25.99±0.50 cm, 26.62±0.37 cm, and 27.33±0.26 cm) and T_2 (22.07±1.11 cm, 24.86±0.91 cm, 26.19±1.06 cm, 26.75±0.95 cm, and 27.77±0.93cm) which included FYM and *Azotobacter* respectively. The lowest leaf length with petiole (20.19±0.37 cm, 22.28±0.34 cm, 23.12±0.46 cm, 23.79±0.38 cm and 24.85±0.21 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf length with petiole at low and high-altitude regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf length with petiole at both sites. Furthermore, at 30 DAT, the leaf length with petiole at LA region was 43.78% higher than in the HA region. However, no significant change was observed in leaf length with petiole during 45 and 75 DAT at both the locations. However, after 60 and 90 days of transplanting, the leaf length with petiole grown in the HA region was found to be increased by 4.34% and 9.64%, respectively, as compared to the plants grown at the LA region. The altitudes and treatments significantly affected the leaf length with petiole at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was significant at 45 DAT.

4.1.3.2 Cauliflower cultivar WS 909

Table 4.10 revealed that the leaf length with petiole of cauliflower was found to be significantly affected by all the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) at both HA and LA locations.

Table 4.10 Comparative effect of location and treatments on leaf length with petiole (cm) of *Brassica oleracea* var. botrytis cultivar WS 909

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	10.79 ± 0.42^{c}	10.76±0.27^{b}	16.74 ± 0.74^{b}	16.54±0.45^{b}	24.91 ± 1.37^{b}	26.48±0.89^{b}	32.87 ± 1.21^{b}	32.33±1.35^{b}	39.87 ± 1.62^{b}	41.08±1.04^{b}
T_2	9.99 ± 0.42^{b}	10.01±0.39^{b}	15.92 ± 0.86^{b}	15.99±0.48^{b}	27.97 ± 0.73^{b}	26.25±0.48^{b}	31.34 ± 1.04^{b}	30.76±0.81^{b}	38.63 ± 1.00^{b}	39.97±0.50^{b}
T_3	11.20 ± 0.31^{c}	11.19±0.25^{c}	18.58 ± 0.53^{c}	18.55±0.33^{c}	29.16 ± 0.41^{c}	30.28±0.55^{c}	35.70 ± 0.81^{c}	34.85±0.23^{c}	43.96 ± 0.80^{c}	44.77±1.36^{c}
T_4	8.63 ± 0.38^{a}	8.60±0.31^{a}	13.28 ± 0.34^{a}	13.21±0.07^{a}	23.23 ± 0.46^{a}	23.07±0.96^{a}	26.32 ± 1.22^{a}	25.27±1.24^{a}	30.94 ± 1.27^{a}	29.85±1.45^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	24.91± 1.85^{b}	23.89±0.97^{b}	27.89 ± 1.77^{b}	27.41±1.28^{b}	30.16 ± 1.43^{b}	29.25±0.59^{b}	33.69 ± 1.13^{b}	31.38±0.67^{b}	37.26 ± 1.22^{b}	35.60±0.96^{b}
T_2	23.10 ± 0.94^{b}	22.57±0.99^{b}	27.62 ± 1.77^{b}	26.7±1.01^{b}	29.14 ± 1.63^{b}	29.93±1.51^{b}	32.44 ± 1.48^{b}	32.20±1.45^{b}	35.72 ± 1.06^{b}	35.28±0.35^{b}
T_3	28.47 ± 0.61^{c}	26.98±0.41^{c}	31.42 ± 0.53^{c}	30.79±0.94^{c}	33.01 ± 0.32^{c}	33.19±1.29^{c}	37.66 ± 0.39^{c}	35.25±1.27^{c}	42.17 ± 0.26^{c}	39.73±1.82^{c}
T_4	19.82 ± 1.65^{a}	18.01±0.83^{a}	22.16 ± 1.12^{a}	22.05±0.56^{a}	23.73 ± 0.91^{a}	24.05±0.79^{a}	26.09 ± 0.48^{a}	25.94±1.16^{a}	29.24 ± 0.64^{a}	28.27±1.07^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	10.77±0.34^{b}	24.40±1.41^{b***}	16.65±0.59^{b}	27.65±1.53^{b***}	25.69±1.13^{b}	29.70±0.70^{b**}	32.6±1.27^{b}	32.53±0.70^{b}	40.47±1.32^{b**}	36.43±0.32^{b}
T_2	10.00±0.40^{b}	22.83±0.47^{b***}	15.95±0.66^{b}	27.16±1.35^{b***}	25.61±0.31^{b}	29.54±1.57^{b*}	31.05±0.83^{b}	32.32±1.45^{b}	39.30±0.73^{b**}	35.50±0.52^{b}
T_3	11.19±0.27^{c}	27.72±0.50^{c***}	18.57±0.37^{c}	31.11±0.41^{c***}	29.72±0.41^{c}	33.10±0.64^{c**}	35.27±0.52^{c}	36.46±0.44^{c*}	44.36±1.04^{c*}	40.95±1.03^{c}
T_4	8.62±0.34^{a}	18.92±1.10^{a***}	13.24±0.20^{a}	22.10±0.55^{a***}	23.15±0.71^{a}	23.90±0.20^{a}	25.80±1.23^{a}	26.02±0.43^{a}	30.39±1.34^{a}	28.75±0.46^{a}
ALT	***		***		***		NS		***	
TRE	***		***		***		***		***	
ALT×TRE	***		*		*		NS		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf length with petiole (11.19±0.27cm, 18.57±0.37 cm, 29.72±0.41cm, 35.27±0.52cm, and 44.36±1.04cm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (10.77±0.34cm, 16.65±0.59cm, 25.69±1.13cm, 32.6±1.27cm, and 40.47±1.32cm) and T_2 (10.00±0.40cm, 15.95±0.66 cm, 25.61±0.31cm, 31.05±0.83cm, and 39.30±0.73cm) which included FYM and *Azotobacter* respectively. The lowest leaf length with petiole (8.62±0.34cm, 13.24±0.20cm, 23.15±0.71cm, 25.80±1.23cm and 30.39±1.34cm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf length with petiole (27.72±0.50cm, 31.11±0.41cm, 33.10±0.64cm, 36.46±0.44cm, and 40.95±1.03cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (24.40±1.41cm, 27.65±1.53cm, 29.70±0.70cm, 32.53±0.70cm, and 36.43±0.32cm) and T_2 (22.83±0.47cm, 27.16±1.35cm, 29.54±1.57cm, 32.32±1.45cm, and 35.50±0.52cm) which included FYM and *Azotobacter* respectively. Lowest leaf length with petiole (18.92±1.10cm, 22.10±0.55cm, 23.90±0.20cm, 26.02±0.43cm and 28.75±0.46cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf length with petiole at HA and LA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf length with petiole at both sites. Furthermore, at 30, 45, and 60 DAT, the leaf length with petiole at LA region was 147.65%, 67.56%, and 11.37% higher than in the HA region. However, no significant change was observed in leaf length with petiole during 75 DAT at both the locations. At 90 days of transplanting, the leaf length with petiole grown in the HA region was found to be increased by 8.33%, respectively, as compared to the plants grown at the LA region. The altitudes significantly affected the leaf length with petiole at different days after transplanting except 75 DAT. The interaction between altitude and treatment (ALT×TRE) was also found significant ($P < 0.05$) except at 75 and 90 DAT.

4.1.3.3 Knol-khol cultivar White Vienna

The leaf length with petiole of knol-khol was found to be significantly affected by all the four treatments (T_1, T_2, T_3, and T_4) at both HA and LA locations. The data are present in Table 4.11.

Table 4.11 Comparative effect of location and treatments on leaf length with petiole (cm) of *Brassica oleracea* var. gongylodes cultivar White Vienna

High altitude (HA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	13.97 ± 0.49^{b}	13.91±0.36^{b}	22.00 ± 0.38^{b}	21.84±0.42^{b}	27.16 ± 0.56^{b}	27.76±0.88^{b}
T_2	14.03 ± 0.87^{b}	13.99±0.92^{b}	20.94 ± 1.06^{b}	20.9±0.79^{b}	25.27 ± 1.11^{b}	26.51±0.85^{b}
T_3	18.93 ± 0.83^{c}	18.90±0.67^{c}	24.67 ± 0.99^{c}	24.62±0.79^{c}	30.08 ± 1.40^{c}	31.01±1.33^{c}
T_4	11.74 ± 0.64^{a}	11.62±0.58^{a}	16.94 ± 0.70^{a}	16.91±0.59^{a}	20.66 ± 0.96^{a}	21.03±0.63^{a}
Low altitude (LA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	27.97 ± 1.03^{b}	26.58±0.94^{b}	30.23± 0.90^{bc}	30.12±0.61^{b}	31.24 ± 0.88^{bc}	30.43±0.51^{b}
T_2	26.76 ± 2.23^{b}	26.47±1.27^{b}	28.17 ± 1.74^{b}	29.18±1.45^{b}	29.14 ± 1.87^{b}	29.52±0.39^{b}
T_3	31.47 ± 1.98^{c}	29.02±1.35^{c}	32.80 ± 1.85^{c}	32.31±0.14^{c}	33.50 ± 1.72c	33.07±0.93^{c}
T_4	23.52 ± 0.71^{a}	20.45±0.37^{a}	24.60 ± 0.84^{a}	23.86±1.20^{a}	25.27 ± 0.87^{a}	25.55±1.28^{a}
Pooled						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	13.94±0.42^{b}	27.27±0.73^{b***}	21.92±0.4^{b}	30.17±0.19^{c***}	27.45±0.72^{b}	30.84±0.65^{c**}
T_2	14.01±0.89^{b}	26.61±1.47^{b***}	20.92±0.92^{b}	28.67±1.13^{b***}	25.89±0.97^{b}	29.34±0.87^{b**}
T_3	18.91±0.75^{c}	30.24±0.77^{c***}	24.65±0.89^{c}	32.56±0.89^{d***}	30.55±1.33^{c}	33.28±1.12^{d}
T_4	11.68±0.61^{a}	21.98±0.45^{a***}	16.93±0.65^{a}	24.23±0.55^{a***}	20.85±0.79^{a}	25.41±0.21^{a***}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	*		NS		NS	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf length with petiole (18.91±0.75 cm, 24.65±0.89 cm and 30.55±1.33 cm) was recorded at different days after transplanting (30, 45, and 60 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (13.94±0.42 cm, 21.92±0.4 cm, and 27.45±0.72 cm) and T_2 (14.01±0.89 cm, 20.92±0.92 cm, and 25.89±0.97 cm) which included FYM and *Azotobacter* respectively. The lowest leaf length with petiole (11.68±0.61cm, 16.93±0.65 cm, and 20.85±0.79 cm at 30, 45, and 60 DAT respectively) was observed in control. Similarly, at LA maximum leaf length with petiole (30.24±0.77 cm, 32.56±0.89 cm, and 33.28±1.12 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, and 60 days after transplanting followed by the treatment T_1 (27.27±0.73 cm, 30.17±0.19 cm, and 30.84±0.65 cm) and T_2 (26.61±1.47 cm, 28.67±1.13 cm, and 29.34±0.87 cm) which included FYM and *Azotobacter* respectively. Lowest leaf length with petiole (21.98±0.45 cm, 24.23±0.55 cm and 25.41±0.21 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf length with petiole at HA and LA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf length with petiole at both sites. Furthermore, at 30 and 45 DAT, the leaf length with petiole at LA region was 59.92% and 32.09% higher than in the HA region. However, no significant change was observed in leaf length with petiole during 60 DAT at both the locations. The altitudes and treatments significantly affected the leaf length with petiole at different days after transplanting except. The interaction between altitude and treatment (ALT×TRE) was also found significant ($p \leq 0.05$) except at 45 and 60 DAT.

Among the treatments, T_3 treatment exhibited a significant influence on leaf length as compared to T_1, T_2 and control at both the locations. It has been reported that bio-nitrogen treatments alone or in combination with organic manure resulted in enhancement of vegetative growth as compared to untreated plants (Saffeullah *et al*., 2021). These findings align well with previous studies by Upadhyay *et al*., 2012; Bahadur *et al*., 2006 on cabbage; Meena *et al*., 2017 on broccoli. Moreover, during the initial stage of the plant growth (30 DAT) the maximum leaf length with petiole was recorded in cabbage, cauliflower and knol-khol at LA as compared to HA grown crop. It could be due to the effect of abiotic stress condition at high altitude. Our results are in good agreement with Singh *et al.,* (2011b). At 60 DAT, in knol-khol maximum leaf length was observed at LA as compared to HA. It might be caused due to a variety of environmental variables and physiological reactions in the plant. However, At 90 DAT, the maximum leaf length with petiole was recorded in cabbage (33.21±0.89 cm) and cauliflower (44.36±1.04 cm) at HA, respectively, whereas, minimum leaf length with petiole was recorded in LA grown cabbage (30.29±0.44 cm) and cauliflower (40.95±1.03 cm). It could be due to the strong effect of FYM and *Azotobacter* and high rate of photosynthesis at high altitude location than low altitude. Singh *et al*. (2011b) and Allen *et al*. (2016) reported that plant cultivated in high light intensity and ultra violet radiation resulted in increased leaf size.

4.1.4 Leaf width (cm) of cruciferous vegetable at different days after transplanting

4.1.4.1 Cabbagecultivar Videshi

All four treatments (T_1, T_2, T_3, and T_4) were found to have a significant effect on cabbage leaf width at both HA and LA locations. Table 4.12 contains the data.

Table 4.12 Comparative effect of location and treatments on leaf width (cm) of *Brassica oleracea* var. capitata cultivar Videshi

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	9.66 ± 0.16^{b}	9.57±0.21^{b}	18.37 ± 0.15^{b}	18.32±0.20^{c}	20.91 ± 0.63^{b}	20.51±0.67^{b}	22.99 ± 0.53^{b}	22.68±0.25^{b}	23.54 ± 0.25^{b}	23.13±0.54^{b}
T_2	9.51 ± 0.02^{b}	9.55±0.17^{b}	18.08 ± 0.17^{b}	17.95±0.07^{b}	21.03 ± 0.46^{b}	20.71±0.42^{b}	22.71 ± 1.21^{b}	22.39±1.32^{b}	23.41 ± 0.36^{b}	23.18±0.27^{b}
T_3	10.07 ± 0.17^{c}	10.02±0.07^{c}	19.53 ± 0.32^{c}	19.50±0.21^{d}	23.48 ± 0.46^{c}	23.16±0.45^{c}	25.37 ± 0.26^{c}	25.12±0.27^{c}	27.11 ± 0.26^{c}	26.84±0.25^{c}
T_4	8.11 ± 0.15^{a}	7.99±0.07^{a}	13.60 ± 0.57^{a}	13.44±0.15^{a}	18.30 ± 0.12^{a}	18.16±0.05^{a}	20.47 ± 0.27^{a}	20.22±0.40^{a}	22.33 ± 0.07^{a}	22.16±0.05^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	13.22 ± 0.67^{b}	12.56±0.48^{b}	14.59 ± 0.80^{b}	14.30±0.98^{b}	17.10 ± 0.41^{bc}	16.46±0.44^{b}	17.71 ± 0.34^{bc}	16.94±0.42^{b}	18.67 ± 0.49^{b}	17.53±0.29^{b}
T_2	13.11 ± 0.48^{b}	12.21±0.30^{b}	14.27 ± 0.49^{b}	14.49±0.63^{b}	16.36 ± 1.09^{b}	16.48±0.37^{b}	16.93 ± 1.09^{b}	17.07±0.39^{b}	18.00 ± 1.12^{b}	17.82±0.36^{b}
T_3	14.50 ± 0.43^{c}	13.82±0.14^{c}	16.07 ± 0.09^{c}	16.53±0.17^{c}	18.28 ± 0.64^{c}	18.87±0.59^{c}	18.80 ± 0.67^{c}	19.47±0.69^{c}	20.08 ± 67^{c}	20.15±0.74^{c}
T_4	11.54 ± 0.24^{a}	10.22±0.22^{a}	12.73 ± 0.52^{a}	12.29±0.49^{a}	14.23 ± 0.61^{a}	14.15±0.45^{a}	14.69 ± 0.57^{a}	14.60±0.37^{a}	15.64 ± 0.32^{a}	15.24±0.48^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	9.61±0.17^{b}	12.89±0.37^{b***}	18.34±0.08^{***b}	14.44±0.69^{b}	20.71±0.65^{***b}	16.78±0.16^{b}	22.83±0.38^{***b}	17.32±0.05^{b}	23.34±0.39^{***b}	18.10±0.11^{b}
T_2	9.53±0.09^{b}	12.66±0.38^{b***}	18.01±0.12^{***b}	14.38±0.50^{b}	20.87±0.44^{***b}	16.42±0.72^{b}	22.55±1.26^{**b}	17.00±0.73^{b}	23.30±0.31^{***b}	17.91±0.74^{b}
T_3	10.04±0.08^{c}	14.16±0.18^{c***}	19.52±0.26^{***c}	16.30±0.13^{c}	23.32±0.45^{***c}	18.57±0.17^{c}	25.24±0.25^{***c}	19.13±0.15^{c}	26.97±0.25^{***c}	20.11±0.04^{c}
T_4	8.05±0.10^{a}	10.88±0.04^{a***}	13.52±0.36^{*a}	12.51±0.50^{a}	18.23±0.06^{***a}	14.20±0.11^{a}	20.34±0.34^{***a}	14.64±0.14^{a}	22.24±0.06^{***a}	15.45±0.09^{a}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	***		***		NS		NS		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf width (10.04±0.08 cm, 19.52±0.26cm, 23.32±0.45cm, 25.24±0.25 cm, and 26.97±0.25 cm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (9.61±0.17 cm, 18.34±0.08 cm, 20.71±0.65 cm, 22.83±0.38 cm, and 23.34±0.39 cm) and T_2 (9.53±0.09 cm, 18.01±0.12 cm, 20.87±0.44 cm, 22.55±1.26 cm, and 23.30±0.31 cm) which included FYM and *Azotobacter* respectively. The lowest leaf width (8.05±0.10 cm, 13.52±0.36 cm, 18.23±0.06 cm, 20.34±0.34 cm and 22.24±0.06 cm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf width (14.16±0.18 cm, 16.30±0.13 cm, 18.57±0.17 cm, 19.13±0.15 cm and 20.11±0.04 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (12.89±0.37 cm, 14.44±0.69 cm, 16.78±0.16 cm, 17.32±0.05 cm and 18.10±0.11 cm) and T_2 (12.66±0.38 cm, 14.38±0.50 cm, 16.42±0.72 cm, 17.00±0.73 cm, and 17.91±0.74 cm) which included FYM and *Azotobacter* respectively. The minimum leaf width (10.88±0.04 cm, 12.51±0.50 cm, 14.20±0.11 cm, 14.64±0.14 cm and 15.45±0.09 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf width at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf width at both sites. Furthermore, at 30 DAT, the leaf width at LA region was 41.04% higher than in the HA region. However, after 45, 60, 75 and 90 days of transplanting, the leaf width grown in the HA region was found to be increased by 19.75%, 25.58%, 31.94% and 34.11%, respectively, as compared to the plants grown at the LA region. The altitudes and treatments significantly affected the leaf width at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was also significant except 60 and 75 DAT.

4.1.4.2 Cauliflower cultivar WS 909

Table 4.13 shows that all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control) had a significant effect on cauliflower leaf width at both the HA and LA locations.

Table 4.13 Comparative effect of location and treatments on leaf width (cm) of *Brassica oleracea* var. botrytis cultivar WS 909

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	4.83 ± 0.06^{b}	4.85±0.04^{b}	8.96 ± 0.13^{b}	9.08±0.20^{b}	13.16 ± 0.18^{c}	12.70±0.15^{b}	14.62 ± 0.02^{b}	14.45±0.34^{b}	21.92 ± 0.48^{b}	22.02±0.42^{b}
T_2	4.72 ± 0.10^{ab}	4.74±0.05^{b}	8.62 ± 0.40^{b}	8.60±0.43^{b}	12.58 ± 0.22^{b}	12.30±0.19^{b}	14.80 ± 0.63^{b}	14.67±0.55^{b}	21.76 ± 0.23^{b}	22.11±0.39^{b}
T_3	5.51 ± 0.16^{c}	5.59±0.12^{c}	11.37 ± 0.37^{c}	11.31±0.36^{c}	14.32 ± 0.28^{d}	13.93±0.26^{c}	16.18 ± 0.29^{c}	16.12±0.27^{c}	23.01 ± 0.55^{c}	23.27±0.47^{c}
T_4	4.52 ± 0.10^{a}	4.49±0.03^{a}	6.82 ± 0.17^{a}	6.78±0.04^{a}	10.69 ± 0.13^{a}	10.57±0.28^{a}	12.54 ± 0.25^{a}	12.23±0.28^{a}	17.33 ± 0.18^{a}	17.28±0.14^{a}
	Low altitude (LA)									
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	10.08 ± 0.71^{b}	9.83±0.50^{b}	11.68 ± 0.56^{b}	10.89±0.26^{b}	12.56 ± 0.52^{b}	11.58±0.15^{b}	13.70 ± 0.40^{b}	12.62±0.25^{b}	14.97 ± 0.50^{c}	13.84±0.57^{b}
T_2	9.13 ± 0.32^{b}	9.02±0.78^{b}	10.93 ± 0.38^{b}	10.84±0.05^{b}	11.90 ± 0.38^{b}	11.59±0.25^{b}	12.93 ± 0.47^{b}	12.66±0.22^{b}	14.02 ± 0.39^{b}	13.68±0.20^{b}
T_3	11.79 ± 0.78^{c}	10.41±0.30^{c}	13.66 ± 0.55^{c}	12.44±0.02^{c}	14.46 ± 0.57^{c}	13.23±0.15^{c}	15.47 ± 0.55^{c}	15.01±0.42^{c}	16.89 ± 0.50^{d}	16.03±0.37^{c}
T_4	6.42 ± 0.20^{a}	6.95±0.60^{a}	8.81 ± 0.24^{a}	8.60±0.33^{a}	9.26 ± 0.28^{a}	9.31±0.61^{a}	10.48 ± 0.36^{a}	10.16±0.31^{a}	11.44 ± 0.43^{a}	11.43±0.36^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	4.84±0.04^{b}	9.96±0.53^{c***}	9.02±0.15^{b}	11.29±0.41^{b***}	12.93±0.15^{c*}	12.07±0.32^{b}	14.53±0.17^{b***}	13.16±0.16^{b}	21.97±0.38^{b***}	14.4±0.38^{c}
T_2	4.73±0.08^{b}	9.08±0.55^{b***}	8.61±0.41^{b}	10.89±0.18^{b***}	12.44±0.15^{b*}	11.75±0.25^{b}	14.73±0.59^{b**}	12.79±0.31^{b}	21.93±0.29^{b***}	13.85±0.24^{b}
T_3	5.55±0.14^{c}	11.10±0.47^{d***}	11.34±0.29^{c}	13.05±0.28^{c**}	14.13±0.27^{d}	13.84±0.31^{c}	16.15±0.28^{c*}	15.24±0.34^{c}	23.14±0.51^{c***}	16.46±0.24^{d}
T_4	4.51±0.06^{a}	6.69±0.2^{a***}	6.80±0.10^{a}	8.70±0.13^{a***}	10.63±0.20^{a**}	9.29±0.31^{a}	12.39±0.26^{a***}	10.32±0.22^{a}	17.31±0.09^{a***}	11.44±0.04^{a}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	***		NS		*		*		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf width (5.55±0.14 cm, 11.34±0.29 cm, 14.13±0.27cm, 16.15±0.28 cm, and 23.14±0.51 cm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (4.84±0.04 cm, 9.02±0.15 cm, 12.93±0.15 cm, 14.53±0.17 cm, and 21.97±0.38 cm) and T_2 (4.73±0.08 cm, 8.61±0.41 cm, 12.44±0.15cm, 14.73±0.59 cm, and 21.93±0.29 cm) which included FYM and *Azotobacter* respectively. The lowest leaf width (4.51±0.06 cm, 6.80±0.10 cm, 10.63±0.20 cm, 12.39±0.26 cm and 17.31±0.09 cm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf width (11.10±0.47 cm, 13.05±0.28 cm, 13.84±0.31 cm, 15.24±0.34 cm, and 16.46±0.24 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (9.96±0.53 cm, 11.29±0.41 cm, 12.07±0.32 cm, 13.16±0.16 cm, and 14.4±0.38 cm) and T_2 (9.08±0.55 cm, 10.89±0.18 cm, 11.75±0.25 cm, 12.79±0.31 cm, and 13.85±0.24 cm) which included FYM and *Azotobacter* respectively. The lowest leaf width (6.69±0.20 cm, 8.70±0.13 cm, 9.29±0.31 cm, 10.32±0.22 cm and 11.44±0.04 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf width at HA and LA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf width at both locations. Furthermore, at 30 and 45 DAT, the leaf width at LA region was 100% and 15.08% higher than in the HA region. However, no significant change was observed in leaf length with petiole during 60 DAT at both the locations. At 75 and 90 days of transplanting, the leaf width grown in the HA region was found to be increased by 5.97% and 40.99% respectively, as compared to the plants grown at the LA region. The altitudes and treatments significantly affected the leaf width at different days after transplanting.

4.1.4.3 Knol-khol cultivar White Vienna

Table 4.14 indicates that at both HA and LA locations, the leaf width of knol-khol was found to be significantly impacted by each of the four treatments (T1, T2, T3, and T4).

Table 4.14 Comparative effect of location and treatments on leaf width (cm) of *Brassica oleracea* var. gongylodes cultivar White Vienna

High altitude (HA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	5.49 ± 0.07^{c}	5.42±0.05^{b}	9.38 ± 0.20^{b}	9.45±0.07^{b}	10.76 ± 0.33^{b}	10.79±0.19^{b}
T_2	5.21 ± 0.12^{b}	5.31±0.11^{b}	9.61 ± 0.24^{b}	9.66±0.31^{b}	10.60 ± 0.38^{b}	10.67±0.23^{b}
T_3	6.58 ± 0.05^{d}	6.53±0.09^{c}	11.38 ± 0.05^{c}	11.35±0.11^{c}	11.33 ± 0.10^{c}	11.36±0.15^{c}
T_4	4.11 ± 0.08^{a}	4.09±0.10^{a}	7.39 ± 0.13^{a}	7.51±0.10^{a}	7.81 ± 0.30^{a}	7.92±0.13^{a}
Low altitude (LA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	9.37 ± 0.20^{b}	9.36±0.12^{b}	11.31 ± 0.47^{b}	10.88±0.09^{b}	12.06 ± 0.42^{b}	11.18±0.14^{b}
T_2	9.39 ± 0.22^{b}	9.15±0.39^{b}	11.03 ± 0.35^{b}	10.64±0.13^{b}	11.79 ± 0.42^{b}	11.02±0.18^{b}
T_3	10.7 ± 0.03^{c}	10.77±0.09^{c}	12.13 ± 0.06^{c}	12.20±0.24^{c}	12.97 ± 0.09^{c}	12.51±0.19^{c}
T_4	7.47 ± 0.39^{a}	6.71±0.09^{a}	8.96 ± 0.58^{a}	7.85±0.23^{a}	9.72 ± 0.54^{a}	8.18±0.14^{a}
Pooled						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	5.46±0.06^{c}	9.36±0.08^{b***}	9.42±0.07^{b}	11.1±0.24^{b***}	10.77±0.26^{b}	11.62±0.19^{b**}
T_2	5.26±0.11^{b}	9.27±0.24^{b***}	9.63±0.26^{b}	10.84±0.15^{b**}	10.63±0.31^{b}	11.41±0.26^{b*}
T_3	6.56±0.05^{d}	10.52±0.03^{c***}	11.37±0.08^{c}	12.17±0.10^{c***}	11.34±0.12^{c}	12.74±0.14^{c***}
T_4	4.10±0.09^{a}	7.09±0.23^{a***}	7.45±0.11^{a}	8.40±0.20^{a**}	7.87±0.21^{a}	8.95±0.26^{a**}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	***		**		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.
*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf width (6.56±0.05 cm, 11.37±0.08 cm, and 11.34±0.12 cm) was recorded at different days after transplanting (30, 45 and 60 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (5.46±0.06 cm, 9.42±0.07 cm and 10.77±0.26 cm) and T_2 (5.26±0.11 cm, 9.63±0.26 cm and 10.63±0.31 cm) which included FYM and *Azotobacter* respectively. The lowest leaf width (4.10±0.09 cm, 7.45±0.11 cm and 7.87±0.21 cm at 30, 45 and 60 DAT respectively) was observed in control. Similarly, at LA maximum leaf width (10.52±0.03 cm, 12.17±0.10 cm and 12.74±0.14 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45 and 60 days after transplanting followed by the

treatment T_1 (9.36±0.08 cm, 11.1±0.24 cm, and 11.62±0.19 cm) and T_2 (9.27±0.24 cm, 10.84±0.15 cm, and 11.41±0.26 cm) which included FYM and *Azotobacter* respectively. The lowest leaf width (7.09±0.23 cm, 8.40±0.20 cm and 8.95±0.26 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf width at HA and LA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf width at both locations. Furthermore, at 30, 45 and 60 DAT, the leaf width at LA region was 60.37%, 7.04% and 12.35% higher than in the HA region. The altitudes and treatments significantly affected the leaf width at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was found significant ($P < 0.05$) at 30 and 45 DAT.

The leaf width is an important parameter for good head, curd and knob development. In our study (Table 4.12-4.13**)** showed that the treatment (T_3) with FYM+*Azotobacter* significantly ($P<0.05$) increased the leaf width compared to control at both locations (HA & LA). It could be the application of FYM with biofertilizers due to increased microbial biomass and consequently increase in nutrient mineralization. The results were similar to the findings of Verma *et al*. (2014) and Yang *et al*. (2020). Furthermore, at 30 DAT, the maximum leaf width in cabbage, cauliflower and knol-khol was measured at LA grown crop whereas, minimum leaf width was measured at HA grown crop. The reduction of leaf width during the initial stage of plant growth due to abiotic stress conditions at high altitudes was previously discussed. However, at 90 DAT, the maximum leaf width was recorded at HA grown cabbage (26.97±0.25 cm) and cauliflower (23.14±0.51) as compared to LA grown cabbage (20.11±0.04 cm) and cauliflower (16.46±0.24 cm). The leaf width may have increased as a result of increased exposure to sunlight and light intensity. Singh *et al.* (2011b) reported that species of plants native to higher altitude locations may have evolved features specific to their unique environment, such as wider leaves allowing good capture of sunlight and nutrient uptake under demanding conditions. The result also aligns with the findings of Stobdan *et al*. (2018) and Allen (2016).

4.1.5 Leaf area (cm^2) of cruciferous vegetable at different days after transplanting

4.1.5.1 Cabbage cultivar Videshi

The leaf area of cabbage was found to be significantly affected by all the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) both at HA and LA locations (Table 4.15).

At HA, maximum leaf area (137.02±3.98 cm^2, 455.92±16.13 cm^2, 581.85±18.52 cm^2, 678.94±14.54 cm^2, and 811.16±10.11 cm^2) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (119.49±3.12 cm^2, 391.43±16.13 cm^2, 465.93±6.95 cm^2, 561.22±1.36 cm^2, and 623.18±9.41 cm^2) and T_2 (117.76±4.26 cm^2, 386.16±15.24 cm^2, 467.06±7.24 cm^2, 554.9±21.87 cm^2, and 626.3±12.52 cm^2) which included FYM and *Azotobacter* respectively. The lowest leaf area (85.33±0.35 cm^2, 198.93±4.06 cm^2, 336.96±14.67 cm^2, 424.05±20.95 cm^2 and 530.17±8.68 cm^2 at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf area (299.70±1.64 cm^2, 379.32±9.7cm^2, 447.31±5.20 cm^2, 474.59±5.25 cm^2 and 525.87±11.34 cm^2) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (235.63±9.28 cm^2, 305.21±13.59 cm^2, 377.11±6.14 cm^2, 397.43±6.60 cm^2 and 432.89±7.34 cm^2) and T_2 (236.31±12.07 cm^2, 304.16±13.17 cm^2, 367.29±16.74 cm^2, 392.37±14.46 cm^2, and 431.77±18.54 cm^2) which included FYM and *Azotobacter* respectively. The minimum leaf area (176.59±4.58 cm^2, 235.51±10.99 cm^2, 278.63±4.55 cm^2, 299.19±5.31 cm^2 and 331.13±2.34 cm^2) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf area at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf area at both sites. Furthermore, at 30 DAT, the leaf area at LA region was 118.73% higher than in the HA region. However, after 45, 60, 75 and 90 days of transplanting, the leaf area grown in the HA region was found to be increased by 20.19%, 30.08%, 43.06% and 54.25%, respectively, as compared to the plants grown at the LA region. The altitudes and treatments significantly affected the leaf area at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was also found significant.

4.1.5.2 Cauliflower cultivar WS 909

Table 4.16 indicated that the leaf area of cauliflower was found to be significantly affected by all the four treatments (T_1, T_2, T_3, and T_4) at both HA and LA locations.

Table 4.15 Comparative effect of location and treatments on leaf area (cm^2) of *Brassica oleracea* var. capitata cultivar Videshi

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	120.56 ± 5.30^{b}	118.41±1.14^{b}	393.92 ± 18.74^{b}	388.93±13.67^{b}	466.89 ± 7.72^{b}	464.98±6.37^{b}	567.66 ± 6.42^{b}	554.77±5.95^{b}	632.25 ± 12.25^{b}	614.11±7.75^{b}
T_2	118.06 ± 4.73^{b}	117.46±4.28^{b}	388.38 ± 17.94^{b}	383.92±12.68^{b}	468.52 ± 9.30^{b}	465.58±5.52^{b}	560.94 ± 19.89^{b}	548.86±25.21^{b}	631.89 ± 14.00^{b}	620.71±11.15^{b}
T_3	138.00 ± 6.49^{c}	136.03±1.68^{c}	458.18 ± 21.25^{c}	453.64±11.01^{c}	583.20 ± 20.97^{c}	580.49±16.16^{c}	686.28 ± 12.37^{c}	671.61±16.72^{c}	818.63 ± 9.52^{c}	803.69±10.71^{c}
T_4	86.04 ± 0.75^{a}	84.62±0.56^{a}	202.49 ± 7.27^{a}	195.36±1.87^{a}	338.38 ± 13.00^{a}	335.53±16.40^{a}	428.10 ± 19.86^{a}	420.00±22.05^{a}	530.88 ± 9.83^{a}	529.46±7.53^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	242.24 ± 11.34^{l}	229.02±9.75^{b}	304.94 ± 15.38^{b}	305.48±13.65^{l}	389.30 ± 9.52^{c}	364.91±6.08^{b}	407.85 ± 4.66^{b}	387.01±10.98^{l}	452.43 ± 8.41^{b}	413.36±10.63^{b}
T_2	240.15 ± 12.88^{l}	232.46±11.47^{b}	295.06 ± 12.95^{b}	313.27±14.71^{l}	360.82 ± 18.41^{b}	373.77±16.05^{l}	386.88 ± 19.17^{b}	397.87±14.19^{l}	434.75 ± 21.65^{b}	428.79±16.26^{b}
T_3	301.41 ± 5.95^{c}	297.99±9.23^{c}	372.90 ± 9.50^{c}	385.74±12.18^{l}	449.50 ± 17.08^{d}	445.12±17.13^{l}	474.20 ± 19.10^{c}	474.98±18.38^{l}	541.89 ± 22.07^{c}	509.84±22.95^{c}
T_4	181.74 ± 7.70^{a}	171.43±1.61^{a}	236.11 ± 11.59^{a}	234.92±10.44^{l}	277.97 ± 10.90^{a}	279.28±11.64^{l}	300.32 ± 17.42^{a}	298.07±11.44^{l}	337.34 ± 15.32^{a}	324.92±15.62^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	119.49±3.12^{b}	235.63±9.28^{b***}	391.43±16.13^{**b}	305.21±13.59^{b}	465.93±6.95^{***b}	377.11±6.14^{b}	561.22±1.36^{***b}	397.43±6.60^{b}	623.18±9.41^{***b}	432.89±7.34^{b}
T_2	117.76±4.26^{b}	236.31±12.07^{b***}	386.16±15.24^{**b}	304.16±13.17^{b}	467.06±7.24^{***b}	367.29±16.74^{b}	554.9±21.87^{***b}	392.37±14.46^{b}	626.3±12.52^{***b}	431.77±18.54^{b}
T_3	137.02±3.98^{c}	299.70±1.64^{c***}	455.92±16.13^{**c}	379.32±9.7^{c}	581.85±18.52^{***c}	447.31±5.20^{c}	678.94±14.54^{***c}	474.59±5.25^{c}	811.16±10.11^{***c}	525.87±11.34^{c}
T_4	85.33±0.35^{a}	176.59±4.58^{a***}	198.93±4.06^{**a}	235.51±10.99^{a}	336.96±14.67^{**a}	278.63±4.55^{a}	424.05±20.95^{***a}	299.19±5.31^{a}	530.17±8.68^{***a}	331.13±2.34^{a}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	***		***		***		***		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Table 4.16 Comparative effect of location and treatments on leaf area (cm^2) of *Brassica oleracea* var. botrytis cultivar WS 909

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	41.61 ± 2.18^{b}	41.53±1.07^{c}	114.08 ± 4.82^{b}	114.91±3.30^{c}	262.98 ± 12.79^{b}	272.23±7.94^{b}	382.91 ± 14.70^{b}	369.89±16.97^{b}	719.70 ± 25.17^{b}	738.31±20.28^{b}
T_2	38.04 ± 1.84^{ab}	38.06±1.69^{b}	107.06 ± 4.76^{b}	106.89±6.25^{b}	256.45 ± 10.07^{b}	268.88±5.30^{b}	380.99 ± 16.92^{b}	369.30±16.58^{b}	702.94 ± 3.09^{b}	725.01±13.45^{b}
T_3	49.65 ± 2.76^{c}	50.32±1.82^{d}	155.01 ± 3.61^{c}	153.92±2.15^{d}	336.13 ± 11.25^{c}	345.64±10.67^{c}	499.70 ± 21.32^{c}	488.35±15.32^{c}	825.02 ± 26.55^{c}	850.06±26.49^{c}
T_4	35.33 ± 1.56^{a}	34.19±0.89^{a}	69.05 ± 2.68^{a}	69.00±0.51^{a}	193.95 ± 2.68^{a}	190.55±3.37^{a}	282.62 ± 9.82^{a}	267.17±4.77^{a}	497.84 ± 4.64^{a}	484.23±13.04^{a}
	Low altitude (LA)									
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	207.43 ± 8.98^{c}	194.80±8.32^{c}	285.03 ± 14.08^{b}	261.00±9.49^{b}	337.60 ± 16.74^{b}	311.75±13.31^{b}	416.60 ± 19.13^{c}	375.70±11.78^{b}	512.53 ± 20.99^{c}	475.82±18.12^{b}
T_2	179.31 ± 7.99^{b}	177.92±8.27^{b}	269.09 ± 12.59^{b}	255.57±10.56^{b}	314.78 ± 15.98^{b}	310.21±14.83^{b}	379.46 ± 17.41^{b}	364.05±8.44^{b}	457.56 ± 21.29^{b}	459.56±9.33^{b}
T_3	279.88 ± 11.99^{d}	249.77±11.18^{d}	378.18 ± 15.83^{c}	352.71±11.49^{c}	429.41 ± 16.55^{c}	402.07±10.84^{c}	533.79 ± 13.53^{d}	482.80±24.18^{c}	651.82 ± 19.45^{d}	604.85±24.86^{c}
T_4	103.31 ± 3.92^{a}	100.41±1.66^{a}	164.83 ± 6.23^{a}	159.46±6.80^{a}	188.75 ± 6.70^{a}	190.61±9.44^{a}	239.41 ± 9.41^{a}	224.20±2.23^{a}	300.73 ± 11.06^{a}	283.35±13.54^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	41.57±1.60^{c}	201.11±8.60^{c***}	114.5±3.88^{c}	273.01±11.68^{b***}	267.61±10.12^{b}	324.68±7.56^{b**}	376.40±15.80^{b}	396.15±7.27^{c}	729.00±21.02^{b**} *	494.18±11.31^{c}
T_2	38.05±1.75^{b}	178.61±8.06^{b***}	106.97±5.41^{b}	262.33±10.15^{b***}	262.66±7.62^{b}	312.49±14.84^{b**}	375.14±16.71^{b}	371.75±11.17^{b}	713.97±8.17^{b***}	458.56±10.27^{b}
T_3	49.99±2.25^{d}	264.82±10.86^{d***}	154.47±0.96^{d}	365.45±12.20^{c***}	340.89±10.94^{c}	415.74±13.68^{c**}	494.02±18.16^{c}	508.29±16.38^{d}	837.54±26.29^{c***}	628.34±13.4^{d}
T_4	34.76±1.22^{a}	101.86±2.36^{a***}	69.03±1.37^{a}	162.15±0.98^{a***}	192.25±2.93^{a}	189.68±1.37^{a}	274.89±6.30^{a}	231.81±3.60^{a**} *	491.03±6.94^{a***}	292.04±2.71^{a}
ALT	***		***		***		NS		***	
TRE	***		***		***		***		***	
ALT×TRE	***		***		***		**		*	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf area (49.99±2.25 cm^2, 154.47±0.96 cm^2, 340.89±10.94 cm^2, 494.02±18.16 cm^2, and 837.54±26.29 cm^2) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (41.57±1.60 cm^2, 114.5±3.88 cm^2, 267.61±10.12 cm^2, 376.40±15.80 cm^2, and 729.00±21.02 cm^2) and T_2 (38.05±1.75 cm^2, 106.97±5.41 cm^2, 262.66±7.62 cm^2, 375.14±16.71 cm^2, and 713.97±8.17cm^2) which included FYM and *Azotobacter* respectively. The lowest leaf area (34.76±1.22 cm^2, 69.03±1.37 cm^2, 192.25±2.93 cm^2, 274.89±6.30 cm^2 and 491.03±6.94 cm^2 at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf area (264.82±10.86 cm^2, 365.45±12.20 cm^2, 415.74±13.68 cm^2, 508.29±16.38 cm^2 and 628.34±13.4 cm^2) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (201.11±8.60 cm^2, 273.01±11.68 cm^2, 324.68±7.56 cm^2, 396.15±7.27 $cm^2$and 494.18±11.31 cm^2) and T_2 (178.61±8.06 cm^2, 262.33±10.15 cm^2, 367.29312.49±14.84 cm^2, 371.75±11.17 cm^2, and 458.56±10.27 cm^2) which included FYM and *Azotobacter* respectively. The minimum leaf area (101.86±2.36 cm^2, 162.15±0.98 cm^2, 189.68±1.37 cm^2, 231.81±3.60 cm^2 and 292.04±2.71 cm^2) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf area at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf area at both sites. Furthermore, at 30, 45 and 60 DAT, the leaf area at LA region was 429.74%, 136.56% and 21.96% higher than in the HA region. However, after 90 days of transplanting, the leaf area grown in the HA region was found to be increased by 33.29%, respectively, as compared to the plants grown at the LA region. The treatments significantly affected the leaf area at different days after transplanting except 75 DAT. The interaction between altitude and treatment (ALT×TRE) was also found significant ($p \leq 0.05$).

4.1.5.3 Knol-khol cultivar White Vienna

Table 4.17 shows the leaf area of knol-khol, which was found to be substantially affected at both the HA and LA locations by all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control).

Table 4.17 Comparative effect of location and treatments on leaf area (cm^2) of *Brassica oleracea* var. gongylodes cultivar White Vienna

High altitude (HA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	54.47 ± 2.86^{b}	53.78 ± 2.87^{b}	146.53 ± 5.16^{b}	146.02 ± 4.39^{b}	190.98 ± 8.72^{c}	203.30 ± 3.62^{c}
T_2	54.99 ± 3.20^{b}	54.55 ± 0.93^{b}	139.62 ± 6.23^{b}	141.99 ± 3.46^{b}	175.82 ± 6.87^{b}	186.53 ± 8.39^{b}
T_3	83.91 ± 3.99^{c}	83.32 ± 3.84^{c}	189.41 ± 5.59^{c}	188.47 ± 4.71^{c}	240.02 ± 9.98^{d}	252.90 ± 11.86^{d}
T_4	33.29 ± 1.55^{a}	32.87 ± 1.76^{a}	90.80 ± 4.92^{a}	90.87 ± 4.20^{a}	102.96 ± 4.95^{a}	109.11 ± 5.87^{a}
Low altitude (LA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	223.56 ± 10.33^{b}	212.83 ± 8.83^{b}	291.92 ± 11.44^{c}	290.20 ± 8.08^{c}	318.85 ± 10.18^{b}	303.50 ± 12.90^{b}
T_2	217.51 ± 10.48^{b}	205.71 ± 10.17^{b}	269.16 ± 13.23^{b}	270.60 ± 14.77^{b}	300.47 ± 14.09^{b}	289.10 ± 5.40^{b}
T_3	277.01 ± 10.81^{c}	255.65 ± 7.41^{c}	345.85 ± 10.58^{d}	350.16 ± 10.97^{d}	390.04 ± 7.89^{c}	371.99 ± 9.05^{c}
T_4	146.58 ± 6.36^{a}	113.07 ± 1.59^{a}	190.29 ± 9.31^{a}	170.65 ± 3.47^{a}	219.46 ± 10.80^{a}	190.77 ± 4.08^{a}
Pooled						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	54.13 ± 2.84^{b}	$218.20\pm9.26^{b***}$	146.28 ± 4.49^{b}	$291.06\pm7.61^{c**}$	197.14 ± 4.76^{c}	$311.18\pm10.59^{c**}$
T_2	54.77 ± 2.00^{b}	$211.61\pm9.66^{b***}$	140.81 ± 4.74^{b}	$269.88\pm9.77^{b**}$	181.17 ± 6.82^{b}	$294.78\pm7.04^{b***}$
T_3	83.61 ± 3.90^{c}	$266.33\pm5.66^{c***}$	188.94 ± 5.07^{c}	$348.01\pm3.50^{d**}$	246.46 ± 10.75^{d}	$381.02\pm6.91^{d**}$
T_4	33.08 ± 1.63^{a}	$129.82\pm3.68^{a***}$	90.84 ± 4.54^{a}	$180.47\pm4.58^{a**}$	106.04 ± 4.69^{a}	$205.11\pm4.62^{a***}$
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	***		***		**	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference ($P< 0.05$, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***$p\leq0.001$; **$p\leq0.01$; *$p\leq0.05$.*

At HA, maximum leaf area (83.61±3.90 cm^2, 188.94±5.07 cm^2 and 246.46±10.75 cm^2) was recorded at different days after transplanting (30, 45 and 60 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (54.13±2.84 cm^2, 146.28±4.49 cm^2 and 197.14±4.76 cm^2) and T_2 (54.77±2.00 cm^2, 140.81±4.74 cm^2 and 181.17±6.82 cm^2) which included FYM and *Azotobacter* respectively. The

lowest leaf area (33.08±1.63 cm^2, 90.84±4.54 cm^2 and 106.04±4.69 cm^2 at 30, 45 and 60 DAT respectively) was observed in control. Similarly, at LA maximum leaf area (266.33±5.66 cm^2, 266.33±5.66 cm^2 and 381.02±6.91 cm^2) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, and 60 days after transplanting followed by the treatment T_1 (218.20±9.26 cm^2, 291.06±7.61 cm^2, and 311.18±10.59 cm^2) and T_2 (211.61±9.66 cm^2, 269.88±9.77 cm^2, and 294.78±7.04 cm^2) which included FYM and *Azotobacter* respectively. The minimum leaf area (129.82±3.68 cm^2, 180.47±4.58 cm^2 and 205.11±4.62 cm^2) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf area at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf area at both sites. Furthermore, at 30, 45 and 60 DAT, the leaf area at LA region was 218.54%, 84.19% and 54.60% higher than in the HA region. The altitude and treatments significantly affected the leaf area at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was also found significant ($p \leq 0.05$).

4.1.5.4 Radish cultivar Pusa Himani

Leaf area was notably influenced by all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control) at both high altitude (HA) and low altitude (LA) sites. Detailed data can be found in Table 4.18.

Table 4.18 Comparative effect of location and treatments on leaf area (cm^2) of *Raphanus sativus* cultivar Pusa Himani

High altitude (HA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	39.28 ± 1.57^b	39.33±0.85^b	95.47 ± 4.18^b	95.86±3.46^b	123.30 ± 5.03^b	121.93±4.17^b
T_2	38.90 ± 1.83^b	39.21±1.51^b	94.90 ± 4.92^b	95.19±2.53^b	122.60 ± 3.91^b	121.07±3.68^b
T_3	47.04 ± 2.13^c	47.09±0.50^c	125.87 ± 2.29^c	126.24±1.87^c	152.33 ± 5.31^c	150.90±4.99^c
T_4	26.56 ± 0.93^a	26.63±1.00^a	60.54 ± 1.40^a	60.75±1.29^a	85.12 ± 3.46^a	83.75±1.78^a
Low altitude (LA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	94.30 ± 4.79^c	91.36±2.40^b	161.12 ± 5.90^c	158.83±7.12^b	187.93 ± 6.49^b	180.75±6.48^b
T_2	80.89 ± 4.09^b	86.92±1.93^b	148.90 ± 5.78^b	151.28±2.44^b	174.91 ± 6.78^b	178.11±7.90^b
T_3	117.86 ± 5.18^d	116.98±5.11^c	188.55 ± 7.80^d	181.35±6.75^c	228.27 ± 10.59^c	225.76±8.13^c
T_4	55.93 ± 1.75^a	65.47±3.38^a	84.16 ± 3.78^a	91.42±4.78^a	103.45 ± 5.83^a	116.46±3.58^a
Pooled						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	39.31±1.19^b	92.83±3.24^{c***}	95.67±3.68^b	159.98±3.93^{c***}	122.62±4.42^b	184.34±4.62^{b***}
T_2	39.06±1.67^b	83.90±2.14^{b***}	95.04±3.72^b	150.09±3.82^{b***}	121.84±3.77^b	176.51±4.62^{b***}
T_3	47.07±1.11^c	117.43±0.97^{d***}	126.06±1.63^c	184.95±7.19^{d***}	151.61±4.87^c	227.26±5.04^{c***}
T_4	26.59±0.94^a	60.70±2.43^{a***}	60.64±1.34^a	87.78±2.75^{a***}	84.43±2.58^a	109.95±2.95^{a***}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	***		***		***	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At LA, the highest leaf area (117.43±0.97, 184.95±7.19, and 227.26±5.04 cm^2) was recorded at 30, 45, and 60 days after sowing (DAS) in treatment T_3 (FYM+*Azotobacter*), followed by T_1 (92.83±3.24, 159.98±3.93, and 184.34±4.62 cm^2) and T_2 (83.90±2.14, 150.09±3.82, and 176.51±4.62 cm^2), incorporating FYM and *Azotobacter* respectively. The control exhibited the lowest leaf area (60.70±2.43, 87.78±2.75, and 109.95±2.95 cm^2) at these respective time points. Similarly, at HA maximum leaf area (47.07±1.11, 126.06±1.63 and 151.61±4.87 cm^2) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, and 60 days after sowing followed by the treatment T_1 (39.31±1.19, 95.67±3.68 and 122.62±4.42 cm^2) and T_2

(39.06±1.67, 95.04±3.72 and 121.84±3.77 cm^2) which included FYM and *Azotobacter* respectively. The minimum leaf area (26.59±0.94, 60.64±1.34 and 84.43±2.58 cm^2) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf area at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf area at both sites. Furthermore, at 30, 45 and 60 DAS, the leaf area at LA region was 149.48%, 46.72% and 49.90% higher than in the HA region. The altitude and treatments significantly affected the leaf area at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was also found significant (*P <0.05*).

In present study showed that at both locations and on different days after transplanting and sowing, the leaf area was significantly greater in the T_3 treatment compared to the control (T_4). This could be due to improved nutrient availability, enhanced root development, and potential synergistic iteractions between FYM and *Azotobacter*. Another reason is that the T_3 treatment increases leaf area by increasing leaf length and width. Our results are similarly consistent with Verma *et al*. (2014); Siram *et al.* (2023) and Naher *et al.* (2014). Additionally, at 30 (DAT/DAS), cabbage, cauliflower, knol-khol and radish displayed the maximum leaf area in crops grown at LA, contrasting with those at HA. It could be the result of lower temperatures at high altitudes can slow down metabolic processes and growth rates during the early stages of plant development. Moreover, in knol-khol and radish, at 60 DAS, the maximum leaf area was also recorded at LA as compared to HA location. It might have appeared due to the difference in species, crop characteristics and superior plant growth at LA region (Singh et al., 2011a). At 90 DAT, the higher leaf area in cabbage and cauliflower was (811.16±10.11 and 837.54±26.29 cm^2) recorded at HA as compared to LA grown cabbage (525.87±11.34 cm^2) and cauliflower (628.34±13.4 cm^2). It is due to the result of maximum leaf length and width and light intensity was found at high altitude as compared to low altitude. Our result is similarly consisting with Verma *et al.* (2014) and Singh *et al.* (2011b).

4.1.6 Plant spread (cm) of cruciferous vegetable at different days after transplanting

4.1.6.1 Cabbage cultivar Videshi

Table 4.19 demonstrated that at both the HA and LA locations, all four treatments (T_1, T_2, T_3, and T_4) were found to have a significant impact on the plant spread of cabbage.

Table 4.19 Comparative effect of location and treatments on plant spread (cm) of *Brassica oleracea* var. capitata cultivar Videshi

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	14.93 ± 0.48^{b}	14.53±0.44^{b}	26.71 ± 1.04^{b}	25.56±0.59^{b}	30.31 ± 0.46^{b}	29.39±0.63^{b}	31.16 ± 1.22^{b}	30.43±1.26^{b}	34.34 ± 0.12^{c}	33.68±0.28^{c}
T_2	14.57 ± 0.66^{b}	14.45±0.40^{b}	26.72 ± 0.80^{b}	26.01±0.59^{b}	30.03 ± 0.68^{b}	29.22±0.69^{b}	30.82 ± 0.63^{b}	30.51±0.68^{b}	32.74 ± 0.20^{b}	33.19±0.30^{b}
T_3	16.33 ± 0.28^{c}	16.31±0.19^{c}	28.17 ± 0.13^{b}	27.89±0.12^{c}	32.08 ± 0.65^{c}	31.43±0.55^{c}	32.82 ± 0.38^{c}	33.12±0.27^{c}	36.84 ± 0.47^{d}	36.33±0.14^{d}
T_4	12.78 ± 0.15^{a}	12.63±0.12^{a}	20.42 ± 0.82^{a}	19.87±0.50^{a}	25.31 ± 0.68^{a}	24.80±0.60^{a}	26.33 ± 0.49^{a}	26.53±0.04^{a}	27.02 ± 0.28^{a}	26.78±0.22^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	21.43 ± 1.37^{b}	21.06±0.69^{b}	23.17 ± 1.04^{b}	24.03±0.37^{b}	26.22 ± 1.16^{b}	25.97±0.66^{b}	27.18 ± 1.12^{b}	26.60±0.65^{b}	28.46 ± 0.92^{b}	27.41±0.55^{b}
T_2	21.26 ± 1.85^{b}	21.06±0.55^{b}	23.20 ± 1.68^{b}	24.23±0.33^{b}	25.80 ± 1.29^{b}	26.17±1.07^{b}	26.74 ± 1.42^{b}	26.92±1.18^{b}	27.98 ± 1.30^{b}	27.81±1.15^{b}
T_3	23.28 ± 0.45^{b}	23.16±0.24^{c}	25.87 ± 0.34^{c}	26.06±0.43^{c}	29.48 ± 0.53^{c}	29.33±0.91^{c}	30.57 ± 0.73^{c}	30.03±0.93^{c}	31.88 ± 0.72^{c}	30.78±0.95^{c}
T_4	17.12 ± 1.65^{a}	16.71±0.88^{a}	19.24 ± 1.28^{a}	19.86±0.28^{a}	22.09 ± 1.71^{a}	22.05±0.73^{a}	22.97 ± 1.79^{a}	22.71±0.75^{a}	24.04 ± 1.84^{a}	23.55±0.78^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	14.73±0.46^{b}	21.25±0.91^{b***}	26.13±0.81^{*b}	23.60±0.68^{b}	29.85±0.55^{**b}	26.09±0.90^{b}	30.79±1.24^{*b}	26.89±0.88^{b}	34.01±0.14^{***c}	27.93±0.73^{b}
T_2	14.51±0.52^{b}	21.16±0.95^{b***}	26.37±0.69^{*b}	23.72±0.87^{b}	29.63±0.68^{**b}	25.98±1.17^{b}	30.67±0.65^{**b}	26.83±1.29^{b}	32.97±0.22^{**b}	27.89±1.21^{b}
T_3	16.32±0.24^{c}	23.22±0.31^{c***}	28.03±0.07^{***c}	25.96±0.28^{c}	31.76±0.58^{*c}	29.41±0.70^{c}	32.97±0.33^{**c}	30.30±0.82^{c}	36.59±0.27^{***d}	31.33±0.79^{c}
T_4	12.7±0.13^{a}	16.92±0.47^{a***}	20.14±0.66^{a}	19.55±0.65^{a}	25.06±0.63^{*a}	22.07±1.19^{a}	26.43±0.26^{**a}	22.84±1.21^{a}	26.90±0.25^{*a}	23.8±1.24^{a}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	**		*		NS		NS		*	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum plant spread (16.32±0.24 cm, 28.03±0.07 cm, 31.76±0.58 cm, 32.97±0.33 cm, and 36.59±0.27 cm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (14.73±0.46 cm, 26.13±0.81 cm, 29.85±0.55 cm, 30.79±1.24 cm, and 34.01±0.14 cm) and T_2 (14.51±0.52 cm, 26.37±0.69 cm, 29.63±0.68 cm, 30.67±0.65 cm, and 32.97±0.22 cm) which included FYM and *Azotobacter* respectively. The lowest plant spread (12.7±0.13 cm, 20.14±0.66 cm, 25.06±0.63 cm, 26.43±0.26 cm and 26.90±0.25 cm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum plant spread (23.22±0.31 cm, 25.96±0.28 cm, 29.41±0.70 cm, 30.30±0.82 cm and 31.33±0.79 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (21.25±0.91 cm, 23.60±0.68 cm, 26.09±0.90 cm, 26.89±0.88 cm and 27.93±0.73 cm) and T_2 (21.16±0.95 cm, 23.72±0.87 cm, 25.98±1.17 cm, 26.83±1.29 cm, and 27.89±1.21 cm) which included FYM and *Azotobacter* respectively. The minimum plant spread (16.92±0.47 cm, 19.55±0.65 cm, 22.07±1.19 cm, 22.84±1.21 cm and 23.8±1.24 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on plant spread at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum plant spread at both sites. Furthermore, at 30 DAT, the plant spread at LA region was 42.28% higher than in the HA region. However, after 45, 60, 75 and 90 days of transplanting, the plant spread grown in the HA region was found to be increased by 7.97%, 7.99%, 8.81% and 16.79%, respectively, as compared to the plants grown at the LA region. The altitudes and treatments significantly affected the plant spread at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was significant (P<0.05) at 30, 45, and 90 DAT.

4.1.6.2 Cauliflower cultivar WS 909

The plant spread of cauliflower was found to be significantly affected by all the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) at both HA and LA locations. The data are present in Table 4.20.

Table 4.20 Comparative effect of location and treatments on plant spread (cm) of *Brassica oleracea* var. botrytis cultivar WS 909

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	10.24 ± 0.47^{b}	10.22±0.28^{b}	16.80 ± 0.64^{b}	16.21±0.37^{b}	27.09 ± 1.22^{b}	26.63±0.75^{b}	28.27 ± 0.09^{b}	29.09±0.68^{b}	38.18 ± 0.33^{b}	39.26±0.70^{b}
T_2	9.23 ± 0.36^{a}	9.32±0.11^{a}	16.02 ± 0.79^{b}	15.91±0.51^{b}	26.68 ± 0.39^{b}	26.33±0.60^{b}	28.03 ± 0.06^{b}	29.04±0.15^{b}	37.59 ± 1.22^{b}	38.34±1.29^{b}
T_3	10.91 ± 0.25^{c}	11.07±0.35^{c}	19.33 ± 0.39^{c}	19.28±0.07^{c}	30.03 ± 1.22^{c}	29.19±1.21^{c}	32.69 ± 1.11^{c}	33.45±0.65^{c}	42.67 ± 0.75^{c}	43.38±0.54^{c}
T_4	8.82 ± 0.25^{a}	8.93±0.17^{a}	13.61 ± 0.34^{a}	13.30±0.18^{a}	23.40 ± 0.60^{a}	22.71±0.54^{a}	26.41 ± 0.35^{a}	26.08±0.40^{a}	28.91 ± 0.37^{a}	28.71±0.29^{a}
	Low altitude (LA)									
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	16.33 ± 1.30b^{c}	18.81±0.40^{b}	18.17 ± 1.21^{b}	21.72±0.92^{b}	22.81 ± 1.43^{b}	24.98±0.37^{b}	29.09 ± 1.70^{b}	29.68±1.25^{b}	31.66 ± 1.31^{b}	32.42±0.38^{c}
T_2	14.65 ± 1.34^{b}	18.74±0.13^{b}	17.26 ± 1.32^{b}	22.06±0.31^{b}	21.73 ± 1.91^{b}	24.94±0.29^{b}	27.86 ± 1.58^{b}	28.92±0.45^{b}	30.41 ± 1.46^{b}	31.26±0.81^{b}
T_3	18.32 ± 1.78^{c}	22.83±0.62^{c}	20.99 ± 1.66^{c}	24.34±0.32^{c}	26.94 ± 0.82^{c}	27.80±1.03^{c}	31.73 ± 0.38^{c}	32.12±0.49^{c}	34.98 ± 0.95^{c}	35.23±0.35^{d}
T_4	11.18 ± 1.57^{a}	15.08±0.61^{a}	12.84 ± 1.14^{a}	16.68±0.43^{a}	16.21 ± 0.62^{a}	18.91±0.32^{a}	20.01 ± 0.40^{a}	20.78±0.54^{a}	23.67 ± 0.40^{a}	24.70±0.75^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	10.23±0.38^{b}	17.57±0.70^{b***}	16.51±0.47^{b}	19.94±1.06^{b**}	26.86±0.99^{b**}	23.89±0.53^{b}	28.68±0.36^{b}	29.38±0.96^{b}	38.72±0.49^{b***}	32.04±0.54^{c}
T_2	9.28±0.19^{a}	16.70±0.64^{b***}	15.97±0.65^{b}	19.66±0.57^{b**}	26.51±0.49^{b**}	23.34±1.05^{b}	28.54±0.10^{b}	28.39±0.83^{b}	37.97±1.26^{b***}	30.84±0.76^{b}
T_3	10.99±0.29^{c}	20.58±1.19^{c***}	19.30±0.17^{c}	22.66±0.94^{c**}	29.61±1.21^{c*}	27.37±0.69^{c}	33.07±0.85^{c}	31.93±0.25^{c}	43.02±0.62^{c***}	35.11±0.65^{d}
T_4	8.88±0.21^{a}	13.13±1.00^{a**}	13.46±0.26^{a}	14.76±0.40^{a**}	23.06±0.56^{a***}	17.56±0.36^{a}	26.25±0.38^{a***}	20.39±0.36^{a}	28.81±0.31^{a***}	24.18±0.52^{a}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	***		*		*		***		**	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum plant spread (10.99±0.29 cm, 19.30±0.17 cm, 29.61±1.21 cm, 33.07±0.85 cm, and 43.02±0.62 cm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (10.23±0.38 cm, 16.51±0.47 cm, 26.86±0.99 cm, 28.68±0.36 cm, and 38.72±0.49 cm) and T_2 (9.28±0.19 cm, 15.97±0.65 cm, 26.51±0.49 cm, 28.54±0.10 cm, and 37.97±1.26 cm) which included FYM and *Azotobacter* respectively. The lowest plant spread (8.88±0.21 cm, 13.46±0.26 cm, 23.06±0.56 cm, 26.25±0.38 cm and 28.81±0.31 cm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum plant spread (20.58±1.19 cm, 22.66±0.94 cm, 27.37±0.69 cm, 31.93±0.25 cm and 35.11±0.65 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (17.57±0.70 cm, 19.94±1.06 cm, 23.89±0.53 cm, 29.38±0.96 cm and 32.04±0.54 cm) and T_2 (16.70±0.64 cm, 19.66±0.57 cm, 23.34±1.05 cm, 28.39±0.83 cm, and 30.84±0.76 cm) which included FYM and *Azotobacter* respectively. The minimum plant spread (13.13±1.00cm, 14.76±0.40 cm, 17.56±0.36 cm, 20.39±0.36 cm and 24.18±0.52 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on plant spread at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum plant spread at both locations. Furthermore, at 30 and 45 DAT, the plant spread at LA region was 87.26% and 17.39% higher than in the HA region. However, after 60 and 90 days of transplanting, the plant spread grown in the HA region was found to be increased by 8.17%, and 23.08%, respectively, as compared to the plants grown at the LA region. The interaction between altitude and treatment (ALT×TRE) was also found significant ($P < 0.05$) at various DAT.

4.1.6.3 Knol-khol cultivar White Vienna

Table 4.21 displays that the plant spread of knol-khol was significantly different at both the HA and LA locations.

Table 4.21 Comparative effect of location and treatments on plant spread (cm) of knol-khol cultivar White Vienna

High altitude (HA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	13.61 ± 0.72^{b}	13.58±0.52^{b}	22.23 ± 1.03^{b}	22.21±0.82^{b}	26.89 ± 0.98^{b}	27.48±1.64^{b}
T_2	13.02 ± 0.71^{b}	12.98±0.42^{b}	21.56 ± 0.88^{b}	21.61±0.6^{b}	25.80 ± 1.07^{b}	26.46±1.04^{b}
T_3	18.80 ± 0.88^{c}	18.78±0.74^{c}	26.08 ± 0.37^{c}	26.06±0.18^{c}	31.89 ± 0.98^{c}	31.91±0.80^{c}
T_4	11.08 ± 0.69^{a}	11.01±0.42^{a}	18.28 ± 0.53^{a}	18.29±0.47^{a}	22.09 ± 0.93^{a}	22.37±0.60^{a}
Low altitude (LA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	26.39 ± 1.08^{c}	24.72±0.86^{b}	28.56 ± 0.97^{c}	29.08±1.78^{b}	30.46 ± 0.69^{c}	30.49±0.85^{b}
T_2	24.32 ± 1.16^{b}	24.73±0.24^{b}	26.08 ± 1.03^{b}	28.48±0.57^{b}	27.79 ± 1.22^{b}	30.85±0.35^{b}
T_3	28.67 ± 0.55^{d}	28.66±0.18^{c}	30.43 ± 0.62^{d}	32.60±1.34^{c}	32.04 ± 1.21^{c}	34.10±1.80^{c}
T_4	21.58 ± 1.01^{a}	21.02±0.89^{a}	23.18 ± 0.81^{a}	23.97±1.18^{a}	24.90 ± 1.31^{a}	25.93±0.87^{a}
Pooled						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	13.59±0.62^{b}	25.56±0.55^{b***}	22.22±0.89^{b}	28.82±0.82^{c***}	27.18±1.31^{b}	30.47±0.70^{b*}
T_2	13.00±0.57^{b}	24.53±0.66^{b***}	21.58±0.74^{b}	27.28±0.71^{b***}	26.13±1.05^{b}	29.32±0.61^{b**}
T_3	18.79±0.81^{c}	28.66±0.18^{c***}	26.07±0.28^{c}	31.52±0.9^{d***}	31.90±0.84^{c}	33.07±1.49^{c}
T_4	11.04±0.55^{a}	21.30±0.91^{a***}	18.28±0.45^{a}	23.57±0.72^{a***}	22.23±0.76^{a}	25.42±1.07^{a***}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	*		NS		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.
*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum plant spread (18.79±0.81 cm, 26.07±0.28 cm and 31.90±0.84 cm) was recorded at different days after transplanting (30, 45 and 60 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (13.59±0.62 cm, 22.22±0.89 cm and 27.18±1.31 cm) and T_2 (13.00±0.57 cm, 21.58±0.74cm and 26.13±1.05 cm) which included FYM and *Azotobacter* respectively. The lowest plant spread (11.04±0.55 cm, 18.28±0.45 cm and 22.23±0.76 cm at 30, 45 and 60 DAT respectively) was observed in control. Similarly, at LA maximum plant spread (28.66±0.18 cm, 31.52±0.9 cm and 33.07±1.49 cm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, and 60 days after transplanting followed by the

treatment T_1 (25.56±0.55 cm, 28.82±0.82 cm and 30.47±0.70 cm) and T_2 (24.53±0.66 cm, 27.28±0.71 cm and 29.32±0.61 cm) which included FYM and *Azotobacter* respectively. The minimum plant spread (21.30±0.91 cm, 23.57±0.72 cm and 25.42±1.07 cm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on plant spread at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum plant spread at both locations. Furthermore, at 30 and 45 DAT, the plant spread at LA region was 52.53% and 20.91% higher than in the HA region. However, no significant change was recorded in plant spread during 60 DAT at both the locations. The altitudes and treatments significantly affected the plant spread at different days after transplanting. The interaction between altitude and treatment (ALT×TRE) was significant ($p \leq 0.05$) at 30 DAT.

In current investigation, at different growth periods, altitude and bio-organic treatments had a significant impact on the spread of brassica plants (Table 4.19 - 4.21). The treatment (T_3) showed the greatest plant spread, whereas the control group showed the least amount of plant spread at both locations. Previously discussed, the combined application of bio-organic treatment increases plat nutrient uptake, which correlates with increased plant growth attributes such as plant spread when compared to other treatments (T_1, T_2, and T_4). Our findings are consistent with Yang *et al*. (2020) and Upadhyay *et al.* (2012). Furthermore, at 30 DAT, the maximum plant spread for cabbage, cauliflower and knol-khol were recorded at LA whereas, minimum plant spread was found in HA grown cruciferous vegetable. Plant spread may have been suppressed due to the abiotic conditions of high altitude regions. However, later stage of plant growth *i.e*. 90 DAT, the higher plant spread was recorded at HA grown cabbage (36.59±0.27 cm) and cauliflower (43.02±0.62 cm) as compared to LA grown cabbage (31.33±0.79 cm) and cauliflower (35.11±0.65 cm). HA locations usually have cooler temperatures than LA regions. Cruciferous vegetables are adjusting to cooler conditions by spreading out more to obtain sunlight and temperature for photosynthesis, and additionally to improve their potential for growth. According to Verma *et al*. (2014) and Allen *et al.* (2016), the crop's plant spread was enhanced by high nutrient availability and light intensity.

4.1.7 Stem diameter (mm) of cruciferous vegetable at different days after transplanting

4.1.7.1 *Brassica oleracea* var. capitata cultivar Videshi

Table 4.22 revealed that all four treatments (T1, T2, T3, and T4) had a significant effect on cabbage stem diameter at both the HA and LA locations.

Table 4.22 Comparative effect of location and treatments on stem diameter (mm) of cabbage cultivar Videshi

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	7.39 ± 0.16^{b}	7.31±0.15^{b}	12.72 ± 0.41^{b}	12.55±0.31^{b}	13.12 ± 0.58^{b}	13.02±0.60^{b}	17.04 ± 0.30^{b}	17.21±0.29^{b}	22.68 ± 0.47^{b}	21.59±0.27^{b}
T_2	7.02 ± 0.12^{b}	6.98±0.14^{b}	12.60 ± 0.13^{b}	12.56±0.26^{b}	12.91 ± 0.21^{b}	12.89±0.24^{b}	16.95 ± 0.35^{b}	17.06±0.49^{b}	22.62 ± 0.15^{b}	21.56±0.33^{b}
T_3	8.46 ± 0.37^{c}	8.41±0.27^{c}	15.18 ± 0.34^{c}	15.00±0.27^{c}	15.83 ± 0.10^{c}	15.75±0.21^{c}	20.14 ± 0.49^{c}	20.35±0.43^{c}	26.23 ± 0.20^{c}	24.28±0.56^{c}
T_4	5.49 ± 0.25^{a}	5.44±0.19^{a}	8.28 ± 0.24^{a}	8.19±0.16^{a}	9.98 ± 0.14^{a}	9.93±0.08^{a}	14.41 ± 0.17^{a}	14.50±0.10^{a}	20.21 ± 0.24^{a}	17.78±0.69^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	12.11 ± 0.79^{b}	10.4±10.40^{b}	12.46±0.80^{b}	12.05±12.05^{b}	13.14 ± 0.81^{b}	13.47±13.47^{b}	15.45 ± 0.78^{b}	14.24±14.24^{b}	17.57 ± 0.72^{b}	15.00±15.00^{b}
T_2	12.19 ± 0.22^{b}	10.75±10.75^{b}	12.62± 0.29^{b}	12.13±12.13^{b}	13.23 ± 0.29^{b}	13.30±13.3^{b}	15.52 ± 0.33^{b}	14.35±14.35^{b}	17.39 ± 0.13^{b}	15.39±15.39^{b}
T_3	14.41 ± 1.52^{c}	11.83±11.83^{c}	14.88±1.49^{c}	14.22±14.22^{c}	15.51 ± 1.51^{c}	15.18±15.18^{c}	17.90 ± 1.23^{c}	16.53±16.53^{c}	20.37 ± 1.11^{c}	17.95±17.95^{c}
T_4	9.86 ± 0.75^{a}	8.29±8.29^{a}	10.47±0.61^{a}	9.90±9.90^{a}	11.01 ± 0.73^{a}	11.00±11.00a	11.83 ± 0.70^{a}	11.78±11.78^{a}	12.98 ± 0.66^{a}	12.65±12.65^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	7.35±0.15^{b}	11.25±0.31^{b***}	12.63±0.36^{b}	12.26±0.39^{b}	13.07±0.58^{b}	13.31±0.49^{b}	17.12±0.30^{**b}	14.85±0.42b	22.14±0.37^{***b}	16.29±0.49^{b}
T_2	7.00±0.13^{b}	11.47±0.27^{b***}	12.58±0.19^{b}	12.37±0.22^{b}	12.90±0.22^{b}	13.26±0.29^{b}	17.01±0.42^{***b}	14.94±0.06b	22.09±0.23^{***b}	16.39±0.13^{b}
T_3	8.43±0.32^{c}	13.12±0.68^{c***}	15.09±0.25^{c}	14.56±0.75^{c}	15.79±0.16^{c}	15.34±1.01^{c}	20.25±0.46^{**c}	17.22±0.82c	25.25±0.34^{***c}	19.16±0.66^{c}
T_4	5.46±0.22^{a}	9.08±0.36^{a***}	8.24±0.20^{a}	10.18±0.20^{a***}	9.96±0.10^{a}	11.01±0.57^{a*}	14.45±0.12^{**a}	11.81±0.59a	19.00±0.24^{***a}	12.82±0.53^{a}
ALT	***		NS		NS		***		***	
TRE	***		***		***		***		***	
ALT×TRE	NS		***		NS		NS		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P <0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum stem diameter (8.43±0.32 mm, 15.09±0.25 mm, 15.79±0.16 mm, 20.25±0.46 mm, and 25.25±0.34 mm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (7.35±0.15 mm, 12.63±0.36 mm, 13.07±0.58 mm, 17.12±0.30 mm, and 22.14±0.37 mm) and T_2 (7.00±0.13 mm, 12.58±0.19 mm, 12.90±0.22 mm, 17.01±0.42 mm, and 22.09±0.23 mm) which included FYM and *Azotobacter* respectively. The lowest stem diameter (5.46±0.22 mm, 8.24±0.20 mm, 9.96±0.10 mm, 14.45±0.12 mm and 19.00±0.24 mm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum stem diameter (13.12±0.68 mm, 14.56±0.75 mm, 15.34±1.01 mm, 17.22±0.82 mm and 19.16±0.66 mm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_2 (11.47±0.27 mm, 12.37±0.22 mm, 13.26±0.29 mm, 14.94±0.06 mm, and 16.39±0.13 mm) and T_1 (11.25±0.31 mm, 12.26±0.39 mm, 13.31±0.49 mm, 14.85±0.42 mm and 16.29±0.49 mm) which included FYM and *Azotobacter* respectively. The minimum stem diameter (9.08±0.36 mm, 10.18±0.20 mm, 11.01±0.57 mm, 11.81±0.59 mm and 12.82±0.53 mm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on stem diameter at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum stem diameter at both sites. Furthermore, at 30 DAT, the plant spread at LA region was 55.63% higher than in the HA region. However, no significant change was observed in stem diameter during 45 and 60 DAT at both the locations. However, after 75 and 90 days of transplanting, the stem diameter grown in the HA region was found to be increased by 17.60% and 31.78%, respectively, as compared to the plants grown at the LA region. The altitudes significantly affected the stem diameter of plant except 45 and 60 DAT. The treatments significantly affected the stem diameter at different days after transplanting.

4.1.7.2 *Brassica oleracea* var. botrytis cultivar WS 909

The stem diameter of cauliflower was found to be significantly affected by all the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) at both HA and LA locations. The data are displays in Table 4.23.

Table 4.23 Comparative effect of location and treatments on stem diameter (mm) of *Brassica oleracea* var. botrytis cultivar WS 909

High altitude (HA)										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	4.20 ± 0.20^{b}	4.23±0.13^{b}	7.71 ± 0.13^{b}	7.70±0.10^{c}	9.85 ± 0.41^{b}	9.86±0.34^{b}	13.51 ± 0.32^{b}	13.08±0.04^{b}	20.58 ± 0.19^{b}	20.17±0.20^{b}
T_2	4.07 ± 0.09^{b}	4.15±0.07^{b}	7.14 ± 0.38^{b}	7.19±0.26^{b}	9.81 ± 0.24^{b}	9.86±0.30^{b}	13.44 ± 0.61^{b}	13.10±0.69^{b}	20.29 ± 0.14^{b}	20.62±0.19^{b}
T_3	5.34 ± 0.06^{c}	5.31±0.07^{c}	9.44 ± 0.45^{c}	9.45±0.33^{d}	11.28 ± 0.21^{c}	11.31±0.06^{c}	17.27 ± 0.57^{c}	16.39±0.91^{c}	24.20 ± 0.43^{c}	24.39±0.58^{c}
T_4	3.73 ± 0.15^{a}	3.72±0.13^{a}	5.64 ± 0.14^{a}	5.66±0.10^{a}	8.45 ± 0.25^{a}	8.43±0.29^{a}	11.53 ± 0.32^{a}	11.04±0.45^{a}	16.68 ± 0.32^{a}	16.41±0.33^{a}
	Low altitude (LA)									
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	9.79 ± 0.66^{b}	7.81±0.24^{b}	10.71 ± 1.06^{b}	10.83±0.24^{b}	11.99 ± 0.86^{b}	11.96±0.03^{b}	14.63 ± 0.95^{b}	14.22±0.08^{b}	16.26 ± 0.87^{b}	16.31±0.42^{b}
T_2	9.10 ± 0.91^{b}	7.92±0.35^{b}	10.00 ± 0.81^{b}	10.76±0.06^{b}	11.15 ± 1.14^{b}	11.84±0.41^{b}	13.44 ± 1.11^{b}	13.77±0.06^{b}	15.52 ± 1.10^{b}	16.49±0.23^{b}
T_3	11.25 ± 0.50^{c}	9.39±0.05^{c}	12.63 ± 0.24^{c}	12.86±0.49^{c}	13.67 ± 0.33^{c}	13.88±0.30^{c}	18.54 ± 0.72^{c}	17.03±0.56^{c}	21.82 ± 0.81^{c}	20.06±0.91^{c}
T_4	6.87 ± 0.27^{a}	6.25±0.72^{a}	7.82 ± 0.45^{a}	7.95±0.01^{a}	8.92 ± 0.21^{a}	8.88±0.24^{a}	11.33 ± 0.47^{a}	11.00±0.31^{a}	12.43 ± 0.53^{a}	12.31±0.23^{a}
Pooled										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	4.21±0.16^{b}	8.80±0.26^{b***}	7.71±0.12^{c}	10.77±0.43^{b***}	9.85±0.36^{b}	11.97±0.41^{b**}	13.3±0.18^{b}	14.43±0.48^{b*}	20.37±0.19^{b***}	16.29±0.61^{b}
T_2	4.11±0.07^{b}	8.51±0.63^{b***}	7.17±0.32^{b}	10.38±0.42^{b***}	9.83±0.27^{b}	11.49±0.76^{b*}	13.27±0.63^{b}	13.61±0.57^{b}	20.46±0.16^{b***}	16.00±0.54^{b}
T_3	5.33±0.02^{c}	10.32±0.23^{c***}	9.45±0.39^{d}	12.74±0.30^{c***}	11.3±0.14^{c}	13.78±0.12^{c***}	16.83±0.74^{c}	17.79±0.64^{c}	24.30±0.50^{c**}	20.94±0.85^{c}
T_4	3.73±0.14^{a}	6.56±0.29^{a***}	5.65±0.12^{a}	7.89±0.23^{a***}	8.44±0.27^{a}	8.90±0.16^{a}	11.29±0.35^{a}	11.16±0.33^{a}	16.55±0.32^{a***}	12.37±0.28^{a}
ALT	***		***		***		*		***	
TRE	***		***		***		***		***	
ALT×TRE	***		*		***		NS		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum stem diameter (5.33±0.02 mm, 9.45±0.39 mm, 11.3±0.14 mm, 16.83±0.74 mm, and 24.30±0.50 mm) was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (4.21±0.16 mm, 7.71±0.12 mm, 9.85±0.36 mm, 13.3±0.18 mm, and 20.37±0.19 mm) and T_2 (4.11±0.07 mm, 7.17±0.32 mm, 9.83±0.27 mm, 13.27±0.63 mm, and 20.46±0.16 mm) which included FYM and *Azotobacter* respectively. The lowest stem diameter (3.73±0.14 mm, 5.65±0.12 mm, 8.44±0.27 mm, 11.29±0.35 mm and 16.55±0.32 mm at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum stem diameter (10.32±0.23 mm, 12.74±0.30 mm, 13.78±0.12 mm, 17.79±0.64 mm and 20.94±0.85 mm) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (8.80±0.26 mm, 10.77±0.43 mm, 11.97±0.41 mm, 14.43±0.48 mm, and 16.29±0.61 mm) and T_2 (8.51±0.63 mm, 10.38±0.42 mm, 11.49±0.76 mm, 13.61±0.57 mm and 16.00±0.54 mm) which included FYM and *Azotobacter* respectively. The minimum stem diameter (6.56±0.29 mm, 7.89±0.23 mm, 8.90±0.16 mm, 11.16±0.33 mm and 12.37±0.28 mm) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on stem diameter at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum stem diameter at both sites. Furthermore, at 30, 45 and 60 DAT, the plant spread at LA region was 93.74%, 34.81% and 21.95% higher than in the HA region. However, no significant change was recorded in stem diameter during 75 DAT at both the locations. At 90 days of transplanting, the stem diameter grown in the HA region was found to be increased by 16.03%, respectively, as compared to the plants grown at the LA region. The altitudes significantly affected the stem diameter of plant except 45 and 60 DAT. The interaction between altitude and treatment (ALT×TRE) was found significant ($p \leq 0.05$) at 30, 45 and 60 DAT.

Tables 4.22 to 4.23 of the current study show that there was a significant difference in stem diameter across all growth stages depending on the treatments (T_1, T_2, T_3, and T_4) and altitude (HA & LA). Among the treatments, T_3 treatment demonstrated superior stem diameter at various days after transplantation at both locations. It could be the result of beneficial soil microbes are fostered by bio-organic farming, and they

are essential to the availability and cycling of nutrients. These microorganisms aid in the breakdown of organic matter into forms that plants can absorb more readily, which improves nutrient uptake and promotes stem development. These findings are consistent with previous reported studies by Bahadur *et al*. (2006), Hasan *et al.* (2018). Moreover, during the initial stage of plant growth (30 DAT), LA-grown cruciferous vegetable such as cabbage and cauliflower exhibited larger stem diameters compared to their counterparts grown at HA. This might be attributed to the adverse abiotic stress conditions prevailing at higher altitudes. Our results align well with those reported by Singh *et al*. (2011b). However, at later stage *i.e.* 90 DAT, the maximum stem diameter was observed in cruciferous vegetable grown at HA, while the minimum stem diameter was noted in LA-grown crop. It could be caused on by stronger winds and more UV light because of thinner atmospheres. Crops may grow thicker stems in response to these circumstances in order to provide structural support and defense against harm. Another factors contributing to these variations include the impact of treatments, soil nutrient composition, and the heightened rate of photosynthesis characteristic of higher altitudes compared to lower ones. Our findings are consistent with those of Allen *et al*. (2016) and Singh *et al*. (2011b).

4.1.8 Leaf chlorophyll content (cci) of cruciferous vegetable at different days after transplanting

4.1.8.1 Cabbage cultivar Videshi

The leaf chlorophyll content of cabbage was found to be significantly affected by all the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter* and control) both at HAand LA locations (Table 4.24).

Table 4.24 Comparative effect of location and treatments on leaf chlorophyll content (cci) of *Brassica oleracea var. capitata* cultivar Videshi

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	27.48 ± 0.34^{b}	27.37±0.34^{b}	47.20 ± 1.38^{b}	45.73±2.02^{b}	50.29 ± 2.03^{b}	49.10±0.90^{b}	54.03 ± 1.05^{b}	52.47±1.14^{b}	58.04 ± 1.14^{b}	56.04±0.62^{b}
T_2	27.40 ± 0.87^{b}	27.31±0.39^{b}	47.42 ± 0.96^{b}	46.68±0.47^{b}	50.16 ± 1.34^{b}	49.00±1.54^{b}	54.00 ± 0.86^{b}	52.72±1.05^{b}	58.39 ± 0.60^{b}	55.71±0.53^{b}
T_3	33.68 ± 1.51^{c}	32.98±0.83^{c}	55.92 ± 2.37^{c}	55.47±2.18^{c}	56.28 ± 1.30^{c}	56.03±1.34^{c}	62.70 ± 2.64^{c}	60.57±2.29^{c}	65.92 ± 2.87^{c}	63.15±1.03^{c}
T_4	22.67 ± 0.99^{a}	22.53±1.10^{a}	38.99 ± 1.12^{a}	38.48±0.84^{a}	43.58 ± 1.85^{a}	42.53±2.25^{a}	48.89 ± 0.97^{a}	46.52±0.61^{a}	50.90 ± 0.41^{a}	48.48±0.83^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	29.84 ± 0.77^{b}	26.81±1.17^{b}	35.23 ± 2.28^{b}	33.49±0.97^{b}	39.52 ± 0.85^{b}	37.16±0.72^{b}	42.62 ± 1.10^{b}	40.97±0.66^{b}	45.86 ± 0.96^{b}	43.87±1.29^{b}
T_2	29.93 ± 2.07^{b}	26.86±0.64^{b}	34.41 ± 2.06^{b}	32.52±1.13^{b}	39.44 ± 0.20^{b}	37.72±0.50^{b}	43.07 ± 0.12^{b}	40.26±1.17^{b}	45.92 ± 1.22^{b}	43.24±0.92^{b}
T_3	34.73 ± 0.15^{c}	30.98±0.43^{c}	40.23 ± 1.72^{c}	38.04±0.40^{c}	42.66 ± 0.57^{c}	41.78±1.21^{c}	46.60 ± 0.56^{c}	44.68±0.69^{c}	51.90 ± 1.33^{c}	48.52±1.43^{c}
T_4	26.43 ± 1.08^{a}	22.73±0.57^{a}	30.39 ± 0.86^{a}	28.84±0.93^{a}	32.16 ± 0.50^{a}	30.63±1.51^{a}	34.47 ± 0.70^{a}	32.69±1.23^{a}	37.42 ± 0.99^{a}	35.60±1.30^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	27.42±0.32^{b}	28.33±0.47^{b}	46.47±1.61^{***b}	34.36±1.56^{b}	49.70±1.47^{***b}	38.34±0.67^{b}	53.25±1.08^{***b}	41.79±0.83^{b}	57.04±0.85^{***b}	44.86±0.84^{b}
T_2	27.35±0.63^{b}	28.40±0.72^{b}	47.05±0.72^{***b}	33.47±0.96^{b}	49.58±1.30^{***b}	38.58±0.23^{b}	53.36±0.95^{***b}	41.67±0.64^{b}	57.05±0.37^{***b}	44.59±1.07^{b}
T_3	33.33±1.16^{c}	32.86±0.30^{c}	55.69±2.26^{***c}	39.13±0.74^{c}	56.16±0.02^{***c}	42.22±0.60^{c}	61.63±2.33^{***c}	45.64±0.53^{c}	64.53±1.81^{***c}	50.21±1.33^{c}
T_4	22.6±1.04^{a}	24.58±0.67^{a}	38.73±0.87^{***a}	29.62±0.37^{a}	43.06±2.04^{***a}	31.39±0.50^{a}	47.71±0.48^{***a}	33.58±0.49^{a}	49.69±0.50^{***a}	36.51±0.67^{a}
ALT	**		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	NS		***		NS		**		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf chlorophyll content (33.33±1.16 cci, 55.69±2.26 cci, 56.16±0.02 cci, 61.63±2.33 cci, and 64.53±1.81 cci was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (27.42±0.32 cci, 46.47±1.61 cci, 49.70±1.47 cci, 53.25±1.08 cci, and 57.04±0.85 cci) and T_2 (27.35±0.63 cci, 47.05±0.72 cci, 49.58±1.30 cci, 53.36±0.95 cci, and 57.05±0.37 cci) which included FYM and *Azotobacter* respectively. The lowest leaf chlorophyll content (22.6±1.04 cci, 38.73±0.87 cci, 43.06±2.04 cci, 47.71±0.48 cci and 49.69±0.50 cci at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf chlorophyll content (32.86±0.30 cci, 39.13±0.74 cci, 42.22±0.60 cci, 45.64±0.53 cci and 50.21±1.33 cci) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_2 (28.40±0.72 cci, 33.47±0.96 cci, 38.58±0.23 cci, 41.67±0.64 cci, and 44.59±1.07 cci) and T_1 (28.33±0.47 cci, 34.36±1.56 cci, 38.34±0.67 cci, 41.79±0.83 cci and 44.86±0.84 cci) which included FYM and *Azotobacter* respectively. The minimum leaf chlorophyll content (24.58±0.67 cci, 29.62±0.37 cci, 31.39±0.50 cci, 33.58±0.49 cci and 36.51±0.67 cci) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf chlorophyll content at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf chlorophyll content at both locations. Furthermore, no significant change was observed in leaf chlorophyll content during 30 DAT, at both HA and LA regions. However, after 45, 60, 75 and 90 days of transplanting, the leaf chlorophyll content in the HA region was found to be increased by 42.32%, 33.02%, 35.04% and 28.52%, respectively, as compared to the plants grown at the LA region. The interaction between altitude and treatment (ALT×TRE) was found significant ($P < 0.05$) at 45 and 75 DAT.

4.1.8.2 Cauliflower cultivar WS 909

Table 4.25 illustrates that at both the HA and LA locations, all four treatments (T_1, T_2, T_3, and T_4) had a significant impact on the chlorophyll content of the cauliflower leaves.

Table 4.25 Comparative effect of location and treatments on leaf chlorophyll content (cci) of *Brassica oleracea* var. botrytis cultivar WS 909

High altitude (HA)										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	26.00 ± 1.38^{b}	25.20±1.42^{b}	42.66 ± 0.89^{b}	42.75±0.69^{b}	44.98 ± 0.74^{b}	44.03±0.47^{b}	49.11 ± 1.08^{b}	45.67±1.37^{b}	54.53 ± 0.81^{b}	52.44±0.91^{b}
T_2	25.58 ± 1.20^{b}	25.23±1.25^{b}	42.87 ± 1.06^{b}	43.00±1.46^{b}	44.26 ± 1.49^{b}	44.46±1.54^{b}	48.14 ± 0.99^{b}	46.09±0.46^{b}	54.42 ± 1.15^{b}	52.64±1.71^{b}
T_3	32.74 ± 1.62^{c}	31.77±0.84^{c}	49.00 ± 1.13^{c}	49.37±1.42^{c}	50.07 ± 0.46^{c}	50.78±0.90^{c}	53.42 ± 1.54^{c}	52.88±1.54^{c}	59.57 ± 0.66^{c}	58.98±0.35^{c}
T_4	21.48 ± 1.11^{a}	21.03±0.17^{a}	38.44 ± 1.41^{a}	38.49±0.69^{a}	38.64 ± 0.24^{a}	39.32±0.17^{a}	41.46 ± 0.77^{a}	40.86±0.69^{a}	45.69 ± 1.04^{a}	43.99±1.51^{a}
	Low altitude (LA)									
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	27.92 ± 1.05^{b}	25.63±0.67^{b}	32.36 ± 0.14^{b}	30.01±0.54^{b}	36.13 ± 0.33^{b}	34.11±0.76^{b}	40.52 ± 1.30^{b}	37.11±1.14^{b}	43.54 ± 0.21^{b}	40.03±0.64^{b}
T_2	27.90 ± 0.74^{b}	25.76±0.83^{b}	32.12 ± 0.41^{b}	30.12±0.26^{b}	36.82 ± 1.15^{b}	33.66±0.43^{b}	40.80 ± 0.84^{b}	37.23±0.54^{b}	43.79 ± 2.06^{b}	40.19±0.84^{b}
T_3	30.66 ± 0.25^{c}	29.67±1.02^{c}	37.14 ± 1.13^{c}	34.72±0.97^{c}	41.62 ± 1.50^{c}	37.34±0.48^{c}	45.41 ± 0.86^{c}	41.90±0.53^{c}	47.03 ± 0.81^{c}	44.88±0.43^{c}
T_4	23.07 ± 0.55^{a}	22.04±0.51^{a}	28.53 ± 0.49^{a}	26.01±0.27^{a}	31.79 ± 0.95^{a}	29.01±1.04^{a}	33.91 ± 1.24^{a}	32.07±1.34^{a}	35.14 ± 1.00^{a}	33.54±1.17^{a}
Pooled										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	25.60±1.33^{b}	26.78±0.79^{b}	42.70±0.79^{b***}	31.18±0.21^{b}	44.51±0.15^{b***}	35.14±0.36b	47.39±0.94^{b***}	38.82±1.09^{b}	53.48±0.77^{b***}	41.79±0.35^{b}
T_2	25.41±1.23^{b}	26.83±0.71^{b}	42.93±1.22^{b***}	31.12±0.28^{b}	44.35±1.51^{b***}	35.24±0.37b	47.12±0.61^{b***}	39.02±0.17^{b}	53.53±1.43^{b***}	41.99±1.27^{b}
T_3	32.26±1.22^{c*}	30.16±0.48^{c}	49.18±1.28^{c***}	35.94±1.05^{c}	50.42±0.55^{c***}	39.48±0.88c	53.15±1.54^{c***}	43.66±0.68^{c}	59.27±0.39^{c***}	45.96±0.36^{c}
T_4	21.25±0.50^{b*}	22.55±0.06^{a}	38.47±1.05^{a***}	27.27±0.38^{a}	38.99±0.08^{a***}	30.40±0.63a	41.16±0.73^{a***}	32.99±1.20^{a}	44.84±1.19^{a***}	34.34±0.18^{a}
ALT	NS		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	**		NS		NS		NS		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf chlorophyll content (32.26±1.22 cci, 49.18±1.28 cci, 50.42±0.55 cci, 53.15±1.54 cci, and 59.27±0.39 cci was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (25.60±1.33 cci, 42.70±0.79 cci, 44.51±0.15 cci, 47.39±0.94 cci, and 53.48±0.77 cci) and T_2 (25.41±1.23 cci, 42.93±1.22 cci, 44.35±1.51 cci, 47.12±0.61 cci, and 53.53±1.43 cci) which included FYM and *Azotobacter* respectively. The lowest leaf chlorophyll content (21.25±0.50cci, 38.47±1.05 cci, 38.99±0.08 cci, 41.16±0.73 cci and 44.84±1.19 cci at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf chlorophyll content (30.16±0.48 cci, 35.94±1.05 cci, 39.48±0.88 cci, 43.66±0.68 cci and 45.96±0.36 cci) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_2 (26.83±0.71cci, 31.12±0.28 cci, 35.24±0.37 cci, 39.02±0.17 cci, and 41.99±1.27 cci) and T_1 (26.78±0.79 cci, 31.18±0.21 cci, 35.14±0.36 cci, 38.82±1.09 cci and 41.79±0.35 cci) which included FYM and *Azotobacter* respectively. The minimum leaf chlorophyll content (22.55±0.06 cci, 27.27±0.38 cci, 30.40±0.63 cci, 32.99±1.20 cci and 34.34±0.18 cci) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf chlorophyll content at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf chlorophyll content at both locations. Furthermore, after 30, 45, 60, 75 and 90 days of transplanting, the leaf chlorophyll content in the HA region was found to be increased by 6.96%, 36.85%%, 27.71%%, 21.75% and 28.97%, respectively, as compared to the plants grown at the LA region. The altitudes significantly affected the leaf chlorophyll content of plant at different days after transplanting except 30 DAT.

4.1.8.3 Knol-khol cultivar White Vienna

At both the HA and LA locations, it was found that all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control) had a significant impact on the leaf chlorophyll content of knol-khol (Table 4.26).

Table 4.26 Comparative effect of location and treatments on leaf chlorophyll content (cci) of *Brassica oleracea* var. gongylodes cultivar White Vienna

High altitude (HA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	16.21 ± 0.75^{b}	16.16±0.58^{b}	26.87 ± 0.43^{b}	26.80±0.33^{b}	31.13 ± 0.76^{b}	30.57±0.55^{b}
T_2	15.38 ± 0.80^{b}	15.32±0.79^{b}	26.77 ± 0.19^{b}	26.73±0.09^{b}	31.16 ± 0.96^{b}	30.67±0.83^{b}
T_3	18.36 ± 0.67^{c}	18.35±0.64^{c}	29.10 ± 0.03^{c}	29.15±0.27^{c}	37.64 ± 0.56^{c}	34.68±0.87^{c}
T_4	10.83 ± 0.44^{a}	10.82±0.44^{a}	20.59 ± 0.49^{a}	20.60±0.53^{a}	23.83 ± 0.75^{a}	23.28±0.67^{a}
Low altitude (LA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	28.90 ± 1.62^{bc}	24.38±1.26^{b}	31.33 ± 1.66^{bc}	26.09±1.11^{b}	34.04 ± 1.30^{b}	30.22±0.61^{b}
T_2	27.30 ± 1.64^{b}	24.80±0.69^{b}	29.38 ± 1.04^{b}	26.08±0.51^{b}	32.21 ± 0.21^{b}	29.81±0.78^{b}
T_3	31.55 ± 0.62^{c}	28.06±1.24^{c}	33.92 ± 1.98^{c}	30.90±0.88^{c}	38.73 ± 1.57^{c}	33.46±0.86^{c}
T_4	22.17 ± 1.61^{a}	21.19±0.33^{a}	26.26 ± 1.06^{a}	23.93±0.44^{a}	28.88 ± 0.96^{a}	24.22±0.72^{a}
Pooled						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	16.18±0.67^{b}	26.64±1.44^{b***}	26.83±0.36^{b}	28.71±0.82^{b*}	30.85±0.65^{b}	32.13±0.37^{c*}
T_2	15.35±0.78^{b}	26.05±0.78^{b***}	26.75±0.06^{b}	27.73±0.70^{b}	30.91±0.84^{b}	31.01±0.37^{b}
T_3	18.35±0.65^{c}	29.80±0.33^{c***}	29.13±0.15^{c}	32.41±0.56^{c***}	36.16±0.60^{c}	36.10±0.37^{d}
T_4	10.83±0.44^{a}	21.68±0.93^{a***}	20.59±0.48^{a}	25.09±0.49^{a***}	23.56±0.67^{a}	26.55±0.16^{a**}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	NS		***		***	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf chlorophyll content (18.35±0.65 cci, 29.13±0.15 cci and 36.16±0.60 cci was recorded at different days after transplanting (30, 45, and 60 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (16.18±0.67 cci, 26.83±0.36 cci and 30.85±0.65 cci) and T_2 (15.35±0.78 cci, 26.75±0.06 cci and 30.91±0.84 cci) which included FYM and *Azotobacter* respectively. The lowest leaf chlorophyll content (10.83±0.44 cci, 20.59±0.48 cci and 23.56±0.67 cci at 30, 45 and

60 DAT respectively) was observed in control. Similarly, at LA maximum leaf chlorophyll content (29.80±0.33 cci, 32.41±0.56 cci and 36.10±0.37 cci) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, and 60 days after transplanting followed by the treatment T_1 (26.64±1.44 cci, 28.71±0.82 cci and 32.13±0.37 cci) and T_2 (26.05±0.78 cci, 27.73±0.70 cci, and 31.01±0.37 cci) which included FYM and *Azotobacter* respectively. The minimum leaf chlorophyll content (21.68±0.93 cci, 25.09±0.49 cci and 26.55±0.16 cci) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf chlorophyll content at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the maximum leaf chlorophyll content at both locations. Furthermore, after 30 and 45 days of transplanting, the leaf chlorophyll content in the LA region was found to be increased by 62.40% and 11.26%, respectively, as compared to the plants grown at the HA region. However, no significant change was recorded in plant spread during 60 DAT at both the locations. The altitudes significantly affected the leaf chlorophyll content of plant at different days after transplanting except 30 DAT. The interaction between altitude and treatment (ALT×TRE) was found significant ($p \leq 0.05$) at 45 and 60 days after transplanting.

4.1.8.4 Radish cultivar Pusa Himani

The chlorophyll content of radish leaves was significantly affected by all four treatments (FYM, *Azotobacter*, *FYM+Azotobacter*, and control) at both HA and LA sites. Comprehensive data are available in Table 4.27.

Table 4.27 Comparative effect of location and treatments on leaf chlorophyll content (cci) of *Raphanus sativus* cultivar Pusa Himani

High altitude (HA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	32.68 ± 1.12^{b}	31.93±1.13^{b}	42.26 ± 0.83^{b}	42.00±0.88^{b}	42.98 ± 0.79^{b}	42.82±0.52^{b}
T_2	33.20 ± 1.43^{b}	32.96±1.25^{b}	42.34 ± 1.30^{b}	42.39±1.34^{b}	42.54 ± 0.52^{b}	43.03±0.90^{b}
T_3	36.92 ± 1.77^{c}	35.53±1.51^{c}	49.20 ± 0.92^{c}	49.41±0.38^{c}	49.32 ± 0.16^{c}	50.11±0.29^{c}
T_4	27.73 ± 1.24^{a}	26.83±0.81^{a}	36.24 ± 0.78^{a}	36.22±0.73^{a}	36.59 ± 1.29^{a}	36.39±0.84^{a}
Low altitude (LA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	22.59 ± 0.93^{b}	21.32±0.56^{b}	25.93 ± 0.78^{b}	25.31±0.46^{b}	29.42 ± 1.54^{b}	28.98±0.86^{c}
T_2	21.07 ± 1.62^{ab}	21.15±1.00^{b}	25.44 ± 1.23^{b}	25.93±0.36^{b}	29.18 ± 0.58^{b}	27.68±0.22^{b}
T_3	25.48 ± 1.73^{c}	23.7±0.61^{c}	28.37 ± 1.11^{c}	28.62±0.40^{c}	33.14 ± 1.02^{c}	32.52±0.42^{d}
T_4	19.02 ± 0.67^{a}	16.36±0.65^{a}	20.57 ± 1.30^{a}	20.98±0.38^{a}	24.47 ± 1.45^{a}	24.69±0.39^{a}
Pooled						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	32.31±1.11^{b***}	21.96±0.18^{b}	42.13±0.86^{b***}	25.62±0.43^{b}	42.9±0.63^{b***}	29.2±0.42^{b}
T_2	33.08±1.33^{b***}	21.11±1.22^{b}	42.37±1.32^{b***}	25.69±0.54^{b}	42.79±0.60^{b***}	28.43±0.20^{b}
T_3	36.23±1.64^{c***}	24.59±0.91^{c}	49.31±0.65^{c***}	28.49±0.75^{c}	49.72±0.16^{c***}	32.83±0.72^{c}
T_4	27.28±1.02^{a***}	17.69±0.01^{a}	36.23±0.71^{a***}	20.77±0.65^{a}	36.49±1.06^{a***}	24.58±0.54^{a}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	NS		***		***	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, the maximum leaf chlorophyll content (36.23±1.64, 49.31±0.65, and 49.72±0.16 cci) was observed at different days after sowing (30, 45, and 60 DAS) in treatment T_3 (FYM+*Azotobacter*), followed by T_1 (32.31±1.11, 42.13±0.86, and 42.9±0.63 cci) and T_2 (33.08±1.33, 42.37±1.32, and 42.79±0.60 cci), which incorporated FYM and *Azotobacter* respectively. The control exhibited the lowest leaf chlorophyll content (27.28±1.02, 36.23±0.71, and 36.49±1.06 cci) at these respective time points. Similarly, at LA the maximum leaf chlorophyll content (24.59±0.91, 28.49±0.75, and 32.83±0.72 cci) was recorded in treatment T_3 (FYM+*Azotobacter*) at

30, 45, and 60 days after sowing, followed by T_1 (21.96±0.18, 25.62±0.43, and 29.2±0.42 cci) and T_2 (21.11±1.22, 25.69±0.54, and 28.43±0.20 cci), which included FYM and *Azotobacter* respectively. The minimum leaf chlorophyll content (17.69±0.01, 20.77±0.65, and 24.58±0.54 cci) was observed in the control group.

However, upon comparison of treatments (T_1, T_2, T_3, and T_4) on leaf chlorophyll content at HA and LA regions, it was evident that treatment T_3 (FYM+*Azotobacter*) consistently resulted in the maximum leaf chlorophyll content at both locations. Furthermore, after 30, 45, and 60 days of sowing, the leaf chlorophyll content in the HA region was found to be increased by 47.30%, 73.08%, and 51.45% respectively, compared to the plants grown at the LA region. The interaction between altitude and treatment (ALT×TRE) was to be significant (P<0.05) at 45 and 60 days after sowing.

Chlorophyll is the primary light-absorbing pigment in plants and is essential for metabolic activity (Verma *et al.*, 2014). In current study maximum leaf chlorophyll content of Brassicaceae vegetables were recorded in treatment (T_3) as compared to (T_1, T_2 and T_4) at both HA and LA locations. Saffeullah *et al.* (2021) reported that high availability of N due to application of FYM and *Azotobacter*significantly increased the chlorophyll content in cabbage. Furthermore, at later stage of plant growth *i.e*. 90 DAT, higher chlorophyll content of cruciferous vegetable such as cabbage, cauliflower, knol-khol and radish was recorded at HA as compared to LA grown crops respectively. It is being proposed that high light intensity might have increased the chlorophyll content in Brassicaceae vegetable leaves at HA as compared to LA. Because there is less air pressure and less atmospheric filtering, high-altitude areas have lower oxygen levels and more intense sunlight. As a result of the high levels of sunshine and reduced oxygen, plants can perform photosynthesis with greater energy, which causes them to produce more chlorophyll. Our findings are consistent with Gao *et al*. (2020), who discovered that white and blue light considerably increased the photosynthetic efficiency and chlorophyll content of Welsh onions.

4.1.9 Leaf anthocyanin content (aci) of cruciferous vegetable at different days after transplanting

4.1.9.1 Cabbage cultivar Videshi

Table 4.28 demonstrated that at both the HA and LA locations, the four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control) had a significant impact on the anthocyanin content of the cabbage leaves.

Table 4.28 Comparative effect of location and treatments on leaf anthocyanin content (aci) of *Brassica oleracea* var. capitata cultivar Videshi

High altitude (HA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	9.70 ± 0.19^{a}	9.62±0.10^{b}	14.90 ± 0.73^{b}	14.64±0.62^{b}	15.11 ± 0.27^{b}	15.07±0.32^{b}	20.13 ± 0.69^{b}	18.58±0.22^{b}	22.11 ± 0.52^{b}	20.83±0.52^{b}
T_2	9.72 ± 0.33^{a}	9.69±0.17^{b}	14.87 ± 0.55^{b}	14.61±0.29^{b}	15.04 ± 0.25^{b}	14.91±0.29^{b}	19.97 ± 0.31^{b}	18.08±0.43^{b}	22.49 ± 0.20^{b}	20.98±0.73^{b}
T_3	11.30 ± 0.19^{b}	11.27±0.22^{c}	17.09 ± 0.29^{c}	16.90±0.18^{c}	17.92 ± 0.24^{c}	17.67±0.21^{c}	22.70 ± 0.43^{c}	20.80±0.56^{c}	25.30 ± 0.21^{c}	24.22±0.57^{c}
T_4	9.31 ± 0.10^{a}	9.19±0.07^{a}	11.69 ± 0.32^{a}	11.49±0.28^{a}	12.02 ± 0.34^{a}	11.98±0.35^{a}	14.58 ± 0.34^{a}	13.86±0.25^{a}	15.63 ± 0.21^{a}	14.94±0.12^{a}
Low altitude (LA)										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	6.82 ± 0.44^{b}	6.40±0.10^{b}	9.14 ± 0.39^{b}	9.09±0.17^{b}	12.31 ± 0.77^{b}	11.38±0.17^{b}	14.30 ± 0.95^{b}	12.33±0.12^{b}	16.12 ± 0.76^{b}	14.20±0.07^{b}
T_2	7.10 ± 0.15^{b}	6.36±0.08^{b}	9.53 ± 0.67^{b}	9.28±0.10^{b}	12.74 ± 0.95^{b}	11.46±0.21^{b}	14.69 ± 0.38^{b}	12.35±0.27^{b}	16.20 ± 0.90^{b}	14.18±0.09^{b}
T_3	8.33 ± 0.26^{c}	7.61±0.10^{c}	11.21 ± 0.66^{c}	10.63±0.12^{c}	14.47 ± 0.49^{c}	13.04±0.45^{c}	16.81 ± 0.45^{c}	14.21±0.17^{c}	19.09 ± 0.31^{c}	16.00±0.06^{c}
T_4	5.30 ± 0.03^{a}	5.22±0.08^{a}	7.38 ± 0.40^{a}	7.20±0.15^{a}	10.26 ± 0.08^{a}	9.7±0.31^{a}	11.49 ± 0.32^{a}	10.47±0.27^{a}	12.78 ± 0.42^{a}	11.69±0.12^{a}
Pooled										
Treatment	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	9.66±0.15^{***b}	6.61±0.26^{b}	14.77±0.67^{***b}	9.12±0.27^{b}	15.09±0.28^{***b}	11.85±0.41^{b}	19.35±0.45^{***b}	13.32±0.50^{b}	21.47±0.51^{***b}	15.16±0.35^{b}
T_2	9.71±0.24^{***b}	6.73±0.05^{b}	14.74±0.42^{***b}	9.41±0.29^{b}	14.98±0.27^{***b}	12.10±0.58^{b}	19.02±0.23^{***b}	13.52±0.13^{b}	21.73±0.44^{***b}	15.19±0.40^{b}
T_3	11.28±0.20^{***c}	7.97±0.17^{c}	17.00±0.21^{***c}	10.92±0.27^{c}	17.79±0.22^{***c}	13.75±0.40^{c}	21.75±0.48^{***c}	15.51±0.25^{c}	24.76±0.36^{***c}	17.54±0.16^{c}
T_4	9.25±0.07^{***a}	5.26±0.05^{a}	11.59±0.30^{***a}	7.29±0.14^{a}	12.00±0.34^{***a}	9.98±0.11^{a}	14.22±0.30^{***a}	10.98±0.05^{a}	15.29±0.07^{***a}	12.23±0.17^{a}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	***		**		***		***		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf anthocyanin content (11.28±0.20 aci, 17.00±0.21 aci, 17.79±0.22 aci, 21.75±0.48 aci, and 24.76±0.36 aci was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (9.66±0.15 aci, 14.77±0.67 aci, 15.09±0.28 aci, 19.35±0.45 aci, and 21.47±0.51 aci) and T_2 (9.71±0.24 aci, 14.74±0.42 aci, 14.98±0.27 aci, 19.02±0.23 aci, and 21.73±0.44 aci) which included FYM and *Azotobacter* respectively. The minimum leaf anthocyanin content (9.25±0.07aci, 11.59±0.30 aci, 12.00±0.34 aci, 14.22±0.30 aci and 15.29±0.07 aci at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf anthocyanin content (7.97±0.17 aci, 10.92±0.27 aci, 13.75±0.40 aci, 15.51±0.25 aci and 17.54±0.16 aci) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_2 (6.73±0.05 aci, 9.41±0.29 aci, 12.10±0.58 aci, 13.52±0.13 aci, and 15.19±0.40 aci) and T_1 (6.61±0.26 aci, 9.12±0.27 aci, 11.85±0.41 aci, 13.32±0.50 aci and 15.16±0.35 aci) which included FYM and *Azotobacter* respectively. The minimum leaf anthocyanin content (5.26±0.05 aci, 7.29±0.14 aci, 9.98±0.11 aci, 10.98±0.05 aci and 12.23±0.17 aci) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf anthocyanin content at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the higher leaf anthocyanin content at both locations. Furthermore, after 30, 45, 60, 75 and 90 days of transplanting, the leaf anthocyanin content in the HA region was found to be increased by 41.53%, 55.68%, 29.38%, 40.23% and 41.16%, respectively, as compared to the plants grown at the LA region. The interaction between altitude and treatment (ALT×TRE) was also found significant ($p \leq 0.05$) at different days after transplanting.

4.1.9.2 Cauliflower cultivar WS 909

At both the HA and LA locations, it was discovered that all four treatments (T_1, T_2, T_3, and T_4) had a significant impact on the anthocyanin content of the cauliflower leaves. Table 4.29 contains the data.

Table 4.29 Comparative effect of location and treatments on leaf anthocyanin content (aci) of *Brassica oleracea* var. botrytis cultivar WS 909

	High altitude (HA)									
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	6.54 ± 0.10^{b}	6.31±0.20^{b}	8.33 ± 0.09^{b}	8.61±0.52^{b}	11.00 ± 0.15^{b}	10.41±0.10^{b}	12.16 ± 0.44^{b}	11.60±0.37^{b}	13.22 ± 0.50^{b}	12.35±0.11^{b}
T_2	6.26 ± 0.38^{b}	6.36±0.27^{b}	7.91 ± 0.60^{b}	8.42±0.34^{b}	10.31 ± 1.08^{b}	10.37±0.17^{b}	11.48 ± 0.92^{b}	11.54±0.18^{b}	12.41 ± 0.82^{b}	12.32±0.08^{b}
T_3	8.42 ± 0.28^{c}	7.36±0.10^{c}	10.19 ± 0.10^{c}	10.52±0.17^{c}	12.23 ± 0.12c	11.84±0.16^{c}	13.53 ± 0.43^{c}	13.26±0.22^{c}	14.72 ± 0.47^{c}	14.00±0.35^{c}
T_4	3.89 ± 0.21^{a}	3.86±0.16^{a}	5.24 ± 0.28^{a}	5.77±0.09^{a}	7.26 ± 0.50^{a}	7.12±0.41^{a}	9.48 ± 0.44^{a}	8.48±0.17^{a}	10.49 ± 0.46^{a}	9.70±0.26^{a}
Low altitude (LA)										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	8.47 ± 0.22^{b}	8.48±0.26^{b}	8.92 ± 0.54^{b}	8.93±0.44^{b}	11.44 ± 0.23^{b}	11.38±0.22^{b}	12.89 ± 0.35^{b}	12.72±0.35^{b}	14.71 ± 0.30^{b}	14.55±0.33^{b}
T_2	8.47 ± 0.23^{b}	8.49±0.05^{b}	8.87 ± 0.54^{b}	8.89±0.50^{b}	11.29 ± 0.30^{b}	11.22±0.14^{b}	12.84 ± 0.25^{b}	12.67±0.31^{b}	14.63 ± 0.12^{b}	14.43±0.26^{b}
T_3	10.52 ± 0.27^{c}	10.54±0.06^{c}	11.29 ± 0.07^{c}	11.36±0.15^{c}	13.09 ± 0.41^{c}	12.98±0.34^{c}	14.89 ± 0.48^{c}	14.35±0.24^{c}	17.01 ± 0.20^{c}	16.73±0.24^{c}
T_4	6.72 ± 0.29^{a}	6.69±0.29^{a}	8.08 ± 0.17^{a}	8.10±0.06^{a}	10.21 ± 0.13^{a}	10.05±0.28^{a}	11.70 ± 0.09^{a}	11.48±0.09^{a}	13.14 ± 0.12^{a}	12.98±0.17^{a}
Pooled										
Treatments	**30 DAT**		**45 DAT**		**60 DAT**		**75 DAT**		**90 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	8.47±0.23^{b***}	6.43±0.11^{b}	8.93±0.47^{b}	8.47±0.24^{b}	11.41±0.23^{b***}	10.70±0.11^{b}	12.81±0.35^{b*}	11.88±0.22^{b}	14.63±0.31^{b***}	12.78±0.26^{b}
T_2	8.48±0.14^{b***}	6.31±0.06^{b}	8.88±0.52^{b}	8.17±0.47^{b}	11.25±0.21^{b*}	10.34±0.49^{b}	12.76±0.28^{b*}	11.51±0.44^{b}	14.54±0.19^{b***}	12.37±0.38^{b}
T_3	10.53±0.15^{c***}	7.89±0.18^{c}	11.33±0.11^{c***}	10.35±0.11^{c}	13.03±0.37^{c*}	12.04±0.14^{c}	14.62±0.35^{c**}	13.39±0.22^{c}	16.87±0.22^{c***}	14.36±0.34^{c}
T_4	6.71±0.29^{a***}	3.87±0.18^{a}	8.09±0.08^{a***}	5.51±0.18^{a}	10.13±0.20^{a***}	7.19±0.24^{a}	11.59±0.09^{a***}	8.98±0.26^{a}	13.06±0.15^{a***}	10.10±0.18^{a}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	**		***		***		***		*	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf anthocyanin content (10.53±0.15 aci, 11.33±0.11 aci, 13.03±0.37 aci, 14.62±0.35 aci, and 16.87±0.22 aci was recorded at different days after transplanting (30, 45, 60, 75 and 90 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (8.47±0.23 aci, 8.93±0.47 aci, 11.41±0.23 aci, 12.81±0.35 aci, and 14.63±0.31 aci) and T_2 (8.48±0.14 aci, 8.88±0.52 aci, 11.25±0.21 aci, 12.76±0.28 aci, and 14.54±0.19 aci) which included FYM and *Azotobacter* respectively. The minimum leaf anthocyanin content (6.71±0.29 aci, 8.09±0.08 aci, 10.13±0.20 aci, 11.59±0.09 aci and 13.06±0.15 aci at 30, 45, 60, 75 and 90 DAT respectively) was observed in control. Similarly, at LA maximum leaf anthocyanin content (7.89±0.18 aci, 10.35±0.11 aci, 12.04±0.14 aci, 13.39±0.22 aci and 14.36±0.34 aci) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45, 60, 75 and 90 days after transplanting followed by the treatment T_1 (6.43±0.11 aci, 8.47±0.24 aci, 10.70±0.11 aci, 11.88±0.22 aci, and 12.78±0.26 aci) and T_2 (6.31±0.06 aci, 8.17±0.47 aci, 10.34±0.49 aci, 11.51±0.44 aci and 12.37±0.38 aci) which included FYM and *Azotobacter* respectively. The minimum leaf anthocyanin content (3.87±0.18 aci, 5.51±0.18 aci, 7.19±0.24 aci, 8.98±0.26 aci and 10.10±0.18 aci) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf anthocyanin content at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the higher leaf anthocyanin content at both locations. Furthermore, after 30, 45, 60, 75 and 90 days of transplanting, the leaf anthocyanin content in the HA region was found to be increased by 33.46%, 9.47%, 8.22%, 9.19% and 17.48%, respectively, as compared to the plants grown at the LA region. The interaction between altitude and treatment (ALT×TRE) was also found significant ($p \leq 0.05$) at different days after transplanting.

4.1.9.3 Knol-khol cultivar White Vienna

Table 4.30 revealed that, at both the HA and LA locations, all four treatments (FYM, *Azotobacter*, FYM+*Azotobacter*, and control) had a significant impact on the anthocyanin content of the leaves of knol-khol.

Table 4.30 Comparative effect of location and treatments on leaf anthocyanin content (aci) of knol-khol cultivar White Vienna

High altitude (HA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	5.90 ± 0.20^{b}	5.84±0.20^{b}	7.50 ± 0.27^{b}	7.47±0.12^{b}	8.76 ± 0.62^{b}	8.69±0.62^{b}
T_2	5.90 ± 0.49^{b}	5.83±0.24^{b}	7.34 ± 0.39^{b}	7.45±0.07^{b}	8.16 ± 0.11^{b}	8.25±0.29^{b}
T_3	6.52 ± 0.44^{b}	6.51±0.13^{c}	8.11 ± 0.12^{c}	8.13±0.07^{c}	11.83 ± 0.58^{c}	11.52±0.50^{c}
T_4	5.11 ± 0.27^{a}	5.09±0.17^{a}	5.58 ± 0.34^{a}	5.60±0.38^{a}	5.97 ± 0.32^{a}	5.84±0.22^{a}
Low altitude (LA)						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	6.46 ± 0.37^{b}	6.51±0.30^{b}	9.16 ± 0.25^{c}	8.14±0.14^{b}	11.97 ± 0.29^{b}	9.38±0.13^{b}
T_2	6.13 ± 0.28^{b}	6.69±0.20^{b}	8.48 ± 0.27^{b}	8.08±0.08^{b}	11.46 ± 0.38^{b}	9.49±0.13^{b}
T_3	7.08 ± 0.15^{c}	7.37±0.09^{c}	10.00 ± 0.38^{d}	9.71±0.27^{c}	13.21 ± 0.35^{c}	10.64±0.21c
T_4	3.71 ± 0.25^{a}	3.66±0.30^{a}	6.29 ± 0.32a	6.01±0.03^{a}	8.61 ± 0.25^{a}	7.21±0.12a
Pooled						
Treatment	**30 DAT**		**45 DAT**		**60 DAT**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	5.87±0.20^{b*}	6.48±0.26^{b}	7.48±0.19^{b***}	8.65±0.09^{c}	8.72±0.62^{b**}	10.67±0.08^{b}
T_2	5.87±0.37^{b}	6.41±0.19^{b}	7.39±0.22^{b**}	8.28±0.11^{b}	8.20±0.17^{b***}	10.47±0.23^{b}
T_3	6.52±0.29^{c*}	7.22±0.09^{c}	8.12±0.10^{c***}	9.86±0.32^{d}	11.68±0.51^{c}	11.92±0.15^{c}
T_4	5.10±0.22^{a***}	3.68±0.12^{a}	5.59±0.36^{a}	6.15±0.14^{a}	5.91±0.26^{a***}	7.91±0.07^{a}
ALT	NS		***		***	
TRE	***		***		***	
ALT×TRE	***		**		***	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf anthocyanin content (6.52±0.29 aci, 8.12±0.10 aci and 11.68±0.51 aci was recorded at different days after transplanting (30, 45 and 60 DAT) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (5.87±0.20 aci, 7.48±0.19 aci and 8.72±0.62 aci) and T_2 (5.87±0.37 aci, 7.39±0.22 aci and 8.20±0.17 aci) which included FYM and *Azotobacter* respectively. The minimum leaf

anthocyanin content (5.10±0.22 aci, 5.59±0.36 aci and 5.91±0.26 aci at 30, 45 and 60 DAT respectively) was observed in control.Similarly, at LA maximum leaf anthocyanin content (7.22±0.09 aci, 9.86±0.32 aci and 11.92±0.15 aci) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45 and 60 days after transplanting followed by the treatment T_1 (6.48±0.26 aci, 8.65±0.09 aci and 10.67±0.08 aci) and T_2 (6.41±0.19 aci, 8.28±0.11 aci and 10.47±0.23 aci) which included FYM and *Azotobacter* respectively. The minimum leaf anthocyanin content (3.68±0.12 aci, 6.15±0.14 aci and 7.91±0.07 aci) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf anthocyanin content at LA and HA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the higher leaf anthocyanin content at both locations. Furthermore, after 30 and 45 days of transplanting, the leaf anthocyanin content in the LA region was found to be increased by 10.74% and 21.43%, respectively, as compared to the plants grown at the HA region. However, no significant change was recorded in plant spread during 60 DAT at both the locations. The interaction between altitude and treatment (ALT×TRE) was also found significant ($p \leq 0.05$) at different days after transplanting.

4.1.9.4 Radishcultivar Pusa Himani

The anthocyanin content of leaves was significantly influenced by all four treatments (T_1, T_2, T_3, and T_4) at both high altitude (HA) and low altitude (LA) sites. Detailed data can be found in Table 4.31.

Table 4.31 Comparative effect of location and treatments on leaf anthocyanin content (aci) of radish cultivar Pusa Himani

High altitude (HA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T1	9.18 ± 0.60^{c}	8.95±0.14^{b}	12.63 ± 0.53^{a}	12.62±0.42^{b}	13.72 ± 0.54^{b}	13.39±0.63^{b}
T2	9.92 ± 0.24^{b}	9.64±0.17^{c}	12.63 ± 0.34^{a}	12.62±0.24^{b}	13.64 ± 0.28^{b}	13.17±0.32^{b}
T3	10.78 ± 0.36^{d}	10.70±0.21^{d}	15.07 ± 0.54^{b}	15.09±0.50^{c}	16.44 ± 0.16^{c}	16.12±0.18^{c}
T4	7.63 ± 0.09^{a}	7.52±0.09^{a}	11.87 ± 0.29^{a}	11.82±0.26^{a}	11.99 ± 0.12^{a}	11.91±0.21^{a}
Low altitude (LA)						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T1	3.74 ± 0.11^{b}	3.78±0.12^{b}	4.60 ± 0.09^{b}	4.26±0.15^{b}	6.56 ± 0.08^{b}	6.58±0.04^{b}
T2	3.54 ± 0.22^{b}	3.62±0.17^{b}	4.51 ± 0.32^{b}	4.35±0.16^{b}	6.31 ± 0.45^{b}	6.72±0.05^{b}
T3	4.40 ± 0.03^{c}	4.58±0.25^{c}	5.64 ± 0.07^{c}	5.51±0.05^{c}	7.61 ± 0.07^{c}	7.89±0.13^{c}
T4	2.63 ± 0.12^{a}	2.79±0.10^{a}	3.28 ± 0.02^{a}	3.45±0.11^{a}	4.09 ± 0.08^{a}	4.85±0.07^{a}
Pooled						
Treatment	**30 DAS**		**45 DAS**		**60 DAS**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**
T1	9.07±0.37^{b***}	3.76±0.08^{b}	12.63±0.48^{a***}	4.43±0.12^{b}	13.55±0.58^{b***}	6.57±0.06^{b}
T2	9.78±0.18^{c***}	3.59±0.19^{b}	12.63±0.28^{a***}	4.43±0.22^{b}	13.41±0.30^{b***}	6.52±0.23^{b}
T3	10.74±0.28^{d***}	4.49±0.14^{c}	15.08±0.52^{b***}	5.58±0.06^{c}	16.28±0.15^{c***}	7.75±0.10^{c}
T4	7.58±0.09^{a***}	2.71±0.06^{a}	11.84±0.27^{a***}	3.36±0.05^{a}	11.95±0.13^{a***}	4.47±0.08^{a}
ALT	***		***		***	
TRE	***		***		***	
ALT×TRE	***		**		***	

*HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant. Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

At HA, maximum leaf anthocyanin content (10.74±0.28, 15.08±0.52 and 16.28±0.15 aci was recorded at different days after sowing (30, 45 and 60 DAS) in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (9.07±0.37, 12.63±0.48 and 13.55±0.58 aci) and T_2 (9.78±0.18, 12.63±0.28 and 13.41±0.30 aci) which included FYM and *Azotobacter* respectively. The minimum leaf anthocyanin content (7.58±0.09, 11.84±0.27 and 11.95±0.13 aci at 30, 45 and 60 DAS respectively) was observed in control. Similarly, at LA maximum leaf anthocyanin content (4.49±0.14, 5.58±0.06 and 7.75±0.10 aci) were also recorded in treatment T_3 (FYM+*Azotobacter*) at 30, 45 and 60 days after sowing followed by the treatment T_1 (3.76±0.08, 4.43±0.12

and 6.57±0.06 aci) and T_2 (3.59±0.19, 4.43±0.22 and 6.52±0.23 aci) which included FYM and *Azotobacter* respectively. The minimum leaf anthocyanin content (2.71±0.06, 3.36±0.05 and 4.47±0.08 aci) was observed in control.

However, when the effect of treatments (T_1, T_2, T_3, and T_4) on leaf anthocyanin content at HA and LA regions was compared, it was found that treatment T_3 (FYM+*Azotobacter*) had the higher leaf anthocyanin content at both locations. Furthermore, after 30, 45 and 60 days of sowing, the leaf anthocyanin content in the HA region was found to be increased by 139.20%, 170.25% and 110.06% respectively, as compared to the plants grown at the LA region. The interaction between altitude and treatment (ALT×TRE) was also found significant ($p \leq 0.05$) at different days after sowing.

Anthocyanin play a crucial role in shielding leaves from the damaging effects of photo-inhibitory light fluxes by absorbing excess photons (Gould, 2004). In our investigation, treatment (T_3) with FYM and *Azotobacter* at various growth stages in both the HA and LA locations had a greater leaf anthocyanin content of Brassicaceae vegetables. It might be the outcome of bio-organic farming, which has a strong emphasis on soil health and gives plants an adequate amount of nutrients, including trace elements and micronutrients that are necessary for the production of anthocyanins content in leaf. Moreover, the maximum leaf anthocyanin content of cruciferous vegetables was recorded at HA as compared to LA grown crop. It is Due to their thin atmospheres, high-altitude environments usually face higher UV radiation levels. As photo-protective substances, anthocyanins capture UV light and shield the leaf tissues from overexposure to radiation. These findings are in agreement with Mahdavian*et al.* (2008), who reported that UV-B and UV-C increased anthocyanin concentration in leaves of *capsicum annum* L.

4.1.10Comparative effect of FYM and *Azotobacter* on yield attributes of cruciferous vegetable grown at HA *vs.*, LA

4.1.10.1 Effect on yield parameters of cabbage cultivar Videshi and cauliflower cultivar WS909

Altitudinal conditions and treatments showed a significant ($p \leq 0.05$) effect on cabbage (Table 4.32 and Figure 4.1) and cauliflower (Table 4.33 and Figure 4.2) yield attributes including number of inner leaves, head/ curd diameter, head/curd length,

head/curd weight per plant, compactness rate and yield (q/ha). The effect of treatments on cabbage and cauliflower at both the locations was validated by One-way ANOVA. The current study showed that the treatment T_3 enhanced the head/curd yield in comparison to control at both the locations. This might be due to the effect of organic manure (FYM) increases the soil biological activity, which aids in nutrient mobilization from applied nutrients. Furthermore, the increase in yield and its attributes caused by *Azotobacter* inoculation could also be linked to an increase in nitrogen fixation which enhanced the vegetative growth and increased the yield attributes of cabbage and cauliflower. These outcomes are consistent with the earlier findings of Verma *et al*. (2014), Bahadur *et al*. (2006) and Upadhyay *et al*. (2012).

Furthermore, the maximum number of inner leaves in cabbage head (64.55±0.48) was found at HA than at LA (61.33±0.73). Head compactness was higher at HA in the treatment T_1 (cabbage) and T_4 (cauliflower). According to Raid *et al* (2009), head compactness of head or curd depends on the ratio of head or curd volume and weight. Additionally, in cabbage and cauliflower, the maximum head/curd diameter (15.86±0.43 and 14.31±0.15 cm), head/curd length (16.84±0.27 and 10.64±0.11 cm), head/curd weight per plant (1662.00±4.17 and 705.06±18.42 g) and yield (494.75±4.97 and 259.05±10.34 q/ha) were found at HA whereas, minimum head/curd diameter (13.04±0.06 and 14.11±0.19 cm), head/curd length (14.02±0.04 and 9.27±0.27 cm), head/curd weight per plant (834.76±25.66 and 466.6±13.47 g) and yield (302.06±11.31 and 209.05±0.72 q/ha) were recorded at LA. Recent studies have demonstrated that longer photoperiod and improved photosynthesis can increase agricultural output at high altitude (Allen, 2016). Additionally, high head/curd yield and better physical properties of cabbage and cauliflower might also be due to superior plant growth parameters at HA which in turn might have resulted in boosting the photosynthetic rate and assimilation of products of biosynthesis in the storage plant tissues such as head thus leading to much better physical indices of cabbage and cauliflower head at HA compared with those grown at LA experimental field (Son *et al*., 2018; Liu *et al.*, 2022).

Table 4.32 Comparative effect of location and treatments on yield parameters of *Brassica oleracea* var. capitata cultivar Videshi

High altitude (HA)												
Treatment	**Number of inner leaf/Head**		**Head Diameter (cm)**		**Head Length (cm)**		**Head weight /plant (g)**		**Compactness**		**Yield (q/ha)**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T1	58.78 ± 0.19^{b}	56.89±0.70^{b}	14.19 ± 0.17^{b}	13.11±0.64^{b}	16.09 ± 0.15^{b}	15.14±0.22^{b}	1320±95.39^{b}	1271.22±54.11^{b}	0.65±0.05^{a}	0.54±0.09^{a}	406.58±1.88^{b}	344.86±16.76^{b}
T2	58.89 ± 0.19^{b}	56.55±0.39^{b}	14.38 ± 0.21^{b}	13.88±0.42^{b}	16.16 ± 0.18^{b}	15.55±0.33^{b}	1354.78±22.3 ^{b}	1244.11±50.35^{b}	0.66±0.04^{a}	0.64±0.05^{a}	401.23±9.64^{b}	335.6±16.68^{b}
T3	66.22 ± 0.51^{c}	62.89±0.51^{c}	16.28 ± 0.10^{c}	15.44±0.77^{c}	17.63 ± 0.05^{c}	16.06±0.51^{c}	1736.22±41.5^{c}	1587.78±49.15^{c}	0.74±0.01^{b}	0.69±0.11^{a}	518.93±15.3^{c}	470.58±7.21^{c}
T4	51.33 ± 0.33^{a}	49.56±0.20^{a}	13.29 ± 0.17^{a}	12.05±0.27^{a}	13.73 ± 0.10^{a}	13.09±0.18^{a}	861.55±6.26^{a}	813.33±29.77^{a}	0.82±0.02^{c}	0.64±0.06^{a}	207.41±5.89^{a}	233.33±6.27^{a}
Low altitude (LA)												
Treatment	**Number of inner leaf/Head**		**Head Diameter (cm)**		**Head Length (cm)**		**Head weight /plant (g)**		**Compactness**		**Yield (q/ha)**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T1	54.11 ± 0.51^{b}	54.11±0.38^{b}	11.52 ± 0.23^{b}	11.96±0.19^{b}	13.16 ± 0.34^{b}	13.09±0.22^{b}	667.97 ± 31.84^{b}	687.99±15.22^{b}	0.68 ± 0.05^{a}	0.79±0.03^{ab}	244.44 ± 9.80^{b}	251.64±9.49^{b}
T2	53.00 ± 0.33^{b}	53.89±0.38^{b}	11.61 ± 0.33^{b}	12.01±0.50^{b}	13.28 ± 0.60^{b}	13.27±0.09^{b}	653.28 ± 18.16^{b}	683.06±26.21^{b}	0.71 ± 0.07^{a}	0.79±0.03^{ab}	241.77 ± 6.66^{b}	250.2±10.5^{b}
T3	61.67 ± 1.00^{c}	61.00±0.67^{c}	12.70 ± 0.06^{c}	13.38±0.14^{c}	14.06 ± 0.07^{c}	13.97±0.14^{c}	816.41 ± 39.30^{c}	853.10±26.69^{c}	0.74 ± 0.03^{a}	0.84±0.04^{b}	301.85 ± 14.81^{c}	302.26±10.03^{c}
T4	47.00 ± 0.88^{a}	46.00±1.2^{a}	10.47 ± 0.32^{a}	10.74±0.19^{a}	11.62 ± 0.20^{a}	11.93±0.23^{a}	526.85 ± 24.01^{a}	524.04±14.2^{a}	0.64 ± 0.08^{a}	0.73±0.08^{a}	194.86 ± 8.76^{a}	194.03±5.53^{a}
Pooled												
Treatment	**Number of inner leaf/Head**		**Head Diameter (cm)**		**Head Length (cm)**		**Head weight /plant (gm)**		**Compactness**		**Yield (q/ha)**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T1	57.84±0.29^{b***}	54.11±0.10^{b}	13.65±0.40^{b***}	11.74±0.12^{b}	15.62±0.18^{b***}	13.12±0.18^{b}	1295.61±42.02^{b***}	677.98±15.10^{b}	0.59±0.07^{a*}	0.73±0.04^{a}	375.72±9.27^{b***}	248.05±3.58^{b}
T2	57.72±0.25^{b***}	53.45±0.25^{b}	14.14±0.31^{b**}	11.81±0.40^{b}	15.86±0.07^{b***}	13.27±0.31^{b}	1299.44±18.72^{b***}	668.17±17.68^{b}	0.65±0.04^{ab}	0.75±0.05^{a}	368.41±9.18^{b***}	245.99±7.42^{b}
T3	64.55±0.48^{c**}	61.33±0.73^{c}	15.86±0.43^{c***}	13.04±0.06^{c}	16.84±0.27^{c***}	14.02±0.04^{c}	1662±4.17^{c***}	834.76±25.66^{c}	0.72±0.05^{b}	0.79±0.02^{a}	494.75±4.97^{c***}	302.06±11.31^{c}
T4	50.44±0.20^{a**}	46.5±0.93^{a}	12.67±0.19^{a***}	10.6±0.23^{a}	13.41±0.14^{a***}	11.78±0.11^{a}	837.45±13.53^{a***}	525.44±17.99^{a}	0.73±0.03^{b}	0.69±0.08^{a}	220.37±6.01^{a**}	194.45±6.81^{a}
ALT	***		***		***		***		**		***	
TRE	***		***		***		***		NS		***	
ALT× TRE	NS		NS		***		***		*		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT x TRE - interaction of altitude and treatment.

Values in columns followed by the same letter (small alphabet) are not significantly different; P<0.05, Duncan's multiple range test between treatments. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments.

*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05, NS = not significant.*

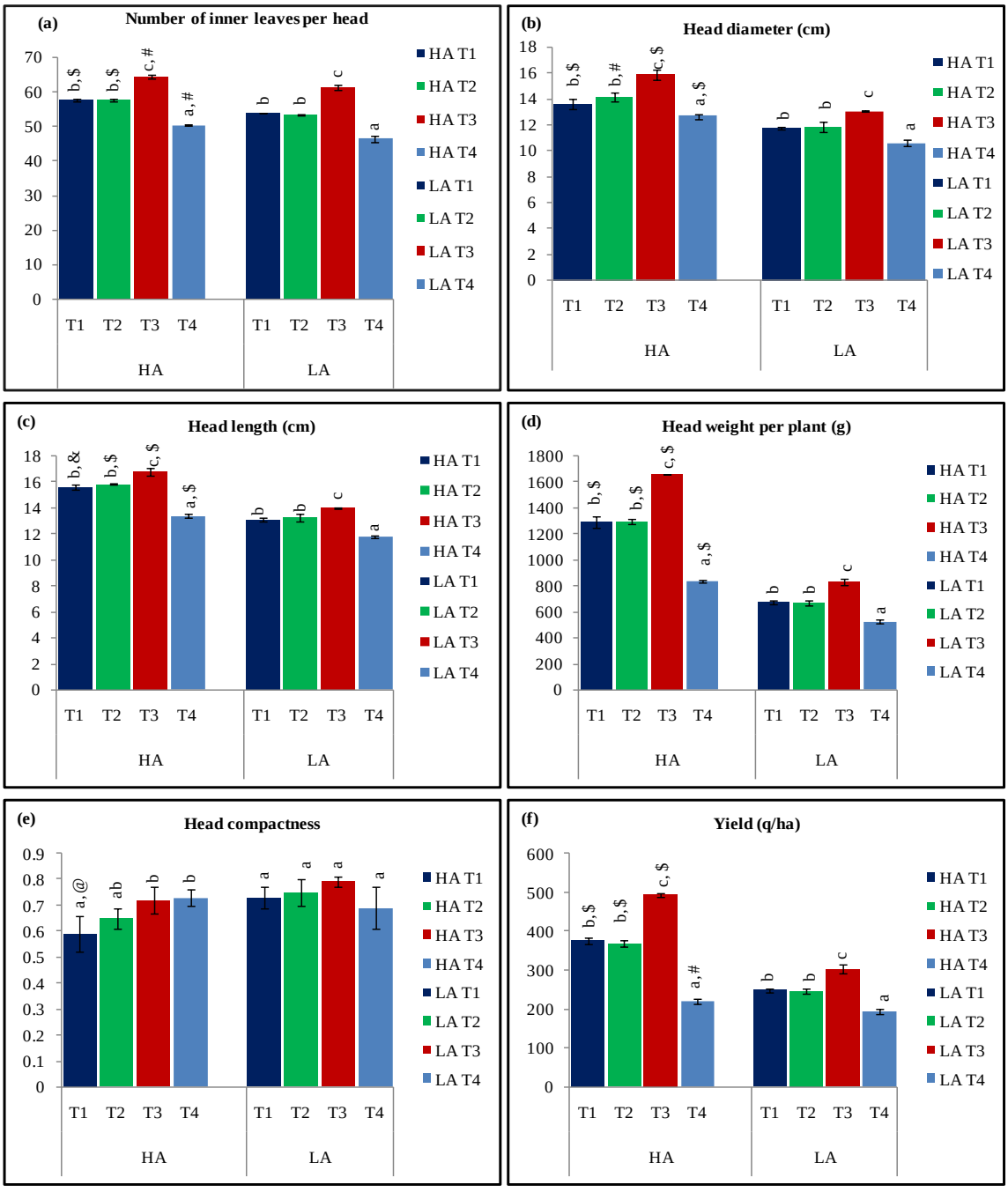

Figure 4.1 Effect of altitudes and treatments on: (a) number of inner leaves per head, (b) head diameter, (c) head length, (d) head weight per plant, (e) head compactness (f) yield. Data presented as mean (±SD) over replicates. Different same letters (lowercase alphabet) indicate significantly different; $p \leq 0.05$, Duncan's multiple range test between treatments. Statistically different between high and low altitude treatments consider at \$ $p \leq 0.001$; # $p \leq 0.01$ and @ $p \leq 0.05$ analyzed by independent t test. HA= High altitude and LA= Low altitude. T_1= FYM @ 150 q/ha, T_2= *Azotobacter* @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ *Azotobacter* @ 8.6 kg/ha and T_4= Control.

Table 4.33 Comparative effect of location and treatments on yield parameters of *Brassica oleracea* var. botrytis cultivar WS909

High altitude (HA)										
Treatment	**Curd diameter (cm)**		**Curd Length (cm)**		**curd weight / plant (g)**		**Compactness**		**Yield (q/ha)**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	12.44 ± 0.06^{b}	12.42±0.08^{b}	9.66 ± 0.23^{b}	9.73±0.2^{b}	520.11 ± 23.80^{b}	505.45±15.79^{b}	1.11 ± 0.04^{b}	1.14±0.07^{b}	193.00 ± 7.72^{b}	189.71±9.59^{b}
T_2	12.27 ± 0.11^{b}	12.28±0.13^{b}	9.62 ± 0.51^{b}	9.66±0.57^{b}	511.00 ± 11.36^{b}	502.78±9.16^{b}	1.09 ± 0.02^{ab}	1.10±0.06^{b}	188.89 ± 4.32^{b}	185.60±8.65^{b}
T_3	14.32 ± 0.32^{c}	14.3±0.06^{c}	10.67 ± 0.10^{c}	10.61±0.14^{c}	707.56 ± 32.18^{c}	702.55±4.95^{c}	1.26 ± 0.06^{c}	1.24±0.02^{c}	261.52 ± 11.86^{c}	256.58±8.83^{c}
T_4	10.82 ± 0.32^{a}	10.44±0.29^{a}	8.25 ± 0.48^{a}	8.20±0.31^{a}	368.89 ± 17.99^{a}	347.34±10.69^{a}	1.02 ± 0.04^{a}	0.99±0.06^{a}	136.21 ± 6.60^{a}	125.10±3.97^{a}
Low altitude (LA)										
Treatment	**Curd diameter (cm)**		**Curd Length (cm)**		**curd weight / plant (g)**		**Compactness**		**Yield (q/ha)**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	12.48 ± 0.47^{b}	11.87±0.34^{b}	7.53 ± 0.12^{b}	7.92±0.05^{b}	322.69 ± 15.30^{c}	323.61±8.34^{b}	1.74 ± 0.09^{b}	1.54±0.16^{b}	179.01 ± 6.17^{c}	182.10±8.97^{b}
T_2	12.17 ± 0.08^{b}	11.66±0.44^{b}	7.43 ± 0.07^{b}	7.94±0.29^{b}	292.84 ± 14.64^{b}	308.87±10.2^{b}	1.76 ± 0.03^{b}	1.56±0.24^{b}	168.31 ± 3.97^{b}	174.9±5.83^{b}
T_3	14.17 ± 0.60^{c}	13.76±0.12^{c}	9.15 ± 0.12^{c}	9.39±0.44^{c}	472.94 ± 21.93^{d}	460.25±11.82^{c}	1.89 ± 0.09^{b}	1.69±0.03^{b}	209.88 ± 6.17^{d}	208.23±4.99^{c}
T_4	8.89 ± 0.63^{a}	8.08±0.23^{a}	6.12 ± 0.18^{a}	6.64±0.22^{a}	181.72 ± 7.89^{a}	187.91±8.49^{a}	1.16 ± 0.20^{a}	0.86±0.06^{a}	105.97 ± 4.71^{a}	106.79±5.56^{a}
Pooled										
Treatment	**Curd diameter (cm)**		**Curd Length (cm)**		**curd weight / plant (g)**		**Compactness**		**Yield (q/ha)**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	12.43±0.06^{b}	12.13±0.23^{b}	9.70±0.21^{b***}	7.73±0.08^{b}	512.78±18.92^{b***}	323.15±6.91^{c}	1.13±0.05^{b**}	1.64±0.12^{b}	191.36±8.59^{b}	180.55±6.70^{c}
T_2	12.28±0.10^{b}	11.84±0.26^{b}	9.64±0.54^{b**}	7.68±0.13^{b}	506.89±8.57^{b***}	300.86±9.74^{b}	1.10±0.03^{b***}	1.66±0.10^{b}	187.24±6.36^{b*}	171.60±4.90^{b}
T_3	14.31±0.15^{c}	14.11±0.19^{c}	10.64±0.11^{c***}	9.27±0.27^{c}	705.06±18.42^{c***}	466.6±13.47^{d}	1.25±0.03^{c***}	1.79±0.05^{b}	259.05±10.34^{c***}	209.05±0.72^{d}
T_4	10.63±0.30^{a**}	8.48±0.26^{a}	8.22±0.40^{a**}	6.38±0.14^{a}	358.11±13.52^{a***}	184.82±2.73^{a}	1.01±0.06^{a}	1.01±0.09^{a}	130.66±5.26^{a**}	106.38±2.17^{a}
ALT	***		***		***		***		***	
TRE	***		***		***		***		***	
ALT×TRE	***		NS		**		***		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT x TRE - interaction of altitude and treatment.

Values in columns followed by the same letter (small alphabet) are not significantly different; P<0.05, Duncan's multiple range test between treatments. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments.

*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05, NS = not significant.*

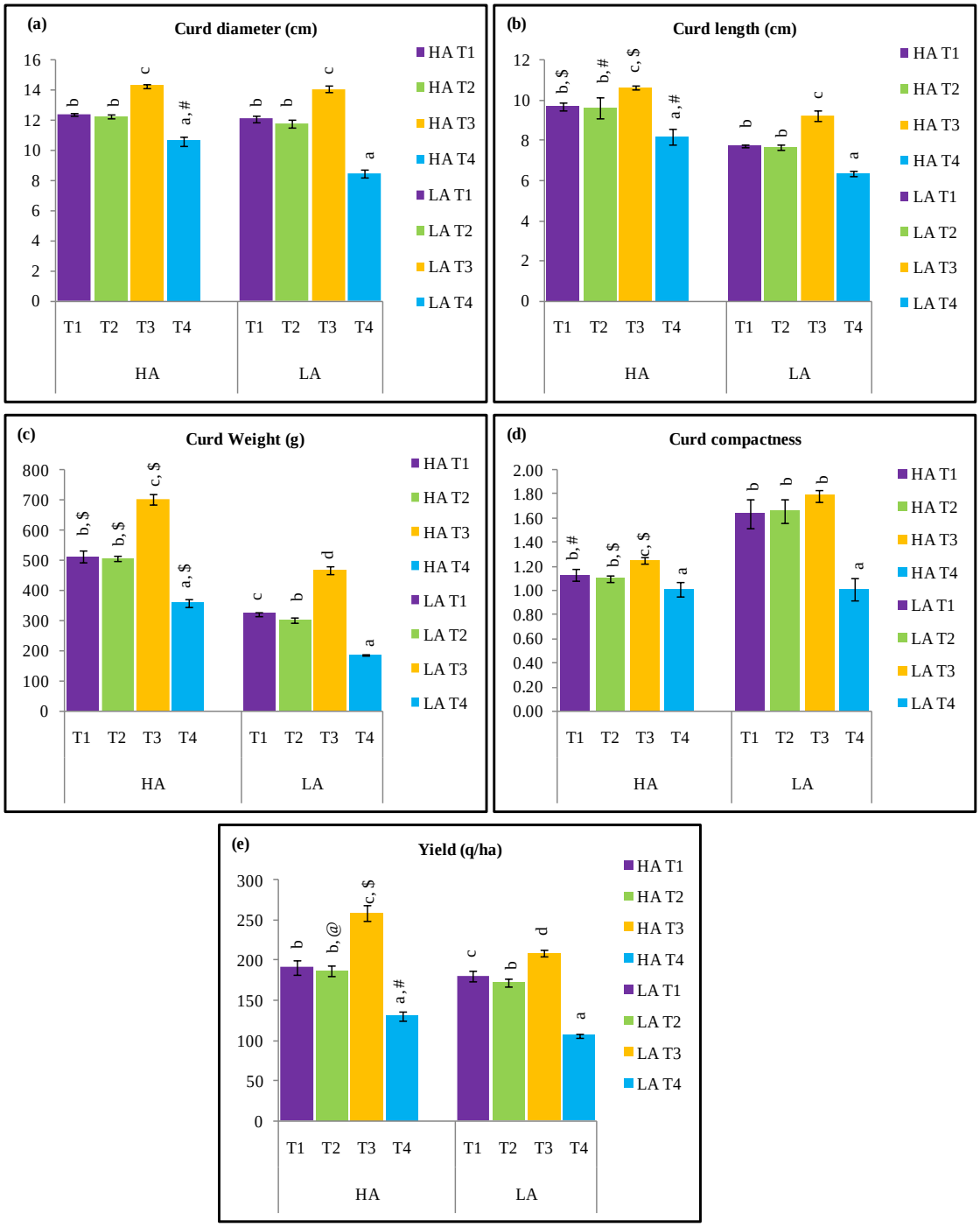

Figure 4.2 Effect of altitudes and treatments on: (a) curd diameter, (b) curd length, (c) curd weight per plant, (d) curd compactness (e) yield. Data presented as mean (±SD) over replicates. Different same letters (lowercase alphabet) indicate significantly different; *p≤0.05*, Duncan's multiple range test between treatments. Statistically different between high and low altitude treatments consider at $ p≤0.001; # p≤0.01 and @ p≤0.05 analyzed by independent t test. HA= High altitude and LA= Low altitude. T_1= FYM @ 150 q/ha, T_2= *Azotobacter* @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ *Azotobacter* @ 8.6 kg/ha and T_4= Control.

4.1.10.2 Knol-khol cultivar White Vienna

Altitudinal conditions and treatments showed a significant effect on kohlrabi yield attributes (Table 4.34 and Figure 4.3) including knob equatorial diameter (mm), knob polar diameter (mm), knob weight per plant (g) and yield (q/ha). The knol-khol yield is affected by the polar and equatorial diameters of the knob as well as the weight of the knob (Shah *et al*., 2019). Based on our results, the T_3 treatment had the maximum knob equatorial diameter (8.8-fold >T_1, 16.8-fold >T_2 and 24.8-fold >T_4, respectively), knob polar diameter (4.4-fold >T_1, 11.5-fold >T_2 and 24.2-fold >T_4, respectively), knob weight per plant (105-fold >T_1, 112-fold >T_2 and 168.8-fold >T_4, respectively) and yield (113.2-fold >T_1, 120.7-fold >T_2 and 174.2-fold >T_4, respectively) at HA grown cabbage. At LA, a similar pattern was found where knob equatorial diameter (12.6-fold >T_1, 11-fold >T_2 and 24.9-fold >T_4, respectively), knob polar diameter (8.3-fold >T_1, 9.7-fold >T_2 and 27.9-fold >T_4, respectively), knob weight per plant (134.2-fold >T_1, 139.1-fold >T_2 and 256.3-fold >T_4, respectively) and yield (148.1-fold >T_1, 152-fold >T_2 and 304.5-fold >T_4, respectively) were found better in T_3. This could be because organic manure (FYM) boosts soil biological activity, which aids in nutrient mobilization from applied fertilizers. Furthermore, the improvement in yield and its attributes caused by *Azotobacter* inoculation could be linked to high nutrient uptake, high photosynthetic rate and increased nitrogen fixation capacity, which promoted vegetative development and yield attributes of knol-khol. These outcomes are consistent with the earlier findings of Shah *et al.* (2019), Bhusan*et al.* (2010), Bahadur *et al*. (2006) and Upadhyay *et al.* (2012).

Further, in T_3 it was found that knob weight per plant (59.8-fold) and yield (137.6-fold) significantly maximum at LA compared to HA grown sample. It could be that superior physical indices of knol-khol may possibly be attributable to superior plant growth features in LA compared to HA produce kohlrabi (Bhusan*et al*., 2010; Son *et al*., 2018).

Table 4.34 Comparative effect of location and treatments on yield parameters of knol-khol cultivar White Vienna

High altitude (HA)								
Treatment	**Knob equatorial diameter (mm)**		**Knob polar diameter (mm)**		**Knob weight /plant (g)**		**Yield (q/ha)**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	84.86 ± 3.84^{c}	78.66±1.94^{c}	89.37 ± 0.91^{c}	80.5±0.76^{c}	267.27 ± 13.51^{b}	252.67±12.61^{b}	263.17 ± 13.10^{b}	252.26±11.13^{b}
T_2	75.82 ± 3.42^{b}	71.63±2.03^{b}	79.72 ± 2.99^{b}	75.92±3.19^{b}	257.40 ± 12.51^{b}	248.67±12.02^{b}	253.50 ± 12.37^{b}	246.91±11.11^{b}
T_3	94.10 ± 3.86^{d}	87.19±2.04^{d}	93.05 ± 0.91^{c}	85.66±1.57^{d}	379.33 ± 13.14^{c}	350.73±8.20^{c}	374.28 ± 12.60^{c}	367.70±8.38^{c}
T_4	68.02 ± 3.23^{a}	63.55±1.40^{a}	67.11 ± 3.19^{a}	63.06±1.56^{a}	204.40 ± 10.12^{a}	188.00±6.84^{a}	201.44 ± 10.05^{a}	191.97±7.12^{a}
Low altitude (LA)								
Treatment	**Knob equatorial diameter (mm)**		**Knob polar diameter (mm)**		**Knob weight /plant (g)**		**Yield (q/ha)**	
	1st year	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**	**1st year**	**2nd year**
T_1	79.27 ± 1.23^{b}	75.21±1.05^{b}	82.75 ± 1.95^{b}	81.94±1.24^{b}	302.69 ± 14.18^{b}	278.52±8.67^{b}	366.46 ± 5.60^{b}	354.53±10.22^{b}
T_2	79.28 ± 2.43^{b}	78.44±2.39^{b}	80.45 ± 3.71^{b}	81.46±1.75^{b}	290.61 ± 14.94^{b}	280.83±12.83^{b}	356.58 ± 3.04^{b}	356.58±8.65^{b}
T_3	93.82 ± 1.89^{c}	85.96±1.74^{c}	95.50 ± 3.06^{c}	85.86±0.52^{c}	465.12 ± 20.21^{c}	384.60±7.78^{c}	519.75 ± 25.69^{c}	497.53±13.75^{c}
T_4	64.59 ± 0.52^{a}	65.4±3.10^{a}	64.30 ± 0.83^{a}	61.24±1.97^{a}	171.89 ± 9.17^{a}	165.18±9.61^{a}	207.61 ± 2.92^{a}	200.62±5.38^{a}
Pooled								
Treatment	**Knob equatorial diameter (mm)**		**Knob polar diameter (mm)**		**Knob weight /plant (g)**		**Yield (q/ha)**	
	HA	**LA**	**HA**	**LA**	**HA**	**LA**	**HA**	**LA**
T_1	81.76±2.37^{c*}	77.24±1.13^{b}	84.94±0.35^{c}	82.34±0.56^{b}	259.97±8.22^{b**}	290.6±6.12^{b}	257.72±7.72^{b***}	360.49±5.63^{b}
T_2	73.78±0.73^{b***}	78.86±0.40^{b}	77.82±2.67^{b}	80.96±1.04^{b}	253.03±11.98^{b*}	285.72±5.44^{b}	250.21±11.74^{b***}	356.58±4.33^{b}
T_3	90.64±1.89^{d}	89.89±0.08^{c}	89.35±1.24^{d}	90.68±1.28^{c}	365.03±9.62^{c**}	424.86±11.83^{c}	370.99±8.90^{c***}	508.64±18.55^{c}
T_4	65.79±2.24^{a}	64.99±1.29^{a}	65.08±2.21^{a}	62.77±1.4^{a}	196.2±5.38^{a**}	168.53±3.67^{a}	196.71±7.26^{a}	204.11±1.86^{a}
ALT	NS		NS		***		***	
TRE	***		***		***		***	
ALT×TRE	***		*		***		***	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT x TRE - interaction of altitude and treatment.

Values in columns followed by the same letter (small alphabet) are not significantly different; P<0.05, Duncan's multiple range test between treatments. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments.

*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05, NS = not significant.*

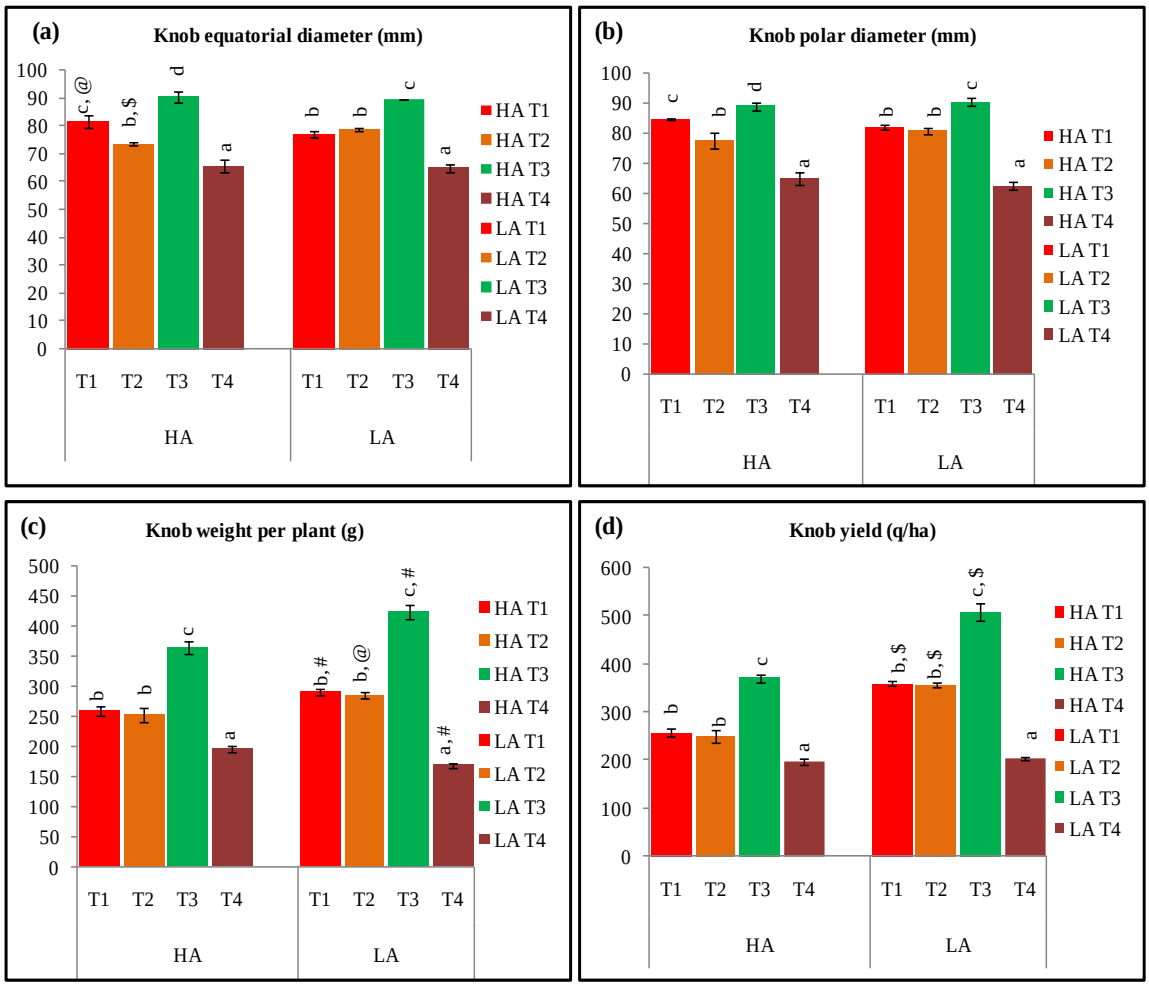

Figure 4.3 Effect of altitudes and treatments on: (a) knob equatorial diameter, (b) knob polar diameter, (c) knob weight per plant, (d) knob yield. Data presented as mean (±SD) over replicates. Different same letters (lowercase alphabet) indicate significantly different; $p \leq 0.05$, Duncan's multiple range test between treatments. Statistically different between high and low altitude treatments consider at \$ $p \leq 0.001$; # $p \leq 0.01$ and @ $p \leq 0.05$ analyzed by independent t test. HA= High altitude and LA= Low altitude. T_1= FYM @ 150 q/ha, T_2= *Azotobacter* @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ *Azotobacter* @ 8.6 kg/ha and T_4= Control.

4.1.10.3 Radish cultivar Pusa Himani

The yield attributes of radish *i.e.* root diameter, root length, root weight and yield was statistically significant ($p \leq 0.05$) between treatments at both the locations (Table 4.35 and Figure 4.4). At HA, root diameter (35.24±0.79 mm), root length (30.37±0.72 cm), root weight per plant (200.20±6.41 g) and root yield (390.64±4.65 q/ha) were recorded in T_3 treatment (FYM+*Azotobacter*) followed by the treatment T_1 (30.11±0.35 mm, 27.69±0.67 cm, 166.73±5.23 g, and 332.61±12.2 q/ha) and T_2

(30.22±0.82 mm, 27.45±0.83 cm, 166.50±1.11 g and 328.09±7.68 q/ha) which included FYM and *Azotobacter* respectively. The control exhibited the lowest values of root diameter (26.12±0.73 mm), root length (24.71±0.48 cm), root weight per plant (107.37±3.7 g) and yield (214.10±2.79 q/ha) was observed in control. Similarly, at LA the T3 treatment (FYM+*Azotobacter*) also displayed the highest values of root diameter (31.9±0.52 mm), root length (30.93±0.54 cm), root weight per plant (155.59±4.72 g) and yield (308.13±8.53 q/ha) followed by the treatment T_1 (28.62±0.84 mm, 27.35±1.00 cm, 133.00±0.83 g and 258.85±4.78 q/ha) and T_2 (29.22±0.60 mm, 26.75±0.85 cm, 127.95±5.52 g and 247.43±6.24 q/ha) which included FYM and *Azotobacter* respectively. The minimum root diameter (23.32±0.79 mm), root length (23.83±0.73 cm), root weight per plant (81.49±3.33 g) and yield (150.11±2.87 q/ha) was observed in control. At both the locations, the T_3 combination with FYM and *Azotobacter* produced better results than the other treatments. The primary cause of the increased root yield in FYM with *Azotobacter* is that it improves nutrient availability and nitrogen fixation, leading to increased plant growth and dry matter production. These factors subsequently enhanced root diameter, length, and weight, which in turn improved root yield. Our result is similarly consisting with Kumar *et al.* (2016) and Siram *et al*. (2023).

Furthermore, the highest measured root diameter (35.24±0.79 mm), root weight per plant (200.20±6.41 g), and yield (390.64±4.65 q/ha) were noted for radish grown in HA as compared to LA, (31.9±0.52 mm, 155.59±4.72 g, and 308.13±8.53 q/ha, respectively). No significant difference was observed in root length at both the locations. The increased root diameter, root weight, and root yield at HA could be the reduction in bulk density and the subsequent increase in soil porosity and water retention capacity resulting from the application of organic manures. Another contributing factor may be the presence of *Azotobacter*, which could enhance the solubilization of plant nutrients particularly at higher altitudes. This, in turn, may lead to greater uptake of NPK from the soil, ultimately resulting in increased yield. Our results are in good agreement with Balbande *et al.* (2023) and Kumar *et al.* (2016).

Table 4.35 Comparative effect of location and treatments on yield parameters of *Raphanus sativus* cultivar Pusa Himani

High altitude (HA)								
	Diameter of root (mm)		Length of root (cm)		Root weight per plant (g)		Yield (q/ha)	
Treatment	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
T_1	30.59 ± 1.12^{b}	29.63±1.48^{b}	27.51 ± 0.60^{a}	27.86±1.40^{b}	167.27 ± 7.60^{b}	166.2±8.10^{b}	333.33 ± 12.35^{b}	331.89±12.07^{b}
T_2	30.59 ± 1.84^{b}	29.85±0.31^{b}	27.63 ± 1.52^{a}	27.28±0.19^{b}	167.13 ± 6.53^{b}	165.87±8.29^{b}	329.22 ± 9.43^{b}	326.95±5.93^{b}
T_3	36.38 ± 1.75^{c}	34.1±0.52^{c}	30.54 ± 1.34^{b}	30.19±0.57^{c}	198.87 ± 3.70^{c}	201.53±9.14^{c}	389.92 ± 4.71^{c}	391.36±4.90^{c}
T_4	25.89 ± 1.27^{a}	26.35±0.73^{a}	25.68 ± 1.27^{a}	23.74±0.51a	103.13 ± 3.95^{a}	111.6±3.61^{a}	212.96 ± 3.09^{a}	215.22±2.50^{a}
Low altitude (LA)								
	Diameter of root (mm)		Length of root (cm)		Root weight per plant (g)		(Yield q/ha)	
Treatment	1st year	2nd year	1st year	2nd year	1st year	2nd year	1st year	2nd year
T_1	29.71 ± 1.73^{b}	27.54±1.25^{b}	28.61 ± 1.47^{b}	26.09±0.59^{b}	133.60 ± 5.84^{b}	132.39±5.05^{b}	260.29 ± 11.68^{b}	257.41±3.75^{b}
T_2	29.85 ± 0.16^{b}	28.59±1.2^{b}	28.36 ± 1.46^{b}	25.14±0.99^{b}	126.19 ± 6.12^{b}	129.71±6.92^{b}	246.91 ± 6.17^{b}	247.94±9.92^{b}
T_3	33.03 ± 0.98^{c*}	30.77±0.45^{c}	32.76 ± 0.75^{c}	29.11±0.32^{c}	162.31 ± 7.21^{c}	148.87±3.01^{c}	312.76 ± 9.43^{c}	303.50±10.59^{c}
T_4	24.16 ± 1.08^{a}	22.48±0.72^{a}	25.94 ± 0.57^{a}	21.71±0.95^{a}	81.52 ± 4.19^{a}	81.47±4.76^{a}	150.41 ± 5.25^{a}	149.8±6.80^{a}
Pooled								
	Diameter of root (mm)		Length of root (cm)		Root weight per plant (g)		Yield (q/ha)	
Treatment	HA	LA	HA	LA	HA	LA	HA	LA
T_1	30.11±0.35^{b*}	28.62±0.84^{b}	27.69±0.67^{b}	27.35±1.00^{b}	166.73±5.23^{b***}	133.00±0.83^{b}	332.61±12.2^{b***}	258.85±4.78^{c}
T_2	30.22±0.82^{b}	29.22±0.60^{b}	27.45±0.83^{b}	26.75±0.85^{b}	166.50±1.11^{b***}	127.95±5.52^{b}	328.09±7.68^{b***}	247.43±6.24^{b}
T_3	35.24±0.79^{c**}	31.9±0.52^{c}	30.37±0.72^{c}	30.93±0.54^{c}	200.20±6.41^{c***}	155.59±4.72^{c}	390.64±4.65^{c***}	308.13±8.53^{d}
T_4	26.12±0.73^{a*}	23.32±0.79^{a}	24.71±0.48^{a}	23.83±0.73^{a}	107.37±3.7^{a***}	81.49±3.33^{a}	214.10±2.79^{a***}	150.11±2.87^{a}
ALT	***		NS		***		***	
TRE	***		***		***		***	
ALT×TRE	NS		NS		*		NS	

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT x TRE - interaction of altitude and treatment.

*Values in columns followed by the same letter (small alphabet) are not significantly different; P < 0.05, Duncan's multiple range test between treatments. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments. Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05, NS = not significant.*

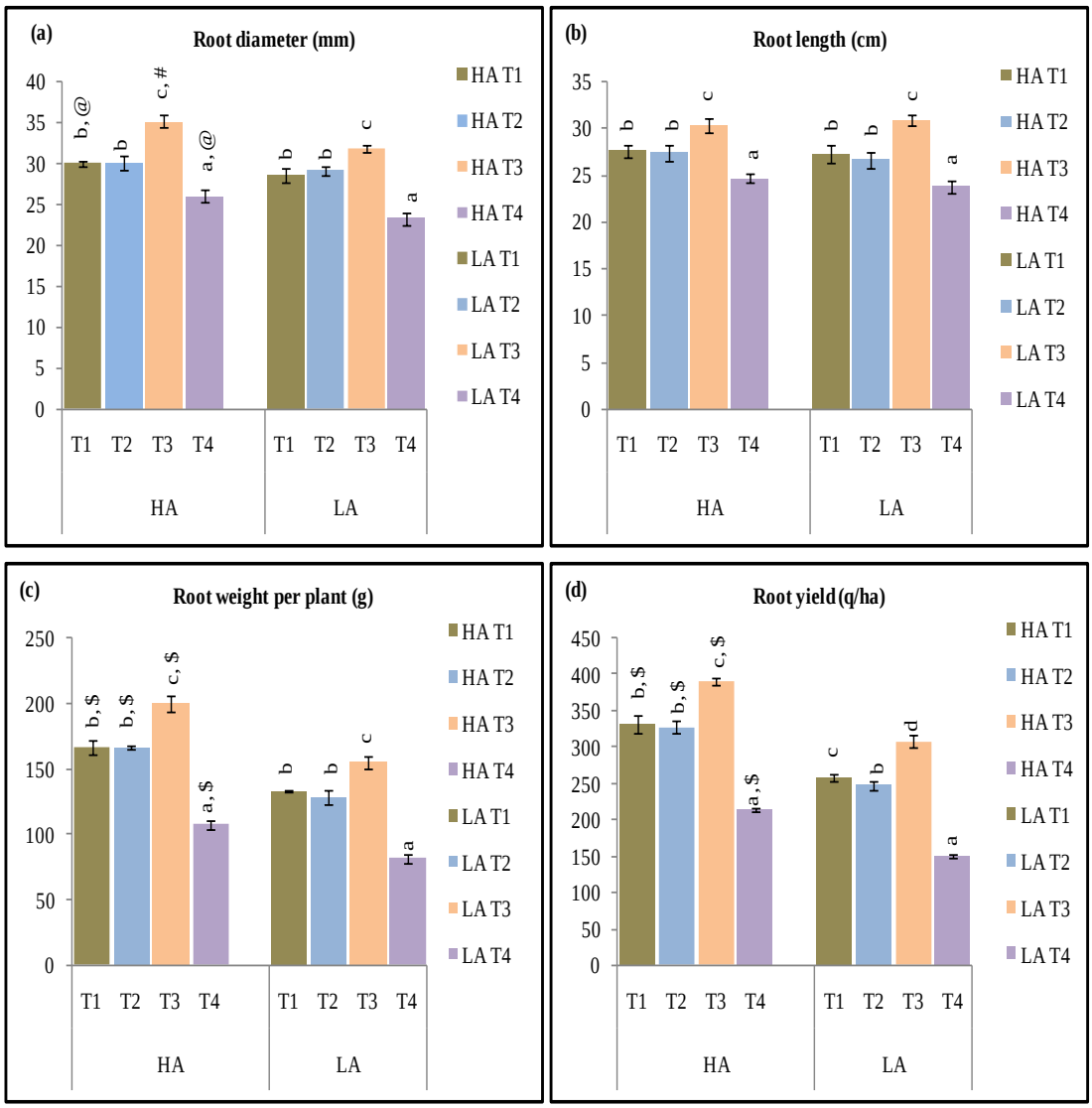

Figure 4.4 Effect of altitudes and treatments on: (a) root diameter, (b) root length, (c) root weight per plant, (d) yield. Data presented as mean (±SD) over replicates. Different same letters (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments. Statistically different between high and low altitude treatments consider at \$ $p \leq 0.001$; # $p \leq 0.01$ and @ $p \leq 0.05$ analyzed by independent t test. HA= High altitude and LA= Low altitude. T_1= FYM @ 150 q/ha, T_2= *Azotobacter* @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ *Azotobacter* @ 8.6 kg/ha and T_4= Control.

4.2 Economics of cruciferous vegetable grown at HA vs. LA

Table 4.36 Economics of different treatments of cruciferous vegetable grown at HA vs. LA

Location	Treatments	B:C Ratio			
		Cabbage	Cauliflower	Knol-khol	Radish
HA	T_1	4.56^b	3.18^b	1.42^b	2.71^b
	T_2	4.97^b	3.45^b	1.50^b	3.05^c
	T_3	6.22^c	4.59^c	2.45^c	3.30^d
	T_4	2.63^a	2.15^a	0.99^a	1.69^a
LA	T_1	1.64^b	1.96^b	1.63^b	1.04^b
	T_2	1.72^b	1.92^b	1.68^b	1.04^b
	T_3	2.16^c	2.38^c	2.68^c	1.39^c
	T_4	1.18^a	0.83^a	0.55^a	0.26^a

HA- high altitude and LA- low altitude, Values presented as means ± SD, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control.
Values in columns followed by the same letter (small alphabet) are not significantly different; $p<0.05$, Duncan's multiple range test between treatments.

Altitudinal situations and treatments had a significant (p<0.05) effect on the benefit-cost ratio of cruciferous vegetables grown at HA and LA locations (Table 4.35). In the current study it was found that treatment T_3 improved the benefit-cost ratio of HA and LA cultivated cabbage (6.22 and 2.16), cauliflower (4.59 and 2.38), knol-khol (2.45 and 2.68), and radish (3.30 and 1.39) as compared to T_1, T_2, and T_4. Furthermore, with the combined application of FYM and *Azotobacter*, highest benefit-cost ratio was observed in HA grown cabbage (6.22), cauliflower (4.59), and radish (3.30) when compared with LA cultivated cruciferous vegetables. It could be the combined application of FYM+*Azotobacter* which greatly enhanced the soil nutrition, growth, and yield at HA cultivated samples, and led to higher profitability (Bahadur *et al.* 2006 and Upadhyay *et al*. 2012). On the other hand, knol-khol grown at LA had a higher benefit-to-cost ratio (2.38) than HA. Better plant growth characteristics and improved yield in LA could be related to the greater physical indices of knol-khol, which might have resulted in increased profitability (Bhusan et al., 2010; Son et al., 2018).

4.3 Comparative effect of FYM and *Azotobacter* on soil fertility and nutritional parameters of cruciferous vegetables grown at HA *vs.*, LA

4.3.1 Effect on soil fertility after crop harvesting

Table 4.37 revealed the chemical characteristics of the soil post-treatment conducted at varying altitudes, high (HA) and low (LA).

Table 4.37 Comparative effect of location and treatments on soil chemical traits after crop harvesting

ALT	TRE	pH	EC (ms/cm)	Organic Carbon (%)	N (Kg/ha)	P (Kg/ha)	K (Kg/ha)	S (mg/kg)	Zn (mg/kg)	Fe (mg/kg)	Mg (mg/kg)	Cu (mg/kg)	Mn (mg/kg)
HA	T_1	7.17±0.05^{a***}	1.30±0.020^{b***}	0.74±0.03^{b***}	59.76±1.47^{b***}	14.62±0.39^{b***}	209.99±1.40^{b***}	139.39±0.97^{b***}	2.23±0.02^{b}	3.10±0.04^{b}	1.60±0.03^{b}	4.06±0.15^{bc}	3.10±0.14^{b***}
	T_2	7.15±0.08^{a***}	1.29±0.02^{b***}	0.74±0.02^{b***}	59.17±2.08^{b***}	14.80±0.32^{b***}	209.32±1.49^{b***}	140.04±1.45^{b***}	2.23±0.01^{b}	3.16±0.04^{bc}	1.56±0.04^{b}	3.92±0.27^{b}	3.14±0.09^{b***}
	T_3	7.10±0.09^{a***}	1.31±0.02^{b***}	0.80±0.01^{c***}	66.90±1.54^{c**}	16.20±0.27^{c***}	213.83±1.56^{c***}	143.18±2.29^{c***}	2.32±0.03^{c}	3.33±0.03^{c}	1.67±0.04^{c}	4.26±0.04^{c}	3.36±0.06^{c***}
	T_4	7.32±0.07^{b***}	1.22±0.01^{a***}	0.67±0.02^{a***}	35.25±1.06^{a**}	12.91±0.44^{a***}	187.5±1.08^{a***}	135.31±1.02^{a***}	2.03±0.08^{a}	2.67±0.19^{a}	1.45±0.04^{a}	3.54±0.10^{a}	2.81±0.05^{a***}
LA	T_1	8.06±0.05^{a}	0.46±0.03^{b}	0.53±0.03^{b}	46.85±1.64^{b}	11.18±0.27^{c}	194.89±2.1^{c}	98.56±1.01^{c}	4±0.1^{b***}	4.98±0.08^{b***}	3.24±0.04^{b***}	5.25±0.08^{b***}	2.11±0.07^{b}
	T_2	8.11±0.03^{ab}	0.45±0.04^{b}	0.54±0.03^{b}	46.31±1.78^{b}	10.29±0.32^{b}	186.78±1.72^{b}	92.5±1.19^{b}	3.94±0.27^{b***}	4.97±0.12^{b***}	3.16±0.04^{b***}	5.15±0.08^{b**}	2.09±0.12^{b}
	T3	8.05±0.07^{a}	0.53±0.02^{c}	0.63±0.02^{c}	56.16±2.51^{c}	12.41±0.4^{d}	204.58±0.81^{d}	103.84±1.66^{d}	4.32±0.03^{c***}	5.19±0.04^{c***}	3.81±0.13^{c***}	5.4±0.1^{c***}	2.29±0.02^{c}
	T_4	8.16±0.03^{b}	0.38±0.01^{a}	0.32±0.02^{a}	29.96±1.48^{a}	8.58±0.45^{a}	172.93±2.54^{a}	85.22±1.91^{a}	3.07±0.15^{a***}	4.08±0.13^{a***}	2.15±0.13^{a***}	4.82±0.06^{a***}	1.71±0.11^{a}
ALT		***	***	***	***	***	***	***	***	***	***	***	***
TRE		**	***	***	***	***	***	***	***	***	***	***	***
ALT ×TRE		NS	NS	***	**	NS	***	***	***	**	***	NS	NS

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @ 150q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @150 q/ha + Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE- interaction of altitude and treatment, NS = not significant.

*Values in columns same letter (lowercase alphabet) indicate no significant difference (P< 0.05, Duncan's multiple range test for treatment comparison). Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

The analysis reveals that treatment T_3 consistently yielded superior outcomes across both altitude conditions, indicating its effectiveness compared to the other treatments. At high altitude (HA), the soil pH fluctuates between 7.10±0.09 and 7.32±0.07, while at low altitude (LA), it ranges from 8.16±0.03 to 8.05±0.07. Notably, the soil pH at LA surpasses that at HA, possibly due to heightened accumulation of base-forming cations like calcium (Ca^{+2}) and magnesium (Mg^{+2}), alongside increased levels of calcium carbonate ($CaCO_3$), as posited by Northcott *et al.* (2009). Furthermore, the electrical conductivity (EC) values span from 1.22±0.01 to 1.31±0.02 at HA and from 0.38±0.01 to 0.53±0.02 at LA soil. At HA, soil EC surpasses that at LA, likely influenced by base leaching, which diminishes soil pH with altitude. These observations align with findings from Smith *et al.* (2002), who linked reduced soil pH to heightened accumulation of soil EC.

Further, it was observed that the control values (treatment T_4) exhibited higher levels of organic carbon, N, P, K, S, and Mn in HA soil compared to LA. Conversely, Zn, Fe, Mg, and Cu were significantly higher in the LA soil. Notably, treatment T_3 demonstrated superior soil parameters both at HA and LA locations among all the treatments (T_1, T_2, T_3, and T_4). At HA, Treatment T_3 showed a significant increase in organic carbon (26.98%), N (19.12%), P (30.54%), K (4.52%), S (37.89%), and Mn (46.72%) compared to LA soil. Conversely, Zn (86.21%), Fe (55.86%), Mg (51.16%) and Cu (26.76%) contents were higher in LA soil. The interaction between altitude and treatment (ALT×TRE) was found significantly different ($p \leq 0.05$) on organic carbon, N, K, S, Zn, Fe, and Mg. Moreover, the augmentation in soil organic carbon content, attributed to increased snow precipitation at HA compared to LA, contributes to soil hyper-aridity in cold desert high altitudes. This phenomenon suppresses microbial and enzymatic activities, leading to reduced soil organic matter decomposition and heightened soil organic carbon accumulation, as suggested by Charan *et al*. (2013) and Saeed *et al*. (2014). These findings are consistent with Sevgi and Tecimen's (2003) observations, indicating an elevation-dependent increase in soil carbon concentration in mountainous regions.

The presence of farmyard manure (FYM) and *Azotobacter* in the soil enhances not only nitrogen availability through biological nitrogen fixation processes (Aasfar et al., 2021) but also phosphorus (P) availability (Velmourougane *et al.*, 2019). Azotobacter exhibits unique characteristics such as cyst formation, which confers

resistance to environmental stresses and positively influences soil chemical properties (Aasfar *et al.,* 2021). Kizilkaya (2009) demonstrated that soil carbon and sulfur contents increase in response to FYM and Azotobacter inoculation, accelerating the mineralization of soil organic residues. Furthermore, the availability of zinc, iron, magnesium, and copper declines with increasing altitude, a trend consistent with micronutrient dynamics observed by Charan *et al*. (2013). The combined application of organic manure and biofertilizers enhances soil nutrient availability by stimulating soil microbial activity, decomposing harmful components, and improving soil structure (Naveed *et al*., 2021). These outcomes are in line with Ahmad et al. (2013) findings, which indicate that under specific environmental and soil conditions, FYM and *Azotobacter* application can enhance soil physicochemical properties.

4.3.2 Effect on nutritional attributes of cruciferous vegetable

Throughout the course of the investigation, cruciferous vegetables (such as cabbage, cauliflower, knol-khol, and radish) were grown in both HA and LA locations. The nutritional parameters of these vegetables, such as TSS, titratable acidity, total carbohydrate, crude fat, total protein content, dietary fiber content, ash, anions, and mineral (macro and micro) content, were significantly influenced by FYM, *Azotobacter*, and their combination as compared to control at both HA and LA locations. The detailed experimental findings are given below:-

4.3.2.1 Effect on TSS ($^{\circ}$B) content of cruciferous vegetable

Increased soluble solid content is frequently linked with enhanced flavor perception (Rahman *et al.,* 2021). The present study investigates the impact of four different treatments (T_1, T_2, T_3 and T_4) on Total soluble solid (TSS) content of cruciferous vegetables (Table 4.38), namely cabbage, cauliflower, knol-khol and radish, grown at HA and LA regions.

Table 4.38 Comparative effect of location and treatments on total soluble solids content (°B) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	T_1	7.77±0.06^{b}	7.73±0.12^{b}	**7.75±0.09^{bC*}**	7.07±0.06^{b}	7.10±0.10^{b}	**7.08±0.08^{bB***}**	8.07±0.06^{b}	8.03±0.12^{b}	**8.05±0.09^{b***}**	4.90±0.10^{b}	4.90±0.10^{b}	**4.90±0.09^{bA***}**
	T_2	7.73±0.12^{b}	7.73±0.06^{b}	**7.73±0.08^{bC**}**	6.90±0.10^{b}	7.07±0.06^{b}	**6.98±0.08^{bB***}**	8.07±0.06^{b}	8.03±0.06^{b}	**8.05±0.05^{b***}**	4.77±0.06^{b}	4.80±0.10^{b}	**4.78±0.08^{bA***}**
	T_3	7.93±0.06^{c}	7.97±0.06^{c}	**7.95±0.05^{cC**}**	7.50±0.10^{c}	7.60±0.10^{c}	**7.55±0.05^{cB***}**	9.07±0.06^{c}	8.93±0.15^{c}	**9.00±0.10^{c***}**	5.30±0.10^{c}	5.30±0.10^{c}	**5.30±0.10^{cA***}**
	T_4	6.80±0.10^{a}	6.83±0.06^{a}	**6.82±0.08^{aC**}**	6.63±0.15^{a}	6.57±0.15^{a}	**6.60±0.13^{aB***}**	7.33±0.15^{d}	7.20±0.26^{a}	**7.27±0.20^{a**}**	4.47±0.15^{a}	4.47±0.06^{a}	**4.47±0.08^{aA***}**
LA	T_1	7.47±0.06^{b}	7.40±0.17^{b}	**7.43±0.12^{bD}**	5.93±0.06^{b}	6.03±0.15^{b}	**5.98±0.08^{bB}**	6.90±0.1a^{b}	6.80±0.10^{b}	**6.85±0.00^{c}**	3.77±0.06^{b}	3.80±0.10^{b}	**3.78±0.08^{bA}**
	T_2	7.47±0.06^{b}	7.40±0.10^{b}	**7.43±0.06^{bD}**	5.93±0.06^{b}	6.03±0.12^{b}	**5.98±0.08^{bB}**	6.63±0.15^{a}	6.63±0.06^{b}	**6.63±0.06^{b}**	3.77±0.06^{b}	3.73±0.12^{b}	**3.75±0.09^{bA}**
	T_3	7.77±0.06^{c}	7.73±0.06^{c}	**7.75±0.05^{cD}**	6.57±0.06^{c}	6.60±0.10^{c}	**6.58±0.08^{cB}**	7.63±0.15^{c}	7.60±0.10^{c}	**7.62±0.12^{d}**	4.13±0.06^{c}	4.07±0.06^{c}	**4.10±0.00^{cA}**
	T_4	6.57±0.06^{a}	6.50±0.10^{a}	**6.53±0.06^{aD}**	5.37±0.06^{a}	5.47±0.15^{a}	**5.42±0.10^{aB}**	6.43±0.06^{a}	6.40±0.10^{a}	**6.42±0.08^{a}**	3.30±0.10^{a}	3.33±0.06^{a}	**3.32±0.03^{aA}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		NS			NS			***			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

The effect of treatments on cruciferous vegetables at both the locations was validated by One-way ANOVA. Notably, treatment T_3 (FYM+*Azotobacter*) exhibited a prominent impact, resulting in higher TSS content ranging from 5.30±0.10 to 9.15±0.07 °B and from 4.10±0.00 to 7.75±0.05 °B at HA and LA grown cruciferous vegetables respectively. This could be due to the effect of organic manure (FYM) inoculation with *Azotobacter* which increases the soil biological activity and enhanced the production of phytohormones such as IAA and gibberellins and increase the TSS content of the crops (Gadagi *et al*., 2004). Our findings are consistent with numerous studies that have found a wide range of beneficial effects of PGPB on promoting TSS content of horticulture crops (Upadhyay *et al*,. 2012 and Sepat *et al*., 2012; Sarkar and Rakshit, 2021). Furthermore, at HA, the TSS content in knol-khol, cabbage, cauliflower and radish was found to be 9.15±0.07 °B, 7.95±0.05 °B, 7.55±0.05 °B and 5.30±0.10 °B respectively which is higher in concentration as compared to LA grown knol-khol (7.46±0.05 °B), cabbage (7.75±0.05 °B), cauliflower (6.58±0.08 °B) and radish (4.10±0.00 °B). However, among cruciferous vegetables the higher TSS content was found in knol-khol (9.15±0.07 °B) at HA location. The interaction between altitude and treatments (ALT×TRE) was found in knol-khol and radish except cabbage and cauliflower which might be due to the difference in variety (Singh *et al*. 2011a) and high rates of photosynthesis and nitrogen availability in soil with great efficiency at HA. In previous reports, Rokaya *et al*., 2016 and Naryal *et al*., 2019**,** have shown that increasing elevation increased the TSS content in mandarin and apricot fruit.

4.3.2.2 Effect on titratable acidity (%) content of cruciferous vegetable

The titratable acidity (TA) values of the edible portion of cruciferous vegetables were statistically significant ($p \leq 0.05$). The results are demonstration in Table 4.39.

Table 4.39Comparative effect of location and treatments on titratable acidity content (%) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	0.21±0.01^{b}	0.21±0.02^{b}	**0.21±0.02^{bC}**	0.14±0.02^{b}	0.19±0.02^{b}	**0.17±0.01^{bB}**	0.24±0.02^{b}	0.22±0.02^{b}	**0.23±0.01^{b**}**	0.13±0.01^{b}	0.14±0.01^{a}	**0.13±0.01^{bA}**
	T2	0.24±0.01^{c}	0.23±0.01^{b}	**0.24±0.01^{cC*}**	0.17±0.02^{b}	0.17±0.01^{b}	**0.17±0.01^{bB}**	0.26±0.02^{b}	0.24±0.03^{b}	**0.25±0.02^{b**}**	0.12±0.01^{b}	0.14±0.01^{a}	**0.13±0.01^{bA}**
	T3	0.29±0.01^{d}	0.28±0.02^{c}	**0.29±0.01^{dC*}**	0.26±0.01^{c}	0.25±0.01^{c}	**0.26±0.01^{cB*}**	0.34±0.04^{c}	0.32±0.03^{c}	**0.33±0.01^{c***}**	0.19±0.01^{c}	0.20±0.02^{b}	**0.19±0.01^{cA}**
	T4	0.17±0.02^{a}	0.16±0.01^{a}	**0.16±0.01^{aB*}**	0.12±0.02^{a}	0.12±0.01^{a}	**0.12±0.01^{aA*}**	0.19±0.02^{a}	0.16±0.01^{a}	**0.18±0.01^{a**}**	0.11±0.01^{a}	0.11±0.01^{a}	**0.11±0.01^{aA**}**
LA	**T1**	0.22±0.02^{b}	0.20±0.03^{b}	**0.21±0.03^{bC}**	0.17±0.02^{b}	0.18±0.01^{b}	**0.17±0.00^{bB}**	0.18±0.02^{b}	0.18±0.02^{b}	**0.18±0.01^{b}**	0.12±0.02^{b}	0.15±0.03^{b}	**0.13±0.02^{bA}**
	T2	0.21±0.02^{b}	0.20±0.03^{b}	**0.21±0.01^{bC}**	0.17±0.04^{bc}	0.20±0.03^{b}	**0.18±0.00^{cB}**	0.18±0.02^{b}	0.18±0.02^{b}	**0.18±0.00^{b}**	0.12±0.01^{b}	0.14±0.03^{b}	**0.13±0.02^{bA}**
	T3	0.26±0.01^{c}	0.26±0.02^{c}	**0.26±0.02^{cB}**	0.21±0.03^{c}	0.27±0.02^{c}	**0.24±0.00^{dB}**	0.27±0.01^{c}	0.26±0.01^{c}	**0.27±0.01^{c}**	0.16±0.01^{c}	0.19±0.01^{c}	**0.18±0.01^{cA}**
	T4	0.12±0.01^{a}	0.12±0.02^{a}	**0.12±0.01^{aB}**	0.08±0.02^{a}	0.11±0.02^{a}	**0.09±0.01^{aA}**	0.12±0.02^{a}	0.12±0.02^{a}	**0.12±0.02^{a}**	0.08±0.02^{a}	0.09±0.01^{a}	**0.08±0.01^{aA}**
ALT		**			**			***			***		
TRE		***			***			***			***		
ALT×TRE		***			**			NS			**		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.
Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.
Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.
*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

High level of acidity indicates that mentioned treatments (T_1, T_2, T_3 and T_4) contain high amount of malic acid in cruciferous vegetable. Notably, One-way ANOVA analysis revealed that the treatment T_3 exhibited the highest titratable acidity content, ranging from 0.19±0.01 to 0.37±0.02% in HA-grown cruciferous vegetables and from 0.18±0.01 to 0.26±0.02% in LA-grown vegetables. The application of organic manure combined with biofertilizer has been linked to increased organic acid levels in fruits (Zhang *et al*., 2011). Moreover, knol-khol displayed the highest percentages of titratable acidity at HA (0.37±0.02%), followed by cabbage (0.29±0.01%), cauliflower (0.26±0.01%), and radish (0.19±0.01%), compared to LA-grown knol-khol (0.25±0.01%), cabbage (0.26±0.02%), cauliflower (0.24±0.00%), and radish (0.18±0.01%). The interaction between altitude and treatments (ALT×TRE) was significant ($p \leq 0.05$) across all experimental vegetables. Titratable acidity levels are influenced by crop type and maturity stages (Singh *et al*., 2011a), with potential effects from nutrient concentrations in organic + biofertilizer treatments in HA soil. These results are consistent with findings by Rahman *et al*. (2021).

4.3.2.3 Effect on total carbohydrate content (µg/g of DPE) of cruciferous vegetable

The human body relies on carbohydrates for numerous physiological functions, including energy provision, triglyceride and cholesterol metabolism, and regulation of blood sugar and insulin levels (Holesh *et al*., 2023). Table 4.40 illustrates the variations in carbohydrate contents among four different treatments (T_1, T_2, T_3, and T_4) of cruciferous vegetables grown at both HA and LA locations.

Table 4.40Comparative effect of location and treatments on total carbohydrate content (µg/g of DPE) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	68.62±0.06^{b}	68.08±0.16^{b}	**68.35±0.07^{bD***}**	56.26±0.06^{b}	56.15±0.17^{b}	**56.21±0.09^{bB***}**	59.70±0.17^{b}	59.78±0.24^{b}	**59.74±0.16^{b***}**	34.38±0.06^{b}	34.81±0.40b	**34.60±0.21^{bA}**
	T2	69.47±0.18^{c}	69.16±0.35^{c}	**69.32±0.11^{cD***}**	56.58±0.18^{c}	56.64±0.11^{b}	**56.61±0.12^{cB***}**	60.02±0.12^{c}	60.21±0.24^{b}	**60.12±0.17^{c***}**	34.76±0.12^{c}	34.95±0.25^{b}	**34.85±0.08^{bA*}**
	T3	73.7±0.17^{d}	73.35±0.38^{d}	**73.52±0.27^{dD**}**	58.62±0.06^{d}	58.51±0.46^{c}	**58.56±0.25^{dB***}**	65.69±0.23^{d}	65.34±0.46^{c}	**65.51±0.28^{d***}**	37.72±0.06^{d}	38.93±0.56^{c}	**38.33±0.25^{cA**}**
	T4	63.83±0.06^{a}	62.42±1.00^{a}	**63.13±0.50^{aD**}**	50.92±0.17^{a}	50.31±0.17^{a}	**50.62±0.01^{aC***}**	47.16±0.06^{a}	47.10±0.11^{a}	**47.13±0.03^{a***}**	29.73±0.06^{a}	29.73±0.73^{a}	**29.73±0.39^{aA}**
LA	**T1**	67.28±0.06^{b}	67.51±0.35^{b}	**67.40±0.19^{bD}**	52.9±0.29^{b}	52.33±0.11^{b}	**52.62±0.17^{bB}**	57.34±0.11^{b}	57.44±0.49^{b}	**57.39±0.27^{b}**	34.17±0.06^{b}	34.69±0.43^{b}	**34.43±0.23^{bA}**
	T2	67.97±0.06^{c}	67.23±0.20^{b}	**67.60±0.13^{bD}**	52.84±0.34^{b}	52.63±0.33^{b}	**52.74±0.12^{bB}**	57.43±0.12^{b}	57.78±0.19^{b}	**57.61±0.15^{b}**	34.59±0.18^{c}	34.64±0.29^{b}	**34.62±0.09^{bA}**
	T3	72.48±0.17^{d}	72.44±0.45^{c}	**72.46±0.14^{cD}**	54.79±0.22^{c}	55.27±0.16^{c}	**55.03±0.19^{cB}**	63.63±0.17^{c}	63.45±0.31^{c}	**63.54±0.12^{c}**	36.20±0.09^{d}	36.91±0.20^{c}	**36.56±0.14^{cA}**
	T4	60.38±0.06^{a}	60.27±1.10^{a}	**60.32±0.57^{aD}**	48.24±0.12^{a}	48.05±0.08^{a}	**48.15±0.09^{aC}**	45.06±0.06^{a}	45.57±0.21^{a}	**45.32±0.13^{a}**	29.05±0.06^{a}	29.76±0.54^{a}	**29.41±0.25^{aA}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			*			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Notably, treatment T_3 exerted a significant impact on total carbohydrate content, ranging from 38.33±0.25 to 73.52±0.27 µg/g of DPE in HA-grown cruciferous vegetables and from 36.56±0.14 to 72.46±0.14 µg/g in LA-grown vegetables. This effect may be attributed to the enhanced availability of nitrogen (N) and phosphorus (P) due to bio-organic manuring, which facilitates physiological processes such as carbohydrate synthesis (Zhang *et al.*, 2011; Upadhyay *et al.*, 2012; Shah *et al.*, 2019). Similarly, research on Chinese cabbage treated with biofertilizers has shown an increase in carbohydrate accumulation (Ji *et al*., 2020). Furthermore, at HA, the highest total carbohydrate content was observed in cabbage (73.52±0.27 µg/g of DPE), followed by knol-khol (67.39±1.10 µg/g of DPE), cauliflower (58.56±0.25 µg/g of DPE), and radish (38.33±0.25 µg/g of DPE), compared to LA grown: cabbage (72.46±0.14 µg/g of DPE), knol-khol (57.51±1.16 µg/g of DPE), cauliflower (55.03±0.19 µg/g of DPE), and radish (36.56±0.14 µg/g of DPE). Notably, cabbage exhibited the highest carbohydrate content among cruciferous vegetables at HA, as confirmed by an independent t-test analysis between HA and LA. The interaction between altitude and treatments (ALT×TRE) significantly influenced carbohydrate content in all experimental vegetables. Factors such as longer photoperiods and higher light intensity at HA may enhance photosynthesis, leading to increased carbohydrate content (Allen, 2016). Additionally, factors like deficit irrigation and dry climatic conditions at HA could contribute to higher carbohydrate content. These findings are consistent with research on mandarins by Rokaya *et al.* (2016) and apricots by Naryal *et al.* (2019). Enhancing carbohydrate levels in organically grown vegetables could potentially ameliorate hypoxemia among HA consumers under extreme conditions.

4.3.2.4Effect on crude fat content (%) of cruciferous vegetable

Crude fat plays a crucial role in plant development and the synthesis of organic substances, with its content directly influenced by the macro and microelement composition of the soil (Yassen *et al.*, 2009). The variations in crude fat contents of four different treatments (T_1, T_2, T_3 and T_4) and location (HA & LA) of studied vegetable showed statistically significant (Table 4.41).

Table 4.41 Comparative effect of location and treatments on crude fat content (%) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	0.24 ± 0.01^{b}	0.25 ± 0.01^{b}	$\mathbf{0.24\pm0.01^{bC}}$	1.01 ± 0.01^{c}	1.01 ± 0.01^{c}	$\mathbf{1.01\pm0.00^{cD**}}$ *	0.21 ± 0.01^{a}	0.24 ± 0.02^{b}	$\mathbf{0.23\pm0.01^{b}}$	0.16 ± 0.01^{b}	0.15 ± 0.01^{b}	$\mathbf{0.16\pm0.01^{bA***}}$
	T2	0.31 ± 0.00^{b}	0.32 ± 0.01^{c}	$\mathbf{0.31\pm0.01^{cC}}$	0.89 ± 0.04^{b}	0.89 ± 0.04^{b}	$\mathbf{0.89\pm0.04^{bD*}}$	0.28 ± 0.01^{b}	0.27 ± 0.02^{bc}	$\mathbf{0.27\pm0.01^{c}}$	0.16 ± 0.00^{b}	0.16 ± 0.02^{bc}	$\mathbf{0.16\pm0.01^{bA***}}$
	T3	0.33 ± 0.01^{d}	0.35 ± 0.02^{d}	$\mathbf{0.34\pm0.01^{dC}}$	1.14 ± 0.02^{d}	1.15 ± 0.01^{d}	$\mathbf{1.14\pm0.02^{dD**}}$	0.31 ± 0.02^{c}	0.30 ± 0.03^{c}	$\mathbf{0.31\pm0.02^{d*}}$	0.17 ± 0.01^{c}	0.18 ± 0.02^{c}	$\mathbf{0.18\pm0.01^{cA**}}$
	T4	0.11 ± 0.00^{a}	0.12 ± 0.02^{a}	$\mathbf{0.12\pm0.01^{aA}}$	0.61 ± 0.01^{a}	0.62 ± 0.03^{a}	$\mathbf{0.61\pm0.02^{aC}}$	0.20 ± 0.00^{a}	0.20 ± 0.01^{a}	$\mathbf{0.20\pm0.01^{a}}$	0.13 ± 0.01^{a}	0.12 ± 0.01^{a}	$\mathbf{0.13\pm0.01^{aA***}}$
LA	**T1**	0.27 ± 0.02^{b}	0.26 ± 0.02^{b}	$\mathbf{0.27\pm0.01^{bC**}}$	1.16 ± 0.03^{c}	1.14 ± 0.02^{b}	$\mathbf{1.15\pm0.02^{cD}}$	0.24 ± 0.00^{b}	0.23 ± 0.02^{ab}	$\mathbf{0.23\pm0.01^{b}}$	0.04 ± 0.01^{b}	0.05 ± 0.02^{a}	$\mathbf{0.04\pm0.02^{aA}}$
	T2	0.31 ± 0.01^{c}	0.32 ± 0.01^{c}	$\mathbf{0.31\pm0.01^{cC}}$	0.95 ± 0.01^{b}	0.95 ± 0.01^{c}	$\mathbf{0.95\pm0.01^{bD}}$	0.25 ± 0.01^{c}	0.24 ± 0.02^{b}	$\mathbf{0.25\pm0.01^{c}}$	0.04 ± 0.01^{b}	0.05 ± 0.01^{a}	$\mathbf{0.04\pm0.01^{aA}}$
	T3	0.37 ± 0.02^{d}	0.34 ± 0.02^{c}	$\mathbf{0.35\pm0.01^{dC}}$	1.21 ± 0.00^{d}	1.20 ± 0.01^{d}	$\mathbf{1.21\pm0.01^{dD}}$	0.27 ± 0.01^{d}	0.28 ± 0.01^{c}	$\mathbf{0.28\pm0.01^{d}}$	0.07 ± 0.01^{c}	0.08 ± 0.02^{b}	$\mathbf{0.07\pm0.01^{bA}}$
	T4	0.14 ± 0.01^{a}	0.15 ± 0.02^{a}	$\mathbf{0.14\pm0.01^{aC*}}$	0.61 ± 0.01^{a}	0.63 ± 0.01^{a}	$\mathbf{0.62\pm0.00^{aD}}$	0.20 ± 0.01^{a}	0.21 ± 0.01^{a}	$\mathbf{0.21\pm0.01^{a}}$	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	$\mathbf{0.02\pm0.01^{aA}}$
ALT		***			***			*			***		
TRE		***			***			***			***		
ALT×TRE		NS			***			**			NS		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Notably, treatment T_3 exhibited higher fat content, ranging from 0.18±0.01 to 1.14±0.02% at HA and 0.07±0.01 to 1.21±0.01% at LA-grown samples. This effect is attributed to the elevated electrical conductivity and nitrogen content in T_3-treated soil at both high and low altitudes, facilitating crude fat synthesis (Vidal *et al.,* 2018). Furthermore, at HA, the maximum percentages of crude fat were found in cauliflower (1.14±0.02%), cabbage (0.34±0.01%), knol-khol (0.26±0.02%) and radish (0.18±0.01%), respectively as compared to LA grown cauliflower (1.21±0.01%), cabbage (0.35±0.01%), knol-khol (0.21±0.01%) and radish (0.07±0.01%). However, among cruciferous vegetables the higher crude fat was found in cauliflower (1.14±0.02%), at HA location, as proved by an independent t-test analysis. The interaction between altitude and treatments (ALT×TRE) was also found on cauliflower. It might be because HA soil has higher levels of electrical conductivity and nitrogen content which amendment the largest concentrations of ions that encourage the synthesis of crude fat/ fatty acid. Our findings correlate well with Vidal *et al.,* 2018 and Abbey *et al.,* 2018.

4.3.2.5 Effect on total protein content (g/100g DW) of cruciferous vegetable

The human body needs protein in order to produce enough of the necessary amino acids. The significance of dietary protein needs at high altitudes takes a backseat to energy requirements, as an energy deficit alone can result in adverse effects such as negative protein balance and muscle mass loss. While there is limited evidence supporting a reduction in protein synthesis due to hypoxia, this phenomenon is typically observed at much greater altitudes (Koivisto-Mork *et al.* 2020). In the present study, a notable disparity in the protein content of cruciferous vegetables was examined across various treatments (T_1, T_2, T_3, and T_4) and locations, as shown in Table 4.42.

Table 4.42 Comparative effect of location and treatments on total protein content (g/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	14.79±0.08^{b}	14.97±0.56^{b}	**14.88±0.30^{Ba*}**	17.46±0.2^{b}	16.56±0.56^{b}	**17.00±0.18^{bC}**	15.94±0.48^{b}	16.16±0.21^{b}	**16.05±0.31^{b***}**	16.25±0.56^{ab}	16.16±0.22^{b}	**16.21±0.39^{bB***}**
	T2	16.52±0.12^{c}	16.64±0.33^{c}	**16.58±0.18^{cB***}**	17.62±0.23^{b}	17.03±0.48^{b}	**17.32±0.24^{bC}**	18.57±0.21^{c}	18.25±0.22^{c}	**18.41±0.10^{c***}**	16.83±0.22^{bc}	16.49±0.14^{b}	**16.66±0.04^{bB***}**
	T3	17.65±0.07^{d}	17.82±0.21^{d}	**17.73±0.08^{dA**}**	18.34±0.06^{c}	18.46±0.41^{c}	**18.40±0.23^{cB*}**	19.19±0.24^{d}	19.61±0.15^{d}	**19.40±0.12^{d***}**	17.46±0.16^{c}	17.71±0.22^{c}	**17.58±0.16^{cA***}**
	T4	14.11±0.31^{a}	12.78±0.55^{a}	**13.45±0.14^{aB**}**	15.26±0.06^{a}	15.24±0.06^{a}	**15.25±0.06^{aC***}**	14.79±0.22^{a}	14.65±0.22^{a}	**14.72±0.20^{a***}**	15.78±0.54^{a}	14.49±0.16^{a}	**15.13±0.33^{aC***}**
LA	**T1**	14.21±0.21^{b}	13.99±0.23^{b}	**14.1±0.11^{bC}**	16.84±0.21^{b}	16.91±0.12^{b}	**16.87±0.16^{bD}**	13.39±0.35^{b}	12.89±0.21^{b}	**13.14±0.08^{b}**	12.29±0.13^{b}	12.00±0.23^{b}	**12.15±0.14^{bB}**
	T2	14.55±0.55^{b}	14.33±0.34^{b}	**14.44±0.41^{bC}**	16.95±0.12^{b}	16.80±0.24^{b}	**16.88±0.14^{bD}**	14.26±0.46^{c}	13.97±0.23^{c}	**14.12±0.34^{c}**	12.66±0.22^{b}	12.22±0.22^{b}	**12.44±0.10^{bB}**
	T3	16.74±0.32^{c}	16.52±0.46^{c}	**16.63±0.35^{cB}**	17.83±0.09^{c}	17.76±0.22^{c}	**17.80±0.15^{cC}**	15.8±0.37^{d}	15.44±0.35^{d}	**15.62±0.31^{d}**	13.97±0.22^{c}	13.61±0.26^{c}	**13.79±0.07^{cA}**
	T4	12.60±0.31^{a}	12.67±0.19^{a}	**12.63±0.25^{aC}**	14.84±0.22^{a}	14.62±0.21^{a}	**14.73±0.10^{aD}**	11.78±0.23^{a}	11.49±0.34^{a}	**11.63±0.14^{a}**	11.21±0.34^{a}	11.28±0.26^{a}	**11.25±0.29^{aB}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			NS			***			NS		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Within the treatments, T_3 displayed superior crude protein content, with the analyzed varieties ranging from 17.58±0.16 to 19.06±0.19 (g/100g DW) at HA and 13.58±0.32 to 17.80±0.15 (g/100g DW) at LA. This effect is likely due to the combined application of FYM+*Azotobacter*, increasing nitrogen content in the soil. These findings align with previous research (Upadhyay et al., 2012; Shah et al., 2019).Furthermore, at HA, the highest crude protein content was observed in knol-khol (19.06±0.19 g/100g DW), cauliflower (18.40±0.23 g/100g DW), cabbage (17.73±0.08 g/100g DW), and radish (17.58±0.16 g/100g DW), respectively, compared to LA grown knol-khol (13.58±0.32 g/100g DW), cauliflower (17.80±0.15 g/100g DW), cabbage (16.63±0.35 g/100g DW), and radish (13.79±0.07 g/100g). Notably, knol-khol exhibited the highest crude protein content among cruciferous vegetables at HA, confirmed by an independent t-test. The interaction between altitude and treatments (ALT×TRE) was significant for cabbage and knol-khol. The increase in protein content with altitude could be attributed to N-rich compounds in highly mineralized soil (NO_3^-), which promote protein formation in plants (Lima *et al*., 2009). These findings are consistent with previous studies on cabbage (Kumar *et al.,* 2015; Upadhyay *et al.,* 2012) and capsicum (Fallovo *et al.,* 2011).

4.3.2.6 Effect on dietary fiber content (%) of cruciferous vegetable

Dietary fiber plays a crucial role in human nutrition, aiding in the regulation of blood sugar levels, supporting bowel health, reducing cholesterol levels, and decreasing the bioavailability of dietary fat (Rahman *et al.,* 2021). The results of the treatments (T_1, T_2, T_3, and T_4) in the study exhibited significant variations in dietary fiber content (Table 4.43).

Table 4.43 Comparative effect of location and treatments on dietary fiber content (%) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	8.84±0.03^{b}	8.76±0.07^{b}	**8.80±0.04^{bcC***}**	8.25±0.05^{b}	8.30±0.12^{b}	**8.27±0.08^{bB}**	10.65±0.02^{b}	10.56±0.06^{b}	**10.61±0.02^{b***}**	7.43±0.07^{b}	7.47±0.14^{b}	**7.45±0.11^{bA**}**
	T2	8.55±0.33^{b}	8.62±0.23^{b}	**8.58±0.20^{bB*}**	8.90±0.03^{c}	8.84±0.04^{c}	**8.87±0.01^{cC*}**	10.61±0.11^{b}	10.53±0.10^{b}	**10.57±0.09^{b***}**	7.42±0.13^{b}	7.49±0.18^{b}	**7.45±0.15^{bA***}**
	T3	9.71±0.33c	9.47±0.19^{c}	**9.59±0.21^{cB*}**	9.80±0.07^{d}	9.72±0.13^{d}	**9.76±0.10^{dC***}**	11.12±0.22^{c}	11.05±0.11^{c}	**11.09±0.17^{c**}**	8.21±0.02^{c}	8.28±0.10^{c}	**8.24±0.04^{cA***}**
	T4	8.01±0.19^{a}	7.93±0.20^{a}	**7.97±0.18^{aC}**	8.06±0.06^{a}	8.04±0.09^{a}	**8.05±0.06^{aC}**	9.59±0.02^{a}	9.17±0.38^{a}	**9.38±0.19^{a}**	7.12±0.02^{a}	6.97±0.04^{a}	**7.04±0.03^{aA***}**
LA	**T1**	8.54±0.02^{c}	8.52±0.06^{b}	**8.53±0.03^{cD}**	8.31±0.02^{b}	8.27±0.08^{a}	**8.29±0.05^{aC}**	9.65±0.07^{b}	9.56±0.16^{b}	**9.60±0.10^{b}**	6.93±0.10^{c}	6.93±0.08^{b}	**6.93±0.07^{bA}**
	T2	8.12±0.03^{b}	8.32±0.08^{b}	**8.22±0.02^{bC}**	9.34±0.01^{c}	9.17±0.30^{b}	**9.14±0.06^{bD}**	9.68±0.02^{b}	9.61±0.13^{b}	**9.64±0.07^{b}**	6.58±0.08^{b}	6.63±0.13^{a}	**6.60±0.09^{aA}**
	T3	9.04±0.27d	9.14±0.29^{c}	**9.09±0.26^{dB}**	9.07±0.04^{d}	9.21±0.12^{b}	**9.26±0.15^{bB}**	10.35±0.02^{c}	10.30±0.06^{c}	**10.33±0.03^{c}**	7.71±0.05^{d}	7.67±0.04^{c}	**7.69±0.04^{cA}**
	T4	7.51±0.02^{a}	7.97±0.04^{a}	**7.74±0.02^{aC}**	8.02±0.27^{a}	8.17±0.34^{a}	**8.09±0.28^{aD}**	9.21±0.07^{a}	9.07±0.14^{a}	**9.14±0.04^{a}**	6.42±0.02^{a}	6.54±0.14^{a}	**6.48±0.08^{aA}**
ALT		NS			NS			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			***			*		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Notably, T_3 outperformed other treatments, demonstrating a fiber content ranging from 8.24±0.04 to 11.06±0.09% at HA and 7.69±0.04 to 10.09±0.11% at LA. The application of organic manures and biofertilizers notably enhanced the fiber content in T_3 treatment (Upadhyay *et al.* 2012). Furthermore, the higher percentages of dietary fiber were found in knol-khol (11.06±0.09%), cauliflower (9.76±0.10%), cabbage (9.59±0.21%), and radish (8.24±0.04%) at HA respectively as compared to LA grown knol-khol (10.09±0.11%), cauliflower (9.26±0.15%), cabbage (9.09±0.26%) and radish (7.69±0.04%). However, among cruciferous vegetables the maximum dietary fiber was found in knol-khol (11.06±0.09%), at HA location. The interaction between altitude and treatments (ALT×TRE) was also found significant ($p \leq 0.05$) in all the experimental vegetables. It might occur as a result of the high levels of nutrients in HA soil and deficit irrigation and dry climatic condition. The use of bio-organic manure results in an increased fiber content of the carrot due to the activity of organic acids released by the microbes (Evers, 1989). Our findings are consistent with earlier studies on butternut squash (Armesto *et al*., 2020) and bell pepper (Abu-Zahra, 2011).

4.3.2.7 Effect on ash content (%) of cruciferous vegetable

The concentration of minerals included in the vegetables is indicated by their ash level. Vegetables with high ash content are thought to have a high mineral content and are therefore very healthy; however, this may be the opposite if the vegetable included hazardous metals, which also influence the ash percentage of vegetables (Mohammed and Umar, 2023). Table 4.44 in the current study demonstrated that there were substantial differences in ash content across the locations (HA *vs.* LA) and treatments (T_1, T_2, T_3, and T_4).

Table 4.44 Comparative effect of location and treatments on ash content (%) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	8.25±0.02^{b}	8.26±0.04^{b}	**8.26±0.03^{bA***}**	9.13±0.01^{b}	9.16±0.02^{b}	**9.14±0.02^{bB**}**	12.45±0.02^{c}	12.38±0.03^{c}	**12.42±0.01^{c}**	15.23±0.04^{b}	15.13±0.05^{b}	**15.18±0.04^{bD***}**
	T2	8.26±0.04^{b}	8.24±0.07^{b}	**8.25±0.05^{bA***}**	9.32±0.04^{c}	9.30±0.04^{c}	**9.31±0.04^{cB***}**	12.18±0.02^{b}	12.25±0.07^{b}	**12.21±0.03^{b}**	15.24±0.01^{b}	15.22±0.03^{c}	**15.23±0.02^{bD**}**
	T3	8.26±0.02^{b}	8.28±0.01^{b}	**8.27±0.00^{bA***}**	9.48±0.04^{d}	9.53±0.05^{d}	**9.51±0.03^{dB***}**	12.77±0.01^{d}	12.83±0.10^{d}	**12.80±0.04^{d}**	15.18±0.08^{b}	15.23±0.04^{c}	**15.21±0.05^{bD***}**
	T4	8.13±0.03^{a}	8.09±0.03^{a}	**8.11±0.02^{aA***}**	8.42±0.07^{a}	8.47±0.12^{a}	**8.45±0.10^{aB*}**	12.02±0.02^{a}	12.00±0.05^{a}	**12.01±0.03^{a}**	14.32±0.01^{a}	14.41±0.04^{a}	**14.37±0.01^{aC***}**
LA	**T1**	7.69±0.01^{b}	7.69±0.01^{b}	**7.69±0.01^{bA}**	9.07±0.02^{b}	9.09±0.03^{b}	**9.08±0.01^{bB}**	11.54±0.02^{b}	11.55±0.02^{b}	**11.55±0.02^{b***}**	14.55±0.01^{b}	14.58±0.03^{b}	**14.56±0.02^{bD}**
	T2	7.66±0.03^{b}	7.68±0.03^{b}	**7.67±0.02^{bA}**	9.11±0.02^{b}	9.08±0.04^{b}	**9.09±0.02^{bB}**	11.65±0.02^{c}	11.57±0.04^{b}	**11.61±0.03^{c***}**	14.98±0.02^{b}	14.60±0.31^{b}	**14.79±0.15^{bD}**
	T3	7.97±0.06^{c}	7.97±0.07^{c}	**7.97±0.06^{cA}**	9.24±0.03^{c}	9.23±0.03^{c}	**9.24±0.03^{cB}**	11.97±0.03^{d}	11.94±0.04^{c}	**11.95±0.04^{d***}**	14.53±0.03^{c}	14.60±0.04^{b}	**14.57±0.03^{cD}**
	T4	7.09±0.06^{a}	7.12±0.03^{a}	**7.10±0.05^{aA}**	8.88±0.03^{a}	8.50±0.06^{a}	**8.69±0.04^{aB}**	11.15±0.02^{a}	11.21±0.07^{a}	**11.18±0.04^{a***}**	13.52±0.01^{a}	13.56±0.05^{a}	**13.54±0.03^{aD}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			***			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.
Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.
Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.
*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Among the treatments, T_3 displayed superior ash content, with the analyzed varieties ranging from 8.27±0.00 to 15.21±0.05% at HA and 7.97±0.06 to 14.57±0.03% at LA. This effect is likely due to the FYM has an abundance of minerals and organic materials. It increases the availability of nutrients in the soil when paired with the nitrogen-fixing bacterium *Azotobacter*. The higher concentration of minerals that may accumulate in the plant tissues as a result of the enhanced nutrient availability might increase the ash content of the plant. These findings align with previous research (Drozdowska *et al*., 2020; Vale *et al*., 2015). Furthermore, at HA, the highest ash content was observed in radish (15.21±0.05%), knol-khol (12.80±0.04%), cauliflower (9.51±0.03%), and cabbage (8.27±0.00%), respectively, compared to LA grown radish (14.57±0.03%), knol-khol (11.95±0.04%), cauliflower (9.24±0.03%), and cabbage (7.97±0.06%). Notably, radish exhibited the highest ash content among cruciferous vegetables at HA, confirmed by an independent t-test. The interaction between altitude and treatments (ALT×TRE) on ash content was also found significant. Singh *et al.* (2011a) reported that ash content is also influenced by crop type and maturity stages. The plant growing at the HA location may have a high mineral absorption, which reflects the increase in ash content with altitude. According to Mohammed and Umar (2023), a plant's high ash content is caused by its high mineral concentration.

4.3.2.8 Effect on anions content (mg/Kg FW) of cruciferous vegetable

Anions, like nitrate, are found naturally in plant-based substances and are considered to human health because of their reactivity and ease of conversion to nitrites (Rahman *et al.,* 2021). The accumulation of anions such as nitrate, phosphate, and sulphate in fresh produce is primarily influenced by the availability of nitrogen, phosphorus, and potassium in the soil. Significant variations in anion content were observed among different varieties across various bio-organic treatments and locations. Among the various treatments, T_3 treatment demonstrated superior results in nitrate content, ranging from 178.75±6.60 to 1879.45±14.01 mg/kg (Table 4.45), phosphate, ranging from 797.21±12.13 to 978.09±5.65 mg/kg (Table 4.46), and sulphate, ranging from 190.18±2.81 to 335.21±5.43 mg/kg (Table 4.47), in cruciferous vegetables grown in high altitude (HA) conditions compared to those grown in low altitude (LA) conditions, where nitrate, phosphate, and sulphate levels were observed to be (162.60±1.94 to 1609.20±10.28 mg/kg, 665.04±12.95 to 910.02±23.66 mg/kg, and

76.75±4.09 to 291.83±12.27 mg/kg), respectively. The accumulation of anions in the T_3 treatment could be the results of abundance of bio-available nitrogen (N), phosphorus (P), and sulfur (S) in the soil (Liu *et al*., 2014). However, this possibility could be mitigated by implementing organic nutrient management practices, as soluble nitrate (N), phosphate (P), and sulphur (S) are gradually released from manure (Goswami *et al*., 2017). Our findings are in line with those of Herencia *et al*. (2011), who found that organic farming increased the anions content of strawberries and carrots.

Table 4.45 Comparative effect of location and treatments on nitrate content (mg/Kg FW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	264.65±14^{b}	224.28±10.27^{b}	**244.46±3.89^{bB}**	134.57±6.73^{b}	139.06±3.89^{b}	**136.81±3.89^{bA**}**	753.57±6.73^{b}	749.09±16.93^{b}	**751.33±10.82^{bC**}**	1715.73±6.73^{b}	1697.78±10.28^{b}	**1706.75±1.95^{bD***}**
	T2	298.29±3.89^{c}	269.13±6.73^{c}	**283.71±5.14^{cB}**	154.30±0.77^{c}	152.51±3.89^{b}	**153.40±2.33^{cA*}**	767.03±6.73^{c}	760.30±13.46^{b}	**763.67±8.90^{bC}**	1706.76±10.28^{b}	1693.3±20.56^{b}	**1700.03±13.60^{bD***}**
	T3	441.83±3.89^{d}	426.13±14.00^{d}	**433.98±6.73^{dB**}**	181.00±5.75^{d}	176.51±9.19^{c}	**178.75±6.60^{dA*}**	933.45±4.33^{d}	928.51±6.73^{c}	**930.98±3.71^{cC***}**	1888.42±10.27^{c}	1870.48±20.19^{c}	**1879.45±14.01^{cD***}**
	T4	208.58±6.73^{a}	174.94±6.73^{a}	**191.76±0.00^{aB*}**	100.70±6.74^{a}	96.44±10.27^{a}	**98.57±7.87^{aA*}**	580.88±3.89^{a}	585.37±6.73^{a}	**583.13±3.89^{Ac**}**	1626.46±4.32^{a}	1590.13±16.93^{a}	**1608.30±10.43^{aD***}**
LA	**T1**	235.49±6.73^{b}	233.25±10.27^{b}	**234.37±8.47^{bB}**	109.89±3.89^{b}	111.24±6.22^{b}	**110.57±5.05^{bA}**	661.62±16.93^{b}	659.38±20.19^{b}	**660.50±18.53^{bC}**	1527.33±13.46^{b}	1529.58±16.93^{b}	**1528.45±15.17^{bD}**
	T2	284.83±3.89^{c}	287.08±7.77^{c}	**285.95±5.83^{cB}**	145.78±3.88^{c}	147.13±5.44^{c}	**146.45±3.11^{cA}**	740.12±13.46^{c}	735.63±16.93^{c}	**737.87±15.17^{cC}**	1522.85±10.27^{b}	1525.09±10.27^{b}	**1523.97±10.09^{bD}**
	T3	423.89±6.73^{d}	419.40±3.89^{d}	**421.65±3.89^{dB}**	161.48±6.73^{d}	163.72±3.89^{d}	**162.60±1.94^{dA}**	850.01±3.89^{d}	847.77±6.73^{d}	**848.89±5.14^{dC}**	1608.07±6.73^{c}	1610.32±14.00^{c}	**1609.20±10.28^{cD}**
	T4	201.85±6.73^{a}	204.09±3.89^{a}	**202.97±5.14^{aB}**	78.50±3.89^{a}	78.05±3.56^{a}	**78.27±3.71^{aA}**	547.24±14^{a}	542.75±10.28^{a}	**544.99±11.65^{aC}**	1455.56±3.89^{a}	1453.32±6.73^{a}	**1454.44±5.14^{aD}**
ALT		NS			***			***			***		
TRE		***			***			***			***		
ALT×TRE		**			*			***			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Table 4.46 Comparative effect of location and treatments on phosphate content (mg/Kg FW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	688.11±19.43^{b}	679.72±5.04^{b}	**683.91±12.19^{bA***}**	730.07±7.27^{c}	720.75±14.01^{b}	**725.41±7.70^{cC}**	713.29±7^{b}	725.88±7.40^{b}	**719.58±1.21^{bC*}**	948.72±8.07^{b}	916.08±13.99^{b}	**932.40±4.04^{bD*}**
	T2	720.28±13.99^{c}	692.31±13.99^{b}	**706.29±7.00^{cA***}**	696.97±21.36^{b}	701.63±21.36^{b}	**699.30±18.50^{bA*}**	720.28±13.99^{b}	715.62±8.08^{b}	**717.95±10.68^{bA**}**	958.97±12.62^{b}	941.73±10.68^{c}	**950.35±10.44^{cB**}**
	T3	811.66±7.04^{d}	802.33±11.31^{c}	**807.00±4.84^{dAB***}**	810.72±20.28^{d}	783.68±10.59^{c}	**797.21±12.13^{dB*}**	823.78±7.4^{c}	815.39±10.08^{c}	**819.58±7.40^{cB**}**	986.01±13.99^{c}	970.16±11.31^{d}	**978.09±5.65^{dD**}**
	T4	581.35±14.08^{a}	552.45±13.99^{a}	**566.90±6.31^{aB***}**	517.48±7.00^{a}	482.52±13.99^{a}	**500.00±9.25^{aA}**	519.82±8.08^{a}	508.16±10.68^{a}	**513.99±9.25^{aA**}**	622.38±13.99^{a}	580.42±13.99^{a}	**601.40±6.99^{aD***}**
LA	**T1**	552.45±13.99^{b}	554.78±14.56^{b}	**553.61±14.13^{bA}**	720.28±13.99^{c}	716.55±19.85^{c}	**718.42±16.86^{cC}**	669±21.36^{c}	671.33±18.5^{c}	**670.17±19.88^{cB}**	864.80±42.73^{b}	855.48±35.2^{b}	**860.14±38.93^{bD}**
	T2	603.73±21.36^{c}	597.67±9.52^{c}	**600.70±13.33^{cA}**	645.69±21.36^{b}	641.03±16.15^{b}	**643.36±18.50^{bB}**	622.38±27.97^{cb}	627.04±21.36^{b}	**624.71±24.56^{bAB}**	841.49±29.12^{b}	836.83±21.36^{b}	**839.16±25.21^{bC}**
	T3	664.34±13.99^{d}	665.74±11.95^{d}	**665.04±12.95^{dA}**	763.17±14.08^{d}	758.51±21.72^{d}	**760.84±17.85^{dB}**	768.3±7.17^{d}	763.63±14.19^{d}	**765.96±10.5^{dB}**	911.42±21.36^{c}	908.62±25.99^{c}	**910.02±23.66^{cC}**
	T4	445.22±21.36^{a}	440.56±13.99^{a}	**442.89±17.6^{aA}**	482.52±13.99^{a}	483.91±14.19^{a}	**483.22±14.04^{aB}**	440.56±13.99^{a}	439.16±14.2^{a}	**439.86±14.04^{aA}**	473.19±8.08^{a}	469.93±11.95^{a}	**471.56±9.80^{aB}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		NS			NS			NS			*		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Notably, radishes cultivated in HA conditions exhibited the highest nitrate and phosphate content (1879.45±14.01 and 978.09±5.65 mg/kg) compared to those grown in LA conditions (1609.20±10.28 and 910.02±23.66 mg/kg) respectively. Furthermore, cauliflower exhibited the highest sulphate content (335.21±5.43 mg/kg) among cruciferous vegetables grown in HA conditions. It might be the outcome of the higher mineral concentration in high altitude soil compared to low altitude soil. Rahman *et al.* (2021) also noted that the environment, variety, harvesting date, growing site, water supply, and soil type all affected the availability of anions in plants.

4.3.2.9 Effect on nitrogen content (mg/100g DW) of cruciferous vegetable

Nitrogen (N) is a vital component of the body, needed for the synthesis of tissue proteins as well as the synthesis of several nitrogenous compounds that are involved in a wide range of processes (hormones, immunological mediators, neurotransmitters, antioxidant defenses, etc.). Therefore, to maintain normal bodily functions, the body's nitrogen content should be both quantitatively and qualitatively normal as well as normally maintained (Tessari, 2006). Nitrogen plays a vital role as a key nutrient for plants, serving as an essential building block for the protein and chlorophyll present in various crucial parts of the plant's anatomy (Sepat *et al.*, 2012). Table 4.48 illustrates variations in total nitrogen contents of four different treatments (T_1, T_2, T_3 and T_4) of cruciferous vegetable grown at HA and LA location. Among the treatment T_3demonstrated a prominent impact on total nitrogen content ranging from 2813.04±26.24 to 3103.98±19.37 (mg/100g) at HA grown cruciferous vegetables and from 2206.42±11.05 to 2847.73±25.05 (mg/100g) at LA respectively. It might be due to nitrogen-fixing bacteria (*Azotobacter*), which aid in stabilizing atmospheric nitrogen in the soil, enhancing its availability to plants. These findings closely align with those reported by Abd AL-Hseen *et al.* (2020) for cauliflower and Ji *et al.* (2020) for cabbage.

Moreover, at HA, the highest total nitrogen content was observed in knol-khol (3103.98±19.37 mg/100g), cauliflower (2943.3±35.51 mg/100g), cabbage (2837.85±12.35 mg/100g), and radish (2813.04±26.24 mg/100g), compared to LA grown counterparts: knol-khol (2499.23±50.25mg/100g), cauliflower (2847.73±25.05 mg/100g), cabbage (2660.51±55.67 mg/100g), and radish (2206.42±11.05 mg/100g).

Among cruciferous vegetables, knol-khol exhibited the highest nitrogen content at HA, confirmed by an independent t-test analysis. The interaction between altitude and treatments (ALT×TRE) influenced nitrogen content in cabbage and knol-khol. Kalisz *et al.* (2018) suggested that different Brassicaceae species' ability to absorb minerals from the soil and distribute them throughout plant tissues contributes to variations in mineral content among varieties. Additionally, higher nitrogen availability in HA soil may also contribute to the elevated nitrogen content observed. These observations underline the intricate relationship between nitrogen availability, soil characteristics, and plant growth, crucial for optimizing nutrient content in cruciferous vegetables.

Table 4.47 Comparative effect of location and treatments on sulphate content (mg/Kg FW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	272.02±3.86^{b}	253.46±8.18^{b}	**262.74±2.74^{bB**}**	312.36±8.18^{b}	312.36±3.09^{c}	**312.36±3.09^{cD***}**	298.08±8.18^{b}	279.16±5.50^{b}	**288.62±6.84^{bC**}**	157.97±2.68^{b}	158.86±8.18^{b}	**158.41±2.78^{bA**}**
	T2	301.65±11.15^{c}	294.51±5.36^{c}	**298.08±5.57^{cC**}**	310.58±5.36^{b}	290.94±8.18^{b}	**300.76±5.57^{bC***}**	292.73±11.15^{b}	277.73±6.45^{b}	**285.23±6.02^{bB***}**	160.64±5.36^{b}	164.21±6.18^{b}	**162.43±4.09^{bA***}**
	T3	323.07±8.18^{d}	321.29±5.36^{d}	**322.18±1.54^{dB*}**	338.42±5.67^{c}	331.99±5.36^{d}	**335.21±5.43^{dC***}**	321.29±5.36^{c}	326.64±5.35^{c}	**323.97±4.64^{cB***}**	192.77±5.36^{c}	187.60±5.63^{c}	**190.18±2.81^{cA**}**
	T4	210.09±4.68^{a}	180.28±8.18^{a}	**195.18±6.29^{aB}**	238.11±2.69^{a}	225.61±6.46^{a}	**231.86±3.86^{aD***}**	216.87±7.08^{a}	220.44±5.57^{a}	**218.65±3.09^{aC***}**	130.30±3.09^{a}	128.51±5.36^{a}	**129.41±3.09^{aA*}**
LA	**T1**	230.25±10.71^{b}	229.36±9.41^{b}	**229.81±10.05^{bC}**	58.90±5.36^{c}	59.97±3.86^{c}	**59.44±4.58^{cA}**	248.10±11.15^{c}	246.32±10.71^{c}	**247.21±10.82^{cD}**	141.01±8.18^{b}	140.12±4.09^{b}	**140.56±6.14^{bB}**
	T2	248.10±11.15^{b}	246.32±9.27^{b}	**247.21±10.13^{bD}**	36.59±4.09^{b}	37.48±2.68^{b}	**37.04±3.37^{bA}**	182.06±5.36^{b}	185.63±3.09^{b}	**183.85±3.09^{bC}**	130.30±3.09^{b}	132.08±6.18^{b}	**131.19±4.64^{bB}**
	T3	292.73±13.47^{c}	290.94±11.15^{c}	**291.83±12.27^{cD}**	75.86±5.57^{d}	77.64±2.68^{d}	**76.75±4.09^{dA}**	267.74±5.36^{d}	268.45±4.33^{d}	**268.09±4.83^{dC}**	167.78±8.18^{c}	169.93±5.90^{c}	**168.86±6.88^{cB}**
	T4	203.48±5.36^{a}	201.70±8.18^{a}	**202.59±6.74^{aD}**	23.2±3.09^{a}	24.63±1.93^{a}	**23.92±2.49^{aA}**	132.08±8.18^{a}	133.87±5.36^{a}	**132.98±6.73^{aC}**	117.80±5.36^{a}	118.34±4.57^{a}	**118.07±4.96^{aB}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			***			*		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Table 4.48 Comparative effect of location and treatments on nitrogen content (mg/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	2366.00±11.83^{b}	2395.41±89.98^{b}	**2380.70±48.29^{bA*}**	2792.54±32.14^{b}	2649.2±88.98^{b}	**2720.86±28.75^{bC}**	2550.04±76.86^{b}	2586.10±32.37^{b}	**2568.07±48.11^{bB***}**	2599.55±90^{ab}	2586.55±34.34^{b}	**2593.05±61.99^{bB***}**
	T2	2643.92±18.65^{c}	2662.80±52.90^{c}	**2653.36±28.26^{cA***}**	2819.04±35.58^{b}	2724.5±76.83^{b}	**2771.77±38.85^{bB}**	2970.55±34.84^{c}	2919.58±35.51^{c}	**2945.07±15.27^{cC***}**	2692.32±35.67^{bc}	2639.08±22.89^{b}	**2665.7±6.57^{bA***}**
	T3	2823.76±10.92^{d}	2851.94±33.41^{d}	**2837.85±12.35^{dA**}**	2933.19±9.25^{c}	2953.41±65.23^{c}	**2943.3±35.51^{cB}**	3070.32±38.61^{d}	3137.64±23.23^{d}	**3103.98±19.37^{dC***}**	2792.96±25.89^{c}	2833.11±34.35^{c}	**2813.04±26.24^{cA***}**
	T4	2258.02±50.17^{a}	2044.86±89.11^{a}	**2151.44±22.08^{aB**}**	2441.72±9.06^{a}	2438.08±10.15^{a}	**2439.90±9.60^{aC**}**	2367±34.55^{a}	2343.82±34.42^{a}	**2355.41±31.51^{aB***}**	2524.08±86.81^{a}	2317.85±25.33^{a}	**2420.96±53.73^{aC***}**
LA	**T1**	2273.470±33.27^{b}	2238.52±36.10^{b}	**2255.99±17.01^{bC}**	2694.55±34.32^{b}	2706.21±19.42^{b}	**2700.38±25.99^{bD}**	2143.14±55.96^{b}	2061.54±32.37^{b}	**2102.34±12.31^{bB}**	1966.43±21.31^{b}	1919.89±35.87^{b}	**1943.16±21.55^{bA}**
	T2	2328.67±87.43^{b}	2293.75±53.75^{b}	**2311.21±65.98^{bB}**	2711.55±19.29^{b}	2688.31±38.27^{b}	**2699.93±22.65^{bC}**	2282.13±73.80^{c}	2235.54±36.05^{c}	**2258.83±54.46^{cB}**	2025.6±33.93^{b}	1955.76±34.48^{b}	**1990.68±15.97^{bA}**
	T3	2677.95±50.86^{c}	2643.07±73.73^{c}	**2660.51±55.67^{cC}**	2853.56±15^{c}	2841.89±35.13^{c}	**2847.73±25.05^{cD}**	2528.37±57.83^{d}	2470.08±56^{d}	**2499.23±50.25^{dB}**	2235.53±34.38^{c}	2177.33±41.27^{c}	**2206.42±11.05^{cA}**
	T4	2015.93±50.12^{a}	2027.6±31.03^{a}	**2021.77±40.44^{aB}**	2374.06±33.81^{a}	2339.14±33.79^{a}	**2356.60±15.11^{aC}**	1884.98±35.41^{a}	1838.45±54.46^{a}	**1861.72±22.05^{aA}**	1793.71±54.65^{a}	1805.34±41.41^{a}	**1799.52±47.43^{aA}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			NS			***			NS		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.3.2.10 Effect on magnesium content (mg/100g DW) of cruciferous vegetable

Magnesium (Mg) plays a crucial role in maintaining human health by regulating blood pressure, muscle tone, heart muscle contraction, neuromuscular function, and glycemic control (Buturi *et al.,* 2021). Insufficient magnesium intake can lead to weak growth, stunted development, and poor bone health (Linkon *et al.,* 2015). The recommended daily magnesium intake ranges from 320 to 420 mg (Buturi *et al.,* 2021). In present study the Mg contents varied significantly among the cruciferous vegetables, location (HA and LA) and treatments (T_1, T_2, T_3 & T_4) (Table 4.49). Within the treatments, T_3 exhibited maximum magnesium content, with the analyzed varieties value ranging from 261.04±1.65 to 369.4±4.72 (mg/100g) at HA and 302.71±6.34 to 461.87±4.82 (mg/100g) at LA. It could be due to the effect of combine application of FYM+*Azotobacter* increases the higher magnesium content in soil. These findings are consistent with previous studies (Upadhyay *et al*., 2012; Rahman *et al.,* 2021). Furthermore, at LA, knol-khol exhibited the highest magnesium content (461.87±4.82 mg/100g), followed by cabbage (409.67±5.61 mg/100g), cauliflower (393.26±8.51 mg/100g), and radish (302.71±6.34 mg/100g), compared to their counterparts at HA: knol-khol (369.4±4.72 mg/100g), cabbage (276.24±4.66 mg/100g), cauliflower (281.84±1.56 mg/100g), and radish (261.04±1.65 mg/100g). However, among cruciferous vegetables the higher magnesium content was analyzed in knol-khol (461.87±4.82 mg/100g) at LA location. The interaction between altitude and treatments (ALT×TRE) was also found significantly ($p \leq 0.05$) on magnesium content. It may have happened as a result of the higher magnesium content in the LA soil. Our research findings align with those of Singh *et al.* (2011a) and Sarkar and Rakshit (2021), highlighting the importance of magnesium in cruciferous vegetables and its variability across different locations and treatments.

Table 4.49 Comparative effect of location and treatments on magnesium content (mg/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	238.38±8.27^{ab}	234.71±2.97^{a}	236.54±5.30^{aA}	268.18±10.41^{bc}	270.07±5.73^{b}	269.13±7.61^{bB}	358.58±2.86^{c}	354.81±13.86^{b}	356.69±5.67^{bC}	248.03±11.81^{b}	236.99±7.51^{b}	242.51±9.19^{bA}
	T2	251.20±12.62^{b}	256.05±6.99^{b}	253.63±9.48^{bB}	252.47±11.37^{b}	260.81±9.59^{b}	256.64±8.07^{bB}	344.43±2.00^{b}	352.14±9.88^{b}	348.29±4.58^{bC}	245.7±9.77^{b}	233.93±1.89^{b}	239.82±4.66^{bA}
	T3	274.30±1.16^{c}	278.17±8.40^{b}	276.24±4.66^{cB}	278.17±2.99^{c}	285.52±5.40^{c}	281.84±1.56^{cB}	370.76±3.61^{d}	368.04±10.38^{b}	369.4±4.72^{cC}	263.15±1.10^{c}	258.92±4.22^{c}	261.04±1.65^{cA}
	T4	233.08±2.78^{a}	227.03±2.68^{a}	230.05±0.51^{aB}	217.46±7.61^{a}	222.69±7.56^{a}	220.07±7.17^{aA}	313.93±1.83^{a}	320.15±10.82^{a}	317.04±4.60^{aC}	227.67±4.61^{a}	223.61±2.79^{a}	225.65±3.67^{aAB}
LA	**T1**	344.96±16.96^{b}	335.86±16.16^{b}	340.41±16.19^{bB***}	344.96±14.13^{b}	348.72±5.82^{b}	346.84±6.81^{bB***}	429.72±12.62^{b}	427.62±8.17^{b}	428.67±8.56^{bC***}	238.13±11.64^{b}	239.21±11.59^{b}	238.67±11.11^{bA}
	T2	350.07±15.02^{b}	348.45±17.55^{b}	349.26±16.18^{bB***}	356.26±6.48^{b}	352.49±17.36^{b}	354.38±11.89^{bB***}	413.3±17.01^{b}	413.03±10.84^{b}	413.17±13.79^{bC**}	247.28±7.24^{b}	249.97±5.26^{b}	248.63±4.25^{bA}
	T3	412.76±12.11^{c}	406.57±16.23^{c}	409.67±5.61^{cC***}	390.97±6.72^{c}	395.54±10.31^{c}	393.26±8.51^{cB***}	467.12±6.48^{c}	456.63±4.06^{c}	461.87±4.82^{cD***}	301.37±10.54^{c}	304.06±3.64^{c}	302.71±6.34^{cA***}
	T4	303.52±4.49^{a}	294.10±6.72^{a}	298.81±4.71^{aB***}	310.51±6.86^{a}	308.09±3.82^{a}	309.31±5.27^{aC***}	376.44±4.93^{a}	382.09±7.32^{a}	379.26±3.39^{aD***}	210.95±4.59^{a}	205.04±2.91^{a}	208.00±1.99^{aA**}
ALT		***			***			***			*		
TRE		***			***			***			***		
ALT×TRE		***			*			**			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.3.2.11 Effect on potassium and sodium content (mg/100g DW) of cruciferous vegetable

The balance of potassium to sodium in food is pivotal in preventing arteriosclerosis and hypertension, as potassium reduces blood pressure while sodium elevates it (Linkon *et al*. 2015). Interestingly, many functions performed by potassium in plants can also be carried out by sodium, as these two elements are structurally and chemically very similar when hydrated (Kalisz *et al*. 2018).

Potassium and sodium contents of selected cruciferous vegetable were significantly different among the treatments (T_1, T_2, T_3 & T_4). Notably, results showed that the treatment T_3 performed maximum K and Na content was ranged from 3088.08±40.09 to 6275.00±54.49 (mg/100g) and 305.68±16.88 to 556.84±14.74 (mg/100g) at HA and 2808.33±62.92 to 4616.84±13.27 (mg/100g) and 166.67±5.20 to 428.33±14.65 (mg/100g) at LA (Table 4.50 and 4.51). This observation could be attributed to enhanced potassium and sodium absorption by vegetables when biofertilizers are applied alongside manures. Kumar *et al*. (2015) and Sarkar and Rakshit (2021) reported similar findings, linking the application of manures and biofertilizers to increased potassium and sodium content in vegetables. Additionally, at HA, the Higher K and Na content was found in radish (6275.00±54.49 and 556.84±14.74 mg/100g), knol-khol (4625.00±108.25 and 314.06±10.85 mg/100g), cauliflower (3318.90±22.73 and 305.68±16.88 mg/100g) and cabbage (3088.08±40.09 and 320.46±7.42 mg/100g) respectively as compared to LA grown radish (4616.84±13.27 and 428.33±14.65 mg/100g), knol-khol (3993.36±47.34 and 195.00±5.45 mg/100g), cauliflower (3125.00±43.30 and 174.58±0.72 mg/100g) and cabbage (2808.33±62.92 and 166.67±5.20 mg/100g). However, among cruciferous vegetables the higher K and Na content were analyzed in radish (6275.00±54.49 and 556.84±14.74 mg/100g) at HA location. The interaction between altitude and treatments (ALT×TRE) was also found significantly ($p \leq 0.05$) on K and Na content. It is due to the result of higher levels of soil microbial diversity and activity at HA soil as compared to LA soil. This finding is consisting with Bahadur *et al.* (2006) and Singh *et al*. (2011a).

Table 4.50 Comparative effect of location and treatments on potassium content (mg/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	2719.50±71.45^{b}	2694.59±41^{b}	**2707.04±54.11^{bA}**	3179.10±88.28^{b}	3137.49±52.14^{b}	**3158.30±69.94^{bB**}**	3508.33±52.04^{b}	3433.33±52.04^{b}	**3489.34±14.17^{bC}**	5016.67±166.46^{b}	4825±50.00^{b}	**4920.83±85.09^{bD**}**
	T2	2845.77±68.5^{b}	2804.14±16.85^{c}	**2824.95±42.55^{cA**}**	3203.01±63.94^{b}	3169.73±45.10^{b}	**3186.37±53.41^{bB**}**	4300±132.29^{c}	4216.67±76.38^{c}	**4258.33±101.04^{cC**}**	5508.33±187.64^{c}	5166.67±152.75^{c}	**5337.50±169.10^{cD***}**
	T3	3104.75±65.47^{c}	3071.41±24.39^{d}	**3088.08±40.09^{dA**}**	3352.17±79.2^{c}	3285.64±37.43^{c}	**3318.90±22.73^{cB**}**	4683.33±160.73	4566.67±62.92^{d}	**4625.00±108.25^{dC***}**	6341.67±52.04^{d}	6208.33±62.92^{d}	**6275.00±54.49^{dD***}**
	T4	2538.28±62.70^{a}	2513.31±37.05^{a}	**2525.79±46.74^{aA***}**	2900.61±39.68^{a}	2825.81±39.64^{a}	**2863.22±37.65^{aB***}**	3208.33±52.04^{a}	3133.33±52.04^{a}	**3252.37±31.25^{aC*}**	4016.67±160.73^{a}	3933.33±28.87^{a}	**3975±90.14^{dA**}**
LA	**T1**	2708.33±38.19^{bc}	2641.67±62.92^{b}	**2675.00±50.00^{bA}**	2775±66.14^{b}	2791.67±52.04^{b}	**2783.33±59.07^{bB}**	3505.98±36.08^{b}	3472.7±27.59^{b}	**3470.83±26.02^{bC}**	4486.36±81.26^{c}	4453.12±49.43^{c}	**4469.74±60.78^{cD}**
	T2	2608.33±62.92^{b}	2675.00±50^{b}	**2641.67±47.32^{bA}**	3016.67±38.19^{c}	2983.33±38.19^{c}	**3000.00±25.00^{cB}**	3784.11±128.62	3684.29±37.55^{c}	**3734.20±80.53^{cC}**	4177.1±79.01^{b}	4210.37±77.71^{b}	**4193.74±77.03^{bD}**
	T3	2800.00±66.14^{c}	2816.67±62.92^{c}	**2808.33±62.92^{cA}**	3366.67±38.19^{d}	3283.33±52.04^{d}	**3125.00±43.30^{dB}**	4051.59±95.18^{c}	3935.13±41.81^{d}	**3993.36±47.34^{dC}**	4670.88±37.27^{d}	4562.81±46.65^{d}	**4616.84±13.27^{dD}**
	T4	2133.33±87.8^{a}	2066.67±76.38^{a}	**2100.00±76.03^{aA}**	2383.33±80.36^{a}	2333.33±14.43^{a}	**2358.33±38.19^{aB}**	3177.33±63.06^{a}	3163.41±12.55^{a}	**3170.83±26.02^{aC}**	3749.77±51.61^{a}	3815.91±25.93^{a}	**3782.42±84.92^{aD}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		****			****			****			****		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Table 4.51 Comparative effect of location and treatments on sodium content (mg/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	221.22±3.64^{b}	219.56±5.21^{b}	**220.39±1.01^{bA***}**	265.47±13.56^{b}	236.35±10.22^{b}	**250.91±11.86^{bB***}**	262.33±12.62^{b}	251.51±10.28^{b}	**256.92±9.56^{bB***}**	514.39±12.6^{c}	493.56±26.12^{b}	**503.98±18.15^{cC***}**
	T2	273.76±1.57^{c}	262.11±12.68^{c}	**267.94±6.49^{cA***}**	265.38±13.58^{b}	245.42±5.03^{b}	**255.41±9.29^{bA***}**	264.47±13.1^{b}	251.16±2.77^{b}	**257.82±7.09^{bA***}**	453.48±18.77^{b}	465.13±26.28^{b}	**459.30±22.23^{bB***}**
	T3	328.78±6.98^{d}	312.14±9.00^{d}	**320.46±7.42^{dA***}**	311.92±12.25^{c}	299.44±21.9^{c}	**305.68±16.88^{cA***}**	312.82±12.78^{c}	315.31±9.03^{c}	**314.06±10.85^{cA***}**	561±11.77^{d}	552.68±18.29^{c}	**556.84±14.74^{dB***}**
	T4	199.73±4.89^{a}	193.91±3.92^{a}	**196.82±1.89^{aA***}**	213.6±10.06^{a}	210.28±5.22^{a}	**211.94±7.48^{aAB***}**	231.24±6.18^{a}	221.26±6.18^{a}	**226.25±6.18^{aB***}**	411.54±12.47^{a}	394.91±19.14^{a}	**403.23±14.46^{aC***}**
LA	**T1**	122.5±2.50^{b}	125.00±2.5^{b}	**123.75±1.25^{bA}**	169.17±6.29^{b}	163.33±8.78^{b}	**166.25±6.50^{bB}**	178.33±5.77^{b}	175.83±3.82^{b}	**177.08±4.73^{bC}**	293.33±7.64^{b}	288.33±10.41^{b}	**290.83±8.04^{bD}**
	T2	121.67±3.82^{b}	122.50±4.33^{b}	**122.08±4.02^{bA}**	169.17±8.04^{b}	162.50±2.50^{b}	**165.83±5.20^{bB}**	185.83±8.78^{bc}	178.33±6.29^{b}	**182.08±4.02^{bC}**	295±2.50^{b}	281.67±11.27^{b}	**288.33±5.20^{bD}**
	T3	170.83±7.22^{c}	162.50±7.50^{c}	**166.67±5.20^{cA}**	173.33±2.89^{b}	175.83±1.44^{c}	**174.58±0.72^{bA}**	193.33±6.29^{c}	196.67±5.20^{c}	**195.00±5.45^{cB}**	432.50±15.21^{c}	424.17±15.07^{c}	**428.33±14.65^{cC}**
	T4	95.83±3.82^{a}	98.33±5.20^{a}	**97.08±4.02^{aA}**	123.33±2.89^{a}	125.00±5.00^{a}	**124.17±3.82^{aB}**	138.33±6.29^{a}	142.50±6.61^{a}	**140.42±6.17^{aC}**	216.67±7.64^{a}	213.33±10.41^{a}	**215.00±8.66^{aD}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			***			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.3.2.12 Effect on iron content (mg/100g DW) of cruciferous vegetable

Iron is a crucial component of cytochromes, myoglobin, and hemoglobin in the human body, essential for energy synthesis and various metabolic processes including oxygen, DNA, and electron transport. The recommended daily intake of iron ranges between 8 to 18 mg per day (Buturi *et al.,* 2021). Iron deficiency, leading to anemia, is prevalent among children and women due to inadequate dietary intake and represents a widespread mineral deficiency globally (Singh *et al.* 2011a).

In our investigation, treatment T_3 exhibited the highest iron content, ranging from 17.61±0.45 to 11.69±0.28 mg/100g at LA and 14.94±0.31 to 6.46±0.53 mg/100g at HA (Table 4.52). The combination of FYM and *Azotobacter* likely increased soil microorganism populations, impacting plant metabolism and nutrient uptake, thus enhancing iron bioavailability for plant roots. Similar findings were reported by Sarkar and Rakshit (2021) and Bahadur *et al,* (2006), indicating that bio-organic fertilizers significantly increase iron content in cabbage. Moreover, at LA, the higher content of iron was recorded in radish (17.61±0.45 mg/100g), cauliflower (15.73±0.28 mg/100g), knol-khol (14.23±0.20 mg/100g) and cabbage (11.69±0.28 mg/100g) respectively, as compared to HA radish (12.04±0.29 mg/100g), cauliflower (14.94±0.31 mg/100g), knol-khol (12.39±0.18 mg/100g) and cabbage (6.46±0.53 mg/100g). Whereas, in cruciferous vegetables the higher content of iron was found in radish (17.61±0.45 mg/100g) at LA location. Interaction between altitude and treatment was fond in cabbage and cauliflower. It is due to the result of LA soil contain more iron as compared to HA soil. The iron content in vegetables was influenced by available form of iron in soil, crops and maturity stages (Singh *et al.,* 2011a). Therefore, it has been suggested that eating more foods high in iron can help prevent lipid per oxidation and abnormal iron accumulation.

Table 4.52 Comparative effect of location and treatments on iron content (mg/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	4.43±0.41^{b}	4.44±0.92^{ab}	**4.44±0.63^{abA}**	12.77±0.03^{c}	12.33±0.41^{c}	**12.55±0.19^{cC}**	9.96±0.08^{b}	9.45±1.14^{b}	**9.71±0.53^{bB}**	9.64±0.41^{b}	9.87±0.76^{b}	**9.75±0.58^{bB}**
	T2	5.31±0.50^{c}	5.19±0.32^{bc}	**5.25±0.41^{bA}**	7.84±0.19^{b}	9.57±2.65^{b}	**8.71±1.27^{bB}**	9.60±0.45^{b}	9.43±0.58^{b}	**9.52±0.51^{bB}**	10.44±0.35^{c}	9.60±0.69^{b}	**10.02±0.38^{bB}**
	T3	6.67±0.32^{d}	6.25±0.85^{c}	**6.46±0.53^{cA}**	14.88±0.4^{d}	15.00±0.24^{d}	**14.94±0.31^{dC}**	12.7±0.23^{c}	12.08±0.15^{c}	**12.39±0.18^{cB}**	12.1±0.26^{d}	11.98±0.33^{c}	**12.04±0.29^{cB}**
	T4	3.68±0.20^{a}	3.55±0.11^{a}	**3.61±0.11^{aA}**	4.17±0.34^{a}	4.33±0.18^{a}	**4.25±0.23^{aB}**	4.14±0.28^{a}	3.92±0.78^{a}	**4.03±0.53^{aAB}**	8.29±0.41^{a}	8.12±0.14^{a}	**8.20±0.27^{aC}**
LA	**T1**	7.77±0.25^{b}	7.62±0.45^{b}	**7.69±0.21^{cA***}**	14.59±0.60^{c}	13.01±0.45^{c}	**13.8±0.56^{cC*}**	10.89±0.48^{b}	10.35±0.60^{b}	**10.62±0.47^{bB}**	15.93±0.88^{b}	15.04±0.57^{b}	**15.48±0.17^{cD***}**
	T2	7.61±0.27^{b}	7.22±0.24^{b}	**7.41±0.09^{bA***}**	12.58±1.03^{b}	12.75±0.76^{b}	**12.67±0.87^{bC***}**	11.00±0.52^{b}	10.66±0.76^{b}	**10.83±0.58^{bB**}**	14.92±0.46^{b}	14.55±0.81^{b}	**14.73±0.22^{bD***}**
	T3	11.96±0.38^{c}	11.42±0.21^{c}	**11.69±0.28^{dA***}**	15.14±0.68d	16.32±0.61d	**15.73±0.28^{dC**}**	14.11±0.39^{c}	14.35±0.11^{c}	**14.23±0.20^{cB***}**	17.62±0.38^{c}	17.59±0.53^{c}	**17.61±0.45^{dD***}**
	T4	5.95±0.42^{a}	6.32±0.27^{a}	**6.14±0.07^{aB***}**	8.75±0.80^{a}	7.62±0.31^{a}	**8.19±0.26^{aC***}**	4.81±0.25^{a}	4.96±0.39^{a}	**4.88±0.30^{aA}**	13.23±0.46^{a}	12.99±0.54^{a}	**13.11±0.46^{aD***}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			NS			NS		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.
Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.
Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.
Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of

4.3.2.13 Effect on copper content (mg/100g DW) of cruciferous vegetable

Copper is a necessary component of metallo-enzymes, especially oxidases, and aids in the metabolism of iron by changing ferrous ions into ferric states. It is a component of the sole enzyme in brain chemistry that is involved in the synthesis of membrane-bound small-molecule neurotransmitters, dopamine-b-hydroxylase. A person needs 2-3 mg of copper per capita per day (Singh *et al.*, 2011a). It is necessary for numerous physiological functions in plants, including respiration, photosynthesis, the metabolism of carbon and nitrogen, and defense against oxidative stress (Buturi *et al.*, 2021). In our study, T_3 treatment had the maximum amount of Copper in tested cruciferous samples varied between 2.35±0.04 to 1.73±0.05 (mg/100g) at LA and 0.51±0.02 to 0.20±0.01 (mg/100g) at HA (Table 4.53). Our results are similarly consisting with Sarkar and Rakshit, 2021; and Upadhyay *et al.* (2012)reported that bio-organic farming enhances the mineral content of vegetables.Furthermore, at LA, the higher content of Cu was analyzed in radish (2.35±0.04 mg/100g), knol-khol (1.96±0.03 mg/100g), cauliflower (1.93±0.05 mg/100g) and cabbage (1.73±0.05 mg/100g) respectively, as compared to HA grown radish (0.51±0.02 mg/100g), knol-khol (0.20±0.01 mg/100g), cauliflower (0.35±0.01 mg/100g) and cabbage (0.33±0.03 mg/100g) respectively. However among the cruciferous vegetable higher amount of Cu (2.35±0.04 mg/100g) was analyzed in radish at LA location. The interaction between altitude and treatments (ALT×TRE) was also found significantly ($p \leq 0.05$). Earlier discussed that LA soil contain more copper content as compared to HA soil.

Table 4.53 Comparative effect of location and treatments on copper content (mg/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	0.10±0.07^{a}	0.13±0.09^{a}	**0.11±0.08^{aA}**	0.27±0.01^{c}	0.26±0.01^{b}	**0.27±0.00^{cB}**	0.12±0.02^{b}	0.12±0.03^{b}	**0.12±0.02^{cA}**	0.06±0.01^{b}	0.09±0.02^{b}	**0.07±0.01^{bA}**
	T2	0.11±0.08^{a}	0.12±0.07^{a}	**0.11±0.08^{aA}**	0.22±0.02^{b}	0.24±0.04^{b}	**0.23±0.03^{bB}**	0.06±0.01^{a}	0.11±0.04^{ab}	**0.09±0.02^{bA}**	0.07±0.01^{b}	0.09±0.01^{b}	**0.08±0.01^{bA}**
	T3	0.33±0.04^{b}	0.33±0.03^{b}	**0.33±0.03^{bB}**	0.35±0.01^{d}	0.36±0.02^{c}	**0.35±0.01^{dB}**	0.19±0.01^{c}	0.21±0.02^{c}	**0.20±0.01^{dA}**	0.5±0.01^{c}	0.52±0.02^{c}	**0.51±0.02^{cC}**
	T4	0.06±0.01^{a}	0.05±0.02^{a}	**0.05±0.02^{aA}**	0.18±0.02^{a}	0.16±0.02^{a}	**0.17±0.01^{aB}**	0.04±0.01^{a}	0.06±0.02^{a}	**0.05±0.02^{aA}**	0.04±0.01^{a}	0.05±0.01^{a}	**0.04±0.01^{aA}**
LA	**T1**	1.14±0.06^{b}	1.16±0.08^{b}	**1.15±0.07^{bA***}**	1.76±0.03^{b}	1.73±0.05^{b}	**1.75±0.03^{bB***}**	1.88±0.05^{bc}	1.92±0.04^{bc}	**1.90±0.03^{bC***}**	2.03±0.06^{b}	2.10±0.09^{b}	**2.06±0.07^{bD***}**
	T2	1.65±0.07^{c}	1.58±0.09^{c}	**1.62±0.05^{cA***}**	1.85±0.06^{c}	1.78±0.04^{b}	**1.81±0.04^{bB***}**	1.82±0.05^{b}	1.79±0.08^{b}	**1.80±0.07^{bB***}**	2.00±0.08^{b}	1.97±0.10^{b}	**1.98±0.08^{bC***}**
	T3	1.71±0.07^{c}	1.74±0.04^{d}	**1.73±0.05^{dA***}**	1.92±0.04^{c}	1.95±0.07^{c}	**1.93±0.05^{cB***}**	1.94±0.04^{c}	1.98±0.02^{c}	**1.96±0.03^{cB***}**	2.37±0.03^{c}	2.33±0.07^{c}	**2.35±0.04^{cC***}**
	T4	0.98±0.02^{a}	0.97±0.03^{a}	**0.97±0.02^{aA***}**	1.19±0.04^{a}	1.15±0.05^{a}	**1.17±0.03^{aB***}**	1.41±0.05^{a}	1.27±0.13^{a}	**1.34±0.08^{aC***}**	1.70±0.03^{a}	1.73±0.03^{a}	**1.71±0.01^{aD***}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			***			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.3.2.14 Effect on manganese content (mg/100g DW) of cruciferous vegetable

Manganese (Mn) is an essential element of various enzymes that participate in fatty acid and cholesterol synthesis. It also activates a wide range of enzymes, including polymerase and galactotransferase. The recommended daily Mn intake is 2-5 mg per capita (Singh *et al*., 2011a). The investigated cruciferous vegetables contains high amount of Mn at in T_3 treatment at HA location (Table 4.54). Notably, treatment T_3 (FYM+*Azotobacter*) exhibited a prominent impact, resulting in higher amount of Mn content ranging from 3.96±0.13 to 6.28±0.07 mg/100g and from 1.28±0.02 to 2.81±0.07 mg/100g at HA and LA grown cruciferous vegetables respectively. This could be due to the effect of organic manure (FYM) inoculation with *Azotobacter* which increases the soil biological activity and enhanced the uptake of Mn content in plant. Our findings are consistent with Bahadur *et al.,* 2006; Sarkar and Rakshit, 2021.

Furthermore, at HA, the Mn content in knol-khol, radish, cauliflower and cabbage was found to be 6.28±0.07 mg/100g, 5.43±0.07 mg/100g, 4.94±0.03 mg/100g and 3.96±0.13 mg/100g respectively which is higher in concentration as compared to LA grown knol-khol (2.81±0.07 mg/100g), radish (1.97±0.10 mg/100g), cauliflower (1.63±0.03 mg/100g) and cabbage (1.28±0.02 mg/100g). However, among cruciferous vegetables the higher Mn content was found in knol-khol (6.28±0.07 mg/100g) at HA location. The interaction between altitude and treatments (ALT×TRE) was also found significantly ($p \leq 0.05$).It might be due to the difference in variety and high Mn availability in soil with great efficiency at HA (Singh *et al.,* 2011a).

Table 4.54 Comparative effect of location and treatments on manganese content (mg/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
HA	T1	3.24±0.10^{b}	3.22±0.06^{b}	**3.23±0.03^{bA***}**	4.59±0.10^{c}	4.49±0.19^{b}	**4.54±0.13^{bB***}**	5.86±0.02^{c}	5.9±0.18^{b}	**5.88±0.09^{cD***}**	5.14±0.12^{c}	5.01±0.19^{b}	**5.08±0.13^{bC***}**
	T2	3.25±0.15^{b}	3.27±0.30^{b}	**3.26±0.15^{bA***}**	4.33±0.03^{b}	4.6±0.26^{b}	**4.46±0.12^{bB***}**	5.44±0.02^{b}	5.43±0.06^{b}	**5.43±0.03^{bD***}**	4.76±0.21^{b}	4.87±0.29^{b}	**4.82±0.25^{bC***}**
	T3	3.92±0.11^{c}	3.99±0.15^{c}	**3.96±0.13^{cA***}**	4.89±0.05^{d}	4.99±0.05^{c}	**4.94±0.03^{cB***}**	6.18±0.08^{d}	6.37±0.17^{c}	**6.28±0.07^{dD***}**	5.4±0.05^{d}	5.46±0.11^{c}	**5.43±0.07^{cC***}**
	T4	2.82±0.02^{d}	2.69±0.28^{a}	**2.75±0.14^{aA***}**	3.42±0.16^{a}	3.3±0.07^{a}	**3.36±0.05^{aB***}**	4.9±0.02^{a}	4.66±0.44^{a}	**4.78±0.23^{aD***}**	3.88±0.02^{a}	3.81±0.13^{a}	**3.85±0.06^{aC***}**
LA	T1	0.93±0.04^{b}	0.95±0.03^{b}	**0.94±0.03^{bA}**	1.05±0.03^{b}	1.07±0.01^{b}	**1.06±0.01^{bA}**	1.98±0.09^{b}	2.06±0.19^{b}	**2.02±0.13^{bC}**	1.69±0.04^{b}	1.64±0.01a^{b}	**1.66±0.02^{bB}**
	T2	0.94±0.02^{b}	0.95±0.02^{b}	**0.95±0.02^{bA}**	1.12±0.03^{c}	1.10±0.06^{b}	**1.11±0.04^{cB}**	2.10±0.08^{c}	2.03±0.11^{b}	**2.06±0.07^{bD}**	1.71±0.02^{b}	1.69±0.03^{b}	**1.70±0.02^{bC}**
	T3	1.27±0.03^{c}	1.29±0.02^{c}	**1.28±0.02^{cA}**	1.62±0.05^{d}	1.64±0.02^{c}	**1.63±0.03^{dB}**	2.85±0.02^{d}	2.77±0.13^{c}	**2.81±0.07^{cD}**	1.95±0.07^{c}	1.99±0.13^{c}	**1.97±0.10^{cC}**
	T4	0.56±0.01^{a}	0.52±0.08^{a}	**0.54±0.04^{aA}**	0.55±0.02^{a}	0.56±0.03^{a}	**0.55±0.02^{aA}**	1.10±0.05^{a}	1.06±0.08^{a}	**1.08±0.05^{aB}**	1.55±0.01^{a}	1.53±0.04^{a}	**1.54±0.02^{aC}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			**			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.3.2.15 Effect on zinc content (mg/100g DW) of cruciferous vegetable

Zinc (Zn) is a vital mineral for human health, playing a crucial role in protein and nucleic acid synthesis, as well as maintaining enzyme structure and activity. It also influences insulin secretion, glucose uptake, and cell differentiation. The recommended daily intake of zinc is 9-14 mg per capita (Buturi *et al.*, 2021). In plants, Zn is essential for chloroplast development, protein synthesis, and the metabolism of carbohydrates, fats, and nucleic acids (Linkon *et al.*, 2015). Our study revealed that among the treatments (T_1, T_2, T_3, and T_4), cruciferous vegetables were rich sources of zinc under T3 treatment (Table 4.55). Zinc content ranged from 5.40±0.23 to 6.47±0.22 mg/100g at LA and 3.70±0.05 to 5.78±0.13 mg/100g at HA grown cruciferous vegetables. Rahman *et al.* (2021) and Kumar *et al*. (2015) have reported that the combination of organic and plant growth-promoting bacteria increases the micronutrient content in vegetables.

Additionally, radish (6.47±0.22 mg/100g), cauliflower (6.37±0.19 mg/100g), knol-khol (5.65±0.20 mg/100g), and cabbage (5.40±0.23 mg/100g) exhibited higher amounts of Zn at LA compared to radish (5.02±0.19 mg/100g), cauliflower (5.78±0.13 mg/100g), knol-khol (4.41±0.16 mg/100g), and cabbage (3.70±0.05 mg/100g) grown at HA. The interaction between altitude and treatments (ALT×TRE) was also found significant ($p \leq 0.05$) in all the experimental vegetables. It is due to the results of LA soil contain higher Zn as compared to HA soil. Singh *et al*. (2011a) earlier reported that nutrient content affected by variety and maturity stages of the crop. Cruciferous vegetables, particularly radish, identified as rich sources of Zn, can be encouraged in consumer diets to fulfil the body's Zn requirements.

Table 4.55 Comparative effect of location and treatments on zinc content (mg/100g DW) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	3.03±0.16^{b}	3.14±0.21^{b}	**3.09±0.11^{bB}**	4.45±0.23^{b}	4.65±0.34^{b}	**4.55±0.26^{bD}**	2.60±0.11^{b}	2.62±0.12^{b}	**2.61±0.12^{bA}**	3.93±0.13^{b}	4.02±0.32^{b}	**3.97±0.20^{bC}**
	T2	3.31±0.16^{c}	3.13±0.17^{b}	**3.22±0.06^{bA}**	4.42±0.18^{b}	4.52±0.11^{b}	**4.47±0.14^{bB}**	3.11±0.13c	3.28±0.16c	**3.13±0.11^{cA}**	4.35±0.15^{c}	4.14±0.41^{b}	**4.24±0.25^{bB}**
	T3	3.70±0.09^{d}	3.69±0.01^{c}	**3.70±0.05^{cA}**	5.80±0.19^{c}	5.77±0.13^{c}	**5.78±0.13^{cD}**	4.42±0.25^{d}	4.4±0.08d	**4.41±0.16^{dB}**	5.01±0.21^{d}	5.02±0.17^{c}	**5.02±0.19^{cC}**
	T4	2.48±0.06^{a}	2.28±0.15^{a}	**2.38±0.08^{aB}**	2.73±0.05^{a}	2.71±0.13^{a}	**2.72±0.08^{aC}**	1.79±0.07^{a}	1.75±0.10^{a}	**1.77±0.08^{aA}**	3.04±0.18^{a}	2.95±0.09^{a}	**3.00±0.08^{aD}**
LA	**T1**	4.52±0.20^{c}	4.38±0.41^{c}	**4.45±0.30^{cBC**}**	4.79±0.08^{b}	4.73±0.13^{b}	**4.76±0.09^{bC}**	3.60±0.17c	3.45±0.51^{b}	**3.52±0.33^{bA**}**	4.30±0.18^{b}	4.26±0.13^{b}	**4.28±0.15^{bB}**
	T2	3.37±0.05^{b}	3.41±0.07^{b}	**3.39±0.06^{bA*}**	5.11±0.23^{b}	4.95±0.23^{b}	**5.03±0.23^{bC*}**	3.16±0.12b	3.11±0.10b	**3.19±0.09^{bA}**	4.03±0.17^{b}	4.14±0.18^{b}	**4.08±0.11^{bB}**
	T3	5.42±0.26^{d}	5.38±0.20d	**5.40±0.23^{dA***}**	6.4±0.19^{c}	6.35±0.21^{c}	**6.37±0.19^{cB*}**	5.63±0.23^{d}	5.67±0.17c	**5.65±0.2^{cA***}**	6.50±0.18^{c}	6.43±0.25^{c}	**6.47±0.22^{cB***}**
	T4	2.06±0.11^{a}	2.02±0.16^{a}	**2.04±0.13^{aA*}**	3.28±0.17^{a}	3.13±0.12^{a}	**3.20±0.08^{aC**}**	2.66±0.03^{a}	2.73±0.07^{a}	**2.70±0.04^{aB***}**	3.24±0.14^{a}	3.17±0.14^{a}	**3.21±0.13^{ac}**
ALT		***			***			*			***		
TRE		***			***			***			***		
ALT×TRE		***			NS			***			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.4 Comparative effect of FYM and *Azotobacter* on bioactive phytocompound attributes of cruciferous vegetables grown at HA *vs.*, LA

4.4.1 Effect on total phenolic content (µg GAE /mg of DPE) of cruciferous vegetable

Foods derived from plants are rich in polyphenolic compounds, which are effective antioxidants with a plethora of established health benefits, such as anti-inflammatory, anti-mutagenic, and free radical scavenging properties *etc.* (Kumar *et al.*, 2022; Abdel-Shafy and Mansour 2017). In the present study, as has been previously reported by Heimler *et al.* (2006) and Singh *et al.* (2006), presence of significant quantities of polyphenolic compounds was demonstrated in all the cruciferous vegetables samples grown under different conditions (Table 4.56). A noteworthy observation of the current investigation was the impact of different organic agri-treatments (FYM and *Azotobacter* alone or in combination) and distinct altitudinal conditions (HA *vs* LA) on the phenolic content of cruciferous vegetables, namely cabbage, cauliflower, knol-khol, and radish. The total phenolic content (TPC) varied from 9.56±0.15 to 4.18±0.08 µg of gallic acid equivalent (GAE) per milligram of dry powder extract (DPE). One-way ANOVA analysis indicated that treatment T_3 showed the highest response in all the different types of test vegetables *i.e.* cabbage: HA: 9.56±0.15 and LA: 8.91±0.03; cauliflower: HA: 8.68±0.20 and LA: 8.07±0.11; knol-khol: HA: 7.97±0.27 and LA: 6.55±0.01 and radish: HA: 8.96±0.16 and LA: 7.18±0.07 µg of GAE/mg of DPE. Similar trends were followed by T_2 (cabbage: HA: 8.32±0.19, LA: 8.27±0.07; cauliflower: HA: 7.44±0.11, LA: 6.96±0.04; knol-khol: HA: 7.97±0.27, LA: 5.42±0.08; radish: HA: 8.34±0.09, LA: 5.81±0.11 µg of GAE/mg of DPE), T_1 (cabbage: HA: 7.61±0.08, LA: 6.88±0.13; cauliflower: HA: 7.19±0.04, LA: 6.69±0.11; knol-khol: HA: 6.87±0.14, LA: 5.11±0.04; radish: HA: 8.05±0.09, LA: 5.71±0.03 µg of GAE/mg of DPE), and T_4 (cabbage: HA: 6.27±0.15, LA: 5.73±0.09; cauliflower: HA: 6.06±0.15, LA: 5.73±0.02; knol-khol: HA: 5.48±0.05, LA: 4.18±0.08; radish: HA: 6.62±0.01, LA: 4.63±0.05 µg of GAE/mg of DPE), respectively. Notably, cabbage exhibited significantly higher TPC content in T_3 treatment at both locations. Furthermore, an independent t-test analysis for TPC content between the HA and LA locations demonstrated a significantly higher content in the HA region compared to the LA region. Furthermore, significant interaction

between altitude and treatments (ALT×TRE) was found in the TPC values of cabbage, knol-khol and radish. The findings of the current study revealed that the T_3 treatment could maximally boost the TPC values of Brassicaceae vegetables grown at both the locations. The higher content of TPC in the T_3 treatment is most likely due to the cooperative effect of organic manure and plant growth stimulating rhizobacteria (*Azotobacter*) in the biosynthesis that activates the acetate shikimate pathway, resulting in greater phenolics production. These findings are consistent with previous findings of higher TPC levels in organically grown cabbage (Sousa *et al*., 2008), broccoli (Naguib *et al.*, 2012) and cauliflower (Picchi *et al.*, 2012). Also, in another study carried out by Dutta *et al.* (2016), the phenolic content in turmeric rhizomes was also found to be increased when inoculated with rhizobacteria.

However, it is further noteworthy that despite similar agri-treatments, HA grown Brassicaceae vegetable samples showed significantly higher boost in the TPC content than LA grown vegetables. Plants at higher elevations are exposed to abiotic stresses like overwhelmingly intense UV-B radiation, which has a wide range of effects on plant growth, morphology, and physiology especiallytriggering different defensive mechanisms which also includes production of polyphenolic secondary metabolites (Jaakola and Hohtola, 2010; Ben Sassi *et al.*, 2021). There are few reports such as by Kumar *et al*. (2022), where it found that extract of *Eruca sativa* samples from high altitude had more phenolic content as compared to low altitude samples. Thus, co-stimulation of plants with abiotic stresses along with organic agri-techniques could lead to rise of polyphenolic secondary metabolite composition. On the similar lines, Naguib *et al.* (2012) has also reported that higher abiotic stress in organic farming increases the TPC content in organically grown *Brassica olaracea,* var. Italica.

Table 4.56 Comparative effect of location and treatments on total phenolic content (µg GAE /mg of DPE) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	8.53±0.14^{b}	6.69±0.19^{b}	**7.61±0.08^{bC***}**	8.05±0.03^{b}	6.33±0.08^{b}	**7.19±0.04^{bB**}**	7.61±0.14^{b}	6.13±0.38^{b}	**6.87±0.14^{bA***}**	8.71±0.11^{b}	7.38±0.22^{b}	**8.05±0.09^{bD***}**
	T2	9.50±0.25^{c}	7.14±0.37^{c}	**8.32±0.19^{cC}**	8.29±0.07^{c}	6.59±0.30^{b}	**7.44±0.11^{bB**}**	8.05±0.10^{c}	6.01±0.53^{b}	**7.03±0.23^{bA***}**	9.12±0.13^{c}	7.56±0.20^{b}	**8.34±0.09^{cC***}**
	T3	10.63±0.22^{d}	8.49±0.18^{d}	**9.56±0.15^{dC**}**	9.72±0.06^{d}	7.63±0.35^{c}	**8.68±0.20^{cB**}**	8.89±0.08^{d}	7.05±0.61^{c}	**7.97±0.27^{cA***}**	9.71±0.17^{d}	8.20±0.16^{c}	**8.96±0.16^{dB***}**
	T4	6.84±0.15^{a}	5.72±0.16^{a}	**6.27±0.15^{aC**}**	6.64±0.12^{a}	5.48±0.18^{a}	**6.06±0.15^{aB*}**	6.07±0.12^{a}	4.89±0.17^{a}	**5.48±0.05^{aA***}**	7.54±0.05^{a}	5.71±0.03^{a}	**6.62±0.01^{aD***}**
LA	**T1**	7.54±0.22^{b}	6.22±0.05^{b}	**6.88±0.13^{bD}**	7.44±0.21^{b}	5.95±0.02^{b}	**6.69±0.11^{bC}**	5.60±0.06^{b}	4.61±0.04^{b}	**5.11±0.04^{bA}**	5.74±0.08^{b}	5.68±0.05^{b}	**5.71±0.03^{bB}**
	T2	9.83±0.05^{c}	6.73±0.14^{c}	**8.27±0.07^{cD}**	7.59±0.13^{b}	6.33±0.21^{c}	**6.96±0.04^{cC}**	5.94±0.12^{c}	4.89±0.05^{c}	**5.42±0.08^{cA}**	5.82±0.11^{b}	5.81±0.12^{b}	**5.81±0.11^{bB}**
	T3	9.95±0.03^{c}	7.87±0.04^{d}	**8.91±0.03^{dD}**	9.01±0.15^{c}	7.13±0.09^{d}	**8.07±0.11^{dC}**	7.01±0.09^{d}	6.10±0.08^{d}	**6.55±0.01^{dA}**	7.26±0.08^{c}	7.10±0.20^{c}	**7.18±0.07^{cB}**
	T4	6.40±0.09^{a}	5.07±0.10^{a}	**5.73±0.09^{aC}**	6.09±0.11^{a}	5.36±0.08^{a}	**5.73±0.02^{aC}**	4.39±0.09^{a}	3.96±0.14^{a}	**4.18±0.08^{aA}**	4.59±0.06^{a}	4.67±0.06^{a}	**4.63±0.05^{aB}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			NS			*			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.4.2 Effect on total flavonoid content (µg RE/mg of DPE) of cruciferous vegetable

Flavonoids are a sub-category of polyphenolic secondary metabolites that are highly sought after in the nutritionist recommended health promoting diets due to their high efficiency as natural antioxidants as well as preventive and therapeutic properties (Ghasemzadeh and Ghasemzadeh, 2011). The current investigation outlines the impact of different organic agri-treatments on the flavonoids content of Brassicaceae vegetables, namely cabbage, cauliflower, knol-khol, and radish, cultivated at different altitudes. The total flavonoids content (TFC) varied from 14.48±0.41 to 6.96±0.09 µg of rutin trihydrate (RE) per milligram of dry powder extract (DPE) in the current study (Table 4.57). One-way ANOVA analysis revealed that the treatment T_3 maximally boosted the flavonoid contents also as it could increase the TPC levels in all the tested Brassicaceae vegetables *viz.* cabbage: HA: 14.48±0.41 and LA: 10.85±0.03; cauliflower: HA: 12.34±0.10 and LA: 10.52±0.03; knol-khol: HA: 10.65±0.05 and LA: 9.86±0.13 and radish: HA: 9.88±0.17 and LA: 9.14±0.05 µg of RE/mg of DPE. This trend was followed by T_2 (cabbage: HA: 12.55±0.12, LA: 9.74±0.03; cauliflower: HA: 10.94±0.02, LA: 9.54±0.04; knol-khol: HA: 9.42±0.02, LA: 8.77±0.09; radish: HA: 9.15±0.02, LA: 8.40±0.04 µg of RE/mg of DPE), T_1 (cabbage: HA: 11.95±0.12, LA: 9.41±0.15; cauliflower: HA: 10.37±0.04, LA: 9.35±0.04; knol-khol: HA: 9.10±0.07, LA: 8.43±0.12; radish: HA: 8.68±0.02, LA: 8.23±0.05 µg of RE/mg of DPE), and T_4 (cabbage: HA: 9.56±0.19, LA: 7.98±0.16; cauliflower: HA: 9.06±0.03, LA: 8.45±0.02; knol-khol: HA: 7.99±0.07, LA: 7.32±0.05; radish: HA: 7.48±0.07, LA: 6.96±0.09 µg of RE/mg of DPE), respectively. Out of these, cabbage exhibited the highest increase in the TFC level in T_3 treatment at both locations. Overall, cultivation at HA regions supported significantly higher enrichment of TFC that at plains, as proved by an independent t-test analysis for TFC content between the HA and LA. A significant interaction between altitude and treatments (ALT×TRE) was found in TFC of cabbage, cauliflower and radish ($p<0.001$). Similar to the TPC levels, the observed higher content of TFC in the T_3 treatment can be explained by cooperative effect of FYM and *Azotobacter* treatments in the activation of acetate shikimate biosynthetic pathway. These findings are consistent with observations made by earlier researchers that TPC and TFC levels are increased in the bio-organically grown *Brassica oleracea*

var. *capitata* (Sousa *et al.*, 2008), *Brassica oleracea* var. italic (Naguib *et al*., 2012) and *Brassica oleracea* var. *botrytis* (Picchi *et al.*, 2012).

However, as discussed earlier in this manuscript, the take home message in the present study is that that HA grown Brassicaceae vegetables possess significantly higher TFC values than LA grown vegetables. Since these secondary metabolites function as part of plant's defence mechanisms against abiotic stressors like UV radiations, their raised levels in HA grown plants is well justified (Lattanzio *et al*., 2006). This strategy to boost TFC levels in organically grown vegetables could prove to be a boon to grow anti-oxidant rich vegetables at HA for a local consumption under extreme altitude that poses tremendous threat to human health as well.

Table 4.57 Comparative effect of location and treatments on total flavonoid content (µg RE/mg of DPE)of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	13.1±0.1	10.78±0.18b	**11.95±0.12^{bD***}**	11.37±0.17^{b}	9.38±0.09^{b}	**10.37±0.04^{bC***}**	10.24±0.04^{b}	7.95±0.10^{b}	**9.10±0.07^{bB***}**	9.24±0.04^{b}	8.13±0.07^{b}	**8.68±0.02^{bA***}**
	T2	13.97±0.03	11.12±0.25b	**12.55±0.12^{cD***}**	11.84±0.08^{c}	10.04±0.04^{c}	**10.94±0.02^{cC***}**	10.77±0.08^{c}	8.07±0.07^{b}	**9.42±0.02^{cB***}**	9.82±0.14^{c}	8.47±0.11^{c}	**9.15±0.02^{cA***}**
	T3	15.62±0.54	13.34±0.33c	**14.48±0.41^{dD***}**	13.59±0.17^{d}	11.08±0.10^{d}	**12.34±0.10^{dC***}**	11.26±0.18^{d}	10.04±0.28^{c}	**10.65±0.05^{dB***}**	10.42±0.25^{d}	9.34±0.08^{d}	**9.88±0.17^{dA**}**
	T4	10.31±0.2	8.83±0.32a	**9.56±0.19^{aD***}**	9.88±0.10^{a}	8.23±0.09^{a}	**9.06±0.03^{aC***}**	9.31±0.04^{a}	6.66±0.11^{a}	**7.99±0.07^{aB***}**	8.64±0.04^{a}	6.32±0.14^{a}	**7.48±0.07^{aA***}**
LA	**T1**	10.39±0.07	8.42±0.24	**9.41±0.15^{bC}**	9.86±0.07^{b}	8.84±0.11^{b}	**9.35±0.04^{bC}**	9.30±0.20^{b}	7.55±0.17^{b}	**8.43±0.12^{bB}**	8.66±0.07^{b}	7.80±0.12^{b}	**8.23±0.05^{bA}**
	T2	10.79±0.07	8.69±0.04	**9.74±0.03^{cD}**	10.02±0.10^{b}	9.07±0.06^{c}	**9.54±0.04^{cC}**	9.71±0.20^{c}	7.85±0.13^{c}	**8.77±0.09^{cB}**	8.75±0.03^{b}	8.06±0.07^{c}	**8.40±0.04^{cA}**
	T3	11.19±0.07	10.5±0.04	**10.85±0.03^{dD}**	10.68±0.10^{c}	10.36±0.09^{d}	**10.52±0.03^{dC}**	10.62±0.10^{d}	9.10±0.17^{d}	**9.86±0.13^{dB}**	9.64±0.14^{c}	8.64±0.20^{d}	**9.14±0.05^{dA}**
	T4	8.72±0.23	7.23±0.1	**7.98±0.16^{aD}**	9.06±0.07^{a}	7.85±0.07^{a}	**8.45±0.02^{aC}**	8.44±0.04^{a}	6.19±0.07^{a}	**7.32±0.05^{aB}**	7.79±0.14^{a}	6.12±0.06^{a}	**6.96±0.09^{aA}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			NS			**		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.4.3 Effect on antioxidant activitiesof cruciferous vegetable

The antioxidant activity of naturally occurring bioactive phytochemicals has been attributed to numerous mechanisms of action, including hydrogen atom transfer, single electron transfer, and their ability to bind transition metals (Santos-Sanchez *et al.,* 2019; Kumar *et al.,* 2022). The antioxidant benefits of dietary resource can be synergistically enhanced by enrichment of variety of phytochemicals with distinct phenolic groups acting through their unique modes of action (De Brum *et al.,* 2013). In order to assess the anti-oxidant potentials of LA and HA grown Brassicaceae vegetable test samples, combination of two different assays were deployed, *i.e.* DPPH and FRAP, since full antioxidant potential of a sample cannot be determined by a single experiment due to different mechanisms of actions of different anti-oxidant compounds (Shahidi and Zhong, 2015).

4.4.3.1 DPPH assay

As mentioned previously, the antioxidant activity of bioactive substances has been attributed to numerous mechanisms of action, including hydrogen atom transfer, single electron transfer, and their ability to bind transition metals (Kumar *et al.,* 2022). DPPH assay detects the presence of anti-oxidant compounds which reduce the ROS burden *via* mechanism of electron transfer potentials (Pisoschi *et al.,* 2021). Thus, DPPH assay was deployed to assess the effect of organic agri-treatments at different altitude on the free radical scavenging efficacy of various Brassicaceae vegetable samples (results compiled in Table 4.58). In our study, at 30 mg/mL concentration, the percent DPPH content activity varied from 85.97±0.24% to 24.74±0.33%. As expected due to higher TPC and TFC levels, one-way ANOVA analysis showed that treatment T_3 led to maximum DPPH assay based anti-oxidant activities in all the vegetable samples viz. cabbage: HA: 85.97±0.24% and LA: 82.70±0.50%; cauliflower: HA: 85.49±0.20% and LA: 70.25±0.60%; knol-khol: HA: 71.61±0.26% and LA: 64.15±0.47% and radish: HA: 59.68±0.24% and LA: 38.71±0.39%. This trend was followed by T_2 (cabbage: HA: 82.80±0.22%, LA: 80.56±0.85%; cauliflower: HA: 80.95±0.30%, LA: 65.55±0.80%; knol-khol: HA: 67.48±0.65%, LA: 61.13±0.23%; radish: HA: 55.00±0.20%, LA: 35.60±0.51%, T_1 (cabbage: HA: 81.06±0.62%, LA: 78.77±0.58%; cauliflower: HA: 79.52±0.34%, LA: 64.89±0.27%; knol-khol: HA: 65.99±0.38%, LA: 60.10±0.25%; radish: HA:

53.80±0.34%, LA: 34.64±0.06%, and T_4 (cabbage: HA: 65.35±0.25%, LA: 62.23±0.45%; cauliflower: HA: 67.18±0.24%, LA: 61.26±0.15%; knol-khol: HA: 61.62±0.23%, LA: 55.82±0.24%; radish: HA: 32.90±0.22%, LA: 24.74±0.33%, respectively.

Amongst all these Brassicaceae vegetables, cabbage exhibited significantly highest DPPH response in T_3 treatment at HA which correlated with its bioactive phytocompound composition as well. These antioxidant activities were further validated by TPC and TFC patterns in different treatments, revealing a substantial positive correlation (Table 4.55). Clearly, a statistical correlation existed between higher elevations of growth conditions and DPPH assay based anti-oxidant potential. Plant growth-promoting rhizobacteria (PGPR) are responsible for inducing wide spectrum of systemic resistance *via* triggering the expression of battery of genes and pathways to upregulate the accumulation of diverse defensive bioactive molecules (Bhattacharyya and Jha, 2012). Thus, the observation of higher anti-oxidant activities in T_3 plants despite similar growth conditions as T_1, T_2 and T_4 treated plants hints at a collaborative effect of FYM and *Azotobacter* treatments, more so under abiotic stressful environment of HA. These findings are consistent with observations made by other authors where the application of PGPR enhanced the antioxidant capacity of *B. olaracea* L. var. italic (Naguib *et al.*, 2012); *Mentha pulegium* L. (Asghari *et al.*, 2020) and *Glycine max* (Couto *et al.*, 2011).

Table 4.58 Comparative effect of location and treatments on DPPH content (% inhibition) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled	1st year	2nd year	Pooled
HA	T1	81.4±0.72	80.72±0.53b	**81.06±0.62^{bD**}**	81.12±0.27^{b}	77.91±0.42^{b}	**79.52±0.34^{bC***}**	69.02±0.69^{b}	62.97±0.07^{b}	**65.99±0.38^{bB***}**	56.62±0.35^{b}	50.97±0.69^{b}	**53.80±0.34^{bA***}**
	T2	82.97±0.26	82.63±0.23c	**82.80±0.22^{cD*}**	82.22±0.10^{c}	79.69±0.50^{c}	**80.95±0.30^{cC***}**	70.96±0.91^{c}	64.00±0.40^{c}	**67.48±0.65^{cB***}**	57.88±0.24^{c}	52.11±0.26^{c}	**55.00±0.20^{cA***}**
	T3	85.87±0.41	85.11±0.13d	**85.97±0.24^{dD***}**	89.32±0.31^{d}	82.62±0.58^{d}	**85.49±0.20^{dC***}**	74.62±0.23^{d}	68.60±0.44^{d}	**71.61±0.26^{dB***}**	62.73±0.07^{d}	56.63±0.43^{d}	**59.68±0.24^{dA***}**
	T4	66.33±0.25	64.36±0.38	**65.35±0.25^{aC***}**	68.49±0.45^{a}	65.89±0.06^{a}	**67.18±0.24^{aD***}**	64.28±0.36^{a}	58.97±0.37^{a}	**61.62±0.23^{aB***}**	34.49±0.43^{a}	31.31±0.21^{a}	**32.90±0.22^{aA***}**
LA	T1	78.7±0.73	78.84±0.61	**78.77±0.58^{bD}**	65.67±0.36^{b}	64.10±0.24^{b}	**64.89±0.27^{bC}**	61.80±0.17^{b}	58.4±0.34^{b}	**60.10±0.25^{bB}**	37.49±0.29^{b}	31.79±0.24^{b}	**34.64±0.06^{bA}**
	T2	80.79±1.05	80.32±0.68	**80.56±0.85^{cD}**	66.25±0.73^{b}	64.86±0.96^{b}	**65.55±0.80^{bC}**	62.42±0.11^{c}	59.83±0.46^{c}	**61.13±0.23^{cB}**	38.72±0.61^{c}	32.49±0.43^{b}	**35.60±0.51^{cA}**
	T3	82.54±0.25	82.86±0.88	**82.70±0.50^{dD}**	70.95±0.45^{c}	69.56±0.99^{c}	**70.25±0.60^{cC}**	65.41±0.41^{d}	62.89±0.68^{d}	**64.15±0.47^{dB}**	41.40±0.24^{d}	36.02±0.55^{c}	**38.71±0.39^{dA}**
	T4	63.28±0.57	61.17±0.47	**62.23±0.45^{aD}**	61.63±0.30^{a}	60.89±0.46^{a}	**61.26±0.15^{aC}**	56.46±0.05^{a}	55.18±0.44^{a}	**55.82±0.24^{aB}**	26.62±0.27^{a}	22.86±0.85^{a}	**24.74±0.33^{aA}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		NS			***			**			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

4.4.3.2 FRAP assay

The FRAP test was another anti-oxidant assay deployed that determines specific antioxidants which could reduce Fe^{3+}-TPTZ (ferric tripyridyltriazine) into Fe^{2+}-TPTZ (ferrous tripyridyltriazine). The production of the ferrous complex (Fe^{2+}-TPTZ) is estimated as development of the blue-colored complex after reaction incubation (Sharma and Cannoo 2016; Bhardwaj *et al.,* 2020). Plant extracts with a higher reducing capacity are interpreted as having a higher concentration of antioxidant component (El Jemli *et al.,* 2016). The effect of organic agri-treatments and altitudinal conditions on FRAP assay of various Brassicaceae vegetable samples is shown in Table 4.59. FRAP assay results were found to vary from 30.77±0.46 to 8.61±0.12 µg of trolox equivalent (TE) per milligram of dry powder extract (DPE). Similar to DPPH assay, samples collected following treatment T_3 showed the highest response in FRAP assay as well viz. cabbage: HA: 30.77±0.46 and LA: 25.01±0.28; cauliflower: HA: 27.34±0.14 and LA: 22.91±0.25; knol-khol: HA: 20.58±0.19 and LA: 18.79±0.70 and radish: HA: 18.12±0.13 and LA: 13.62±0.25 µg of TE/mg of DPE. This trend was followed by T_2 (cabbage: HA: 26.85±0.34, LA: 22.90±0.65; cauliflower: HA: 24.16±0.16, LA: 19.13±0.18; knol-khol: HA: 19.02±0.59, LA: 17.08±0.47; radish: HA: 16.13±0.35, LA: 11.54±0.32 µg of TE/mg of DPE), T_1 (cabbage: HA: 25.41±0.24, LA: 21.82±0.13; cauliflower: HA: 22.44±0.21, LA: 18.20±0.16; knol-khol: HA: 17.98±0.32, LA: 15.95±0.06; radish: HA: 15.19±0.08, LA: 10.72±0.22 µg TE/mg of DPE), and T_4 (cabbage: HA: 20.67±0.52, LA: 16.75±0.19; cauliflower: HA: 19.57±0.06, LA: 16.27±0.17; knol-khol: HA: 15.03±0.07, LA: 13.09±0.38; radish: HA: 11.12±0.23, LA: 8.61±0.12 µg of TE/mg of DPE), respectively. Cabbage samples outperformed all the other Brassicaceae vegetable samples in FRAP assay, similar to the trends observed in DPPH assay. The results of the reducing power activity were supported by the TPC and TFC values as well as the activities of free radicals (DPPH), which re-emphasizes the existing knowledge of correlation (Table 4.60) between phenolics and antioxidant activities (Gardner *et al.,* 2000; Kumar *et al.,* 2022).

Table 4.59 Comparative effect of location and treatments on FRAP (µg TE/mg of DPE) content of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	27.37±0.31	23.45±0.22b	**25.41±0.24^{bD***}**	26.4±0.08^{b}	18.48±0.38^{b}	**22.44±0.21^{bC***}**	17.72±0.29^{b}	18.23±0.58^{b}	**17.98±0.32^{bB***}**	16.67±0.13^{b}	13.70±0.05^{b}	**15.19±0.08^{bA***}**
	T2	28.53±0.67	25.17±0.42c	**26.85±0.34^{cD***}**	27.86±0.17^{c}	20.46±0.35^{c}	**24.16±0.16^{cC***}**	18.73±0.40^{c}	19.31±0.80^{c}	**19.02±0.59^{cB*}**	17.73±0.24^{c}	14.52±0.46^{c}	**16.13±0.35^{cA***}**
	T3	31.08±0.42	30.47±0.7d	**30.77±0.46^{dD***}**	30.62±0.33^{d}	24.07±0.11^{d}	**27.34±0.14^{dC***}**	20.22±0.21^{d}	20.94±0.53^{d}	**20.58±0.19^{dB*}**	19.45±0.08^{d}	16.79±0.27^{d}	**18.12±0.13^{dA***}**
	T4	21.93±0.92	19.41±0.44a	**20.67±0.52^{aD***}**	22.91±0.15^{a}	16.23±0.27^{a}	**19.57±0.06^{aC***}**	14.82±0.17^{a}	15.24±0.15^{a}	**15.03±0.07^{aB***}**	11.84±0.13^{a}	10.41±0.33^{a}	**11.12±0.23^{aA***}**
LA	**T1**	23.19±0.1	20.45±0.36	**21.82±0.13^{bD}**	19.43±0.19^{b}	16.97±0.30^{b}	**18.20±0.16^{bC}**	16.42±0.19^{b}	15.48±0.29^{b}	**15.95±0.06^{bB}**	10.45±0.02^{b}	10.99±0.46^{b}	**10.72±0.22^{bA}**
	T2	24.36±0.26	21.44±1.07	**22.90±0.65^{cD}**	19.89±0.08^{c}	18.36±0.28^{c}	**19.13±0.18^{cC}**	17.71±0.62^{c}	16.44±0.34^{b}	**17.08±0.47^{cB}**	11.25±0.08^{c}	11.84±0.60^{b}	**11.54±0.32^{cA}**
	T3	26.33±0.18	23.7±0.56	**25.01±0.28^{dD}**	24.10±0.28^{d}	21.71±0.22^{d}	**22.91±0.25^{dC}**	18.60±0.54^{c}	18.99±0.92^{c}	**18.79±0.70^{dB}**	13.41±0.27^{d}	13.83±0.51^{c}	**13.62±0.25^{dA}**
	T4	16.43±0.29	17.07±0.25	**16.75±0.19^{aD}**	17.85±0.13^{a}	14.68±0.25^{a}	**16.27±0.17^{aC}**	12.77±0.53^{a}	13.40±0.28^{a}	**13.09±0.38^{aB}**	8.41±0.43^{a}	8.81±0.50^{a}	**8.61±0.12^{aA}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			NS			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

Table 4.60 Correlation between TPC, TFC, FRAP, and DPPH.

High Altitude				
	TPC	TFC	FRAP	DPPH
TPC	1	.617*	0.56	0.275
TFC		1	.971**	.869**
FRAP			1	.943**
DPPH				1
Low Altitude				
	TPC	TFC	FRAP	DPPH
TPC	1	.782**	.761**	.661*
TFC		1	.987**	.967**
FRAP			1	.976**
DPPH				1

*** significant at the 0.01 and * 0.05level (2-tailed).*

4.4.4 Effect on signature phyto-compounds levels: Kaempferol, indole-3-carbinol and sulforaphane of cruciferous vegetable

Reverse Phase HPLC (RP-HPLC), which is a reliable and popular chromatographic method for quantifying secondary metabolites in plants, was deployed to develop a comparative profile of secondary metabolites from Brassicaceae plants grown at different altitudes (HA vs. LA). The linear regression equations: y = 79691x – 28,706, R^2 = 0.99, y = 32887x + 65,956, R^2 = 0.99 and y = 4105x + 27,823, R^2 = 0.99 were used to calculate the concentration of signature phyto-compounds in Brassicaceae vegetable extracts, for kaempferol (0.122–1000 µg/mL), indole-3-carbinol (0.244–1000 µg/mL) and sulforaphane (7.81–1000 µg/mL) respectively (Table 4.61, 4.62, 4.63).

Kaempferol is an important signature compound of Brassicaceae vegetables. The variations in its levels following various organic agri-treatments and also altitudinal conditions were assessed in the present study (Table 4.61 and Figure 4.5). Its levels were found to vary from 0.92±0.02 to 0.18±0.01 µg/mg of dry powder extract (DPE) among various test samples. Since kaempferol is a natural flavonols, *i.e.* a type of flavonoid, changes in its levels following different treatments showed

trends similar to that of TFC levels *viz.* cabbage: HA: 0.92±0.02 and LA: 0.66±0.01; cauliflower: HA: 0.81±0.01 and LA: 0.59±0.02 and radish: HA: 0.73±0.01 and LA: 0.32±0.01 µg/mg. This trend was followed by T_2 (cabbage: HA: 0.35±0.01, LA: 0.33±0.01; cauliflower: HA: 0.34±0.01, LA: 0.27±0.01; radish: HA: 0.46±0.01, LA: 0.24±0.01 µg/mg), T_1 (cabbage: HA: 0.26±0.01, LA: 0.25±0.01; cauliflower: HA: 0.26±0.00, LA: 0.24±0.00; radish: HA: 0.47±0.01, LA: 0.25±0.00 µg/mg), and T_4 (cabbage: HA: 0.21±0.00, LA: 0.19±0.01; cauliflower: HA: 0.22±0.00, LA: 0.18±0.01; radish: HA: 0.29±0.01, LA: 0.18±0.01 µg/mg), respectively. Cabbage exhibited highest kaempferol content in T_3 treatment at both locations. A statistically significant correlation was observed between HA and boost in kaempferol content in Brassicaceae vegetables. Also altitude and treatments (ALT×TRE) was found to positively interact with kaempferol contents of cabbage, cauliflower and radish ($p \leq 0.001$).

Table 4.61 Comparative effect of location and treatments on kaempferol content (µg/mg of DPE) of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Radish			Knol-khol
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	
HA	**T1**	0.26±0.01^{b}	0.26±0.01^{b}	**0.26±0.01^{bA}**	0.26±0.01^{b}	0.26±0.01^{b}	**0.26±0.00$^{bA\ **}$**	0.47±0.01b	0.46±0.01b	**0.47±0.01^{bB***}**	ND
	T2	0.33±0.01^{c}	0.35±0.01^{c}	**0.35±0.01^{cA*}**	0.34±0.02^{c}	0.35±0.01^{c}	**0.34±0.01^{cA***}**	0.47±0.02b	0.45±0.01b	**0.46±0.01^{bB***}**	
	T3	0.91±0.02^{d}	0.93±0.03^{d}	**0.92±0.02^{dC***}**	0.81±0.01^{d}	0.82±0.01^{d}	**0.81±0.01^{dB***}**	0.74±0.01c	0.72±0.01c	**0.73±0.01^{cA***}**	
	T4	0.20±0.01^{a}	0.22±0.01^{a}	**0.21±0.00^{aC*}**	0.22±0.01^{a}	0.22±0.01^{a}	**0.22±0.00^{aB***}**	0.28±0a	0.29±0.01a	**0.29±0.01^{aA***}**	
LA	**T1**	0.25±0.01^{b}	0.25±0.01^{b}	**0.25±0.01^{bB}**	0.23±0.01^{b}	0.24±0.01^{b}	**0.24±0.00^{bA}**	0.25±0.01c	0.25±0b	**0.25±0.00^{bB}**	ND
	T2	0.34±0.02^{c}	0.32±0.01^{c}	**0.33±0.01^{cA}**	0.26±0.01^{c}	0.27±0.01^{c}	**0.27±0.01^{cB}**	0.24±0b	0.25±0.01b	**0.24±0.01^{bC}**	
	T3	0.66±0.01^{d}	0.66±0.01^{d}	**0.66±0.01^{dA}**	0.57±0.02^{d}	0.60±0.01^{d}	**0.59±0.02^{dB}**	0.32±0.01d	0.32±0.02c	**0.32±0.01^{cC}**	
	T4	0.19±0.01^{a}	0.18±0.01^{a}	**0.19±0.01^{aA}**	0.18±0.01^{a}	0.17±0.01^{a}	**0.18±0.01^{aA}**	0.18±0.01a	0.18±0.01a	**0.18±0.01^{aA}**	
ALT		***			***			***			
TRE		***			***			***			
ALT×TRE		***			***			***			

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, ND = not detect.

Values in columns same letter (lowercase alphabet) indicate significantly different; p≤0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; p≤0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

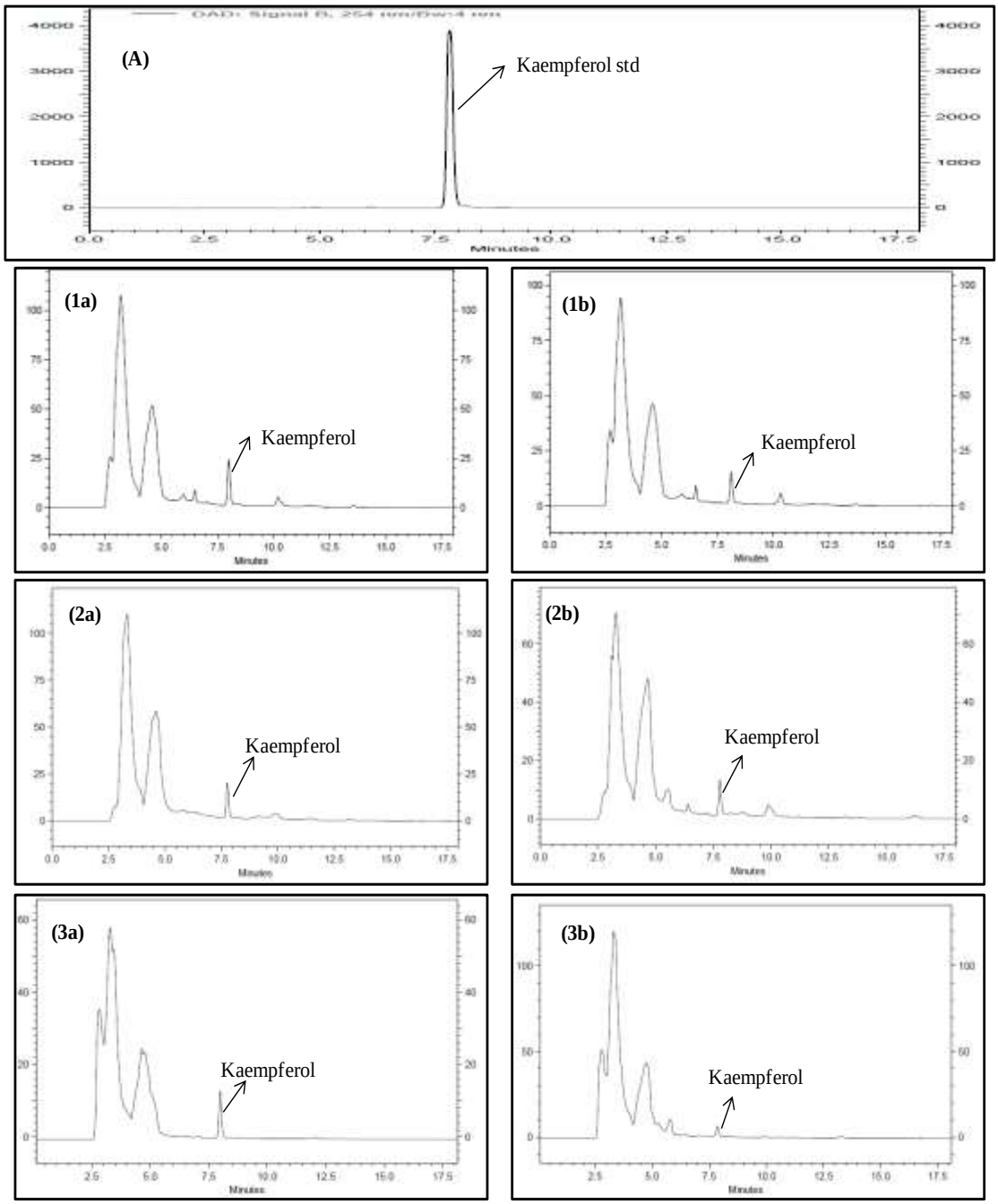

Figure 4.5 RP-HPLC chromatogram of Brassicaceaevegetables (A) Standard peak of kaempferol (1a) cabbage: HA, (1b) cabbage: LA, (2a) cauliflower: HA, (2b) cauliflower: LA, (3a) radish: HA, (3b) radish: LA. HA= High altitude and LA= Low altitude.

Similarly, the variation in the contents of another signature compound of Brassicaceae, i.e. indole-3-carbinol, was assessed with respect to various organic agri-treatments and altitudinal conditions (Table 4.62 and Figure 4.6). The indole-3-carbinol concentration was found to vary from 1.31±0.01 to 0.11±0.01 µg/mg of dry powder extract (DPE). Here again, the treatment T_3 resulted in maximum

accumulation of indole-3-carbinol content in all the Brassicaceae vegetables showing significantly higher contents at HA *viz.* cauliflower: HA: 1.31±0.01 and LA: 0.40±0.01; radish: HA: 1.01±0.03 and LA: 0.85±0.02; knol-khol: HA: 0.91±0.02 and LA: 0.74±0.01; cabbage: HA: 0.65±0.02 and LA: 0.52±0.00 µg/mg. This trend was followed by T_2 (cauliflower: HA: 1.08±0.04 and LA: 0.34±0.01; radish: HA: 0.69±0.04 and LA: 0.61±0.02; knol-khol: HA: 0.64±0.02 and LA: 0.50±0.02; cabbage: HA: 0.45±0.02 and LA: 0.34±0.02 µg/mg), T_1 (cauliflower: HA: 1.03±0.02 and LA: 0.22±0.01; radish: HA: 0.56±0.02 and LA: 0.50±0.02; knol-khol: HA: 0.42±0.01 and LA: 0.30±0.01; cabbage: HA: 0.44±0.01 and LA: 0.31±0.01 µg/mg), respectively. Cauliflower showed maximum accumulation of this phytocompound in comparison to other tested vegetables. Rest correlations with respect to altitude and interactions with different agri-treatments (ALT×TRE) showed similar trends like kaempferol ($p \leq 0.001$ and $p \leq 0.05$).

Table 4.62 Comparative effect of location and treatments on indole-3-carbinol (µg/mg of DPE) content of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	0.44±0.02^{b}	0.44±0.01^{b}	**0.44±0.01^{bA***}**	1.04±0.04^{b}	1.03±0.03^{b}	**1.03±0.02^{bC***}**	0.42±0.01^{b}	0.43±0.01^{b}	**0.42±0.01^{bA***}**	0.56±0.02b	0.56±0.01b	**0.56±0.02^{bB*}**
	T2	0.44±0.02^{b}	0.46±0.02^{b}	**0.45±0.02^{bA**}**	1.07±0.04^{b}	1.08±0.04^{c}	**1.08±0.04^{cC***}**	0.63±0.04^{c}	0.65±0.01^{c}	**0.64±0.02^{cB***}**	0.67±0.04c	0.7±0.03c	**0.69±0.04^{cB**}**
	T3	0.63±0.03^{c}	0.66±0.02^{c}	**0.65±0.02^{cA***}**	1.30±0.01^{c}	1.31±0.02^{d}	**1.31±0.01^{dD***}**	0.89±0.03^{d}	0.92±0.02^{d}	**0.91±0.02^{dB***}**	1.01±0.03d	1.01±0.03d	**1.01±0.03^{dC***}**
	T4	0.26±0.01^{a}	0.26±0.02^{a}	**0.26±0.01^{aB***}**	0.22±0.02^{a}	0.22±0.02^{a}	**0.22±0.02^{aA**}**	0.25±0.01^{a}	0.23±0.01^{a}	**0.24±0.00^{aAB***}**	0.36±0.02a	0.35±0.03a	**0.36±0.02^{aC***}**
LA	**T1**	0.32±0.01^{b}	0.31±0.01^{b}	**0.31±0.01^{bB}**	0.21±0.01^{b}	0.22±0.02^{b}	**0.22±0.01^{bA}**	0.29±0.01^{b}	0.30±0.01^{b}	**0.30±0.01^{bB}**	0.5±0.03b	0.5±0.02b	**0.50±0.02^{bC}**
	T2	0.33±0.03^{b}	0.36±0.01^{c}	**0.34±0.02^{cA}**	0.35±0.00^{c}	0.34±0.01^{c}	**0.34±0.01^{cA}**	0.51±0.01^{c}	0.50±0.01^{c}	**0.50±0.02^{cB}**	0.69±0.06c	0.7±0.01c	**0.61±0.02^{cC}**
	T3	0.52±0.02^{c}	0.53±0.02^{d}	**0.52±0.00^{dB}**	0.39±0.01^{d}	0.40±0.01^{d}	**0.40±0.01^{dA}**	0.73±0.01^{d}	0.74±0.01^{d}	**0.74±0.01^{dC}**	0.85±0.02d	0.85±0.03d	**0.85±0.02^{dD}**
	T4	0.11±0.01^{a}	0.11±0.01^{a}	**0.11±0.01^{aA}**	0.13±0.00^{a}	0.13±0.01^{a}	**0.13±0.01^{aB}**	0.17±0.00^{a}	0.17±0.01^{a}	**0.18±0.01^{aC}**	0.24±0.01a	0.24±0.02a	**0.24±0.01^{aD}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		*			***			***			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment, NS = not significant.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

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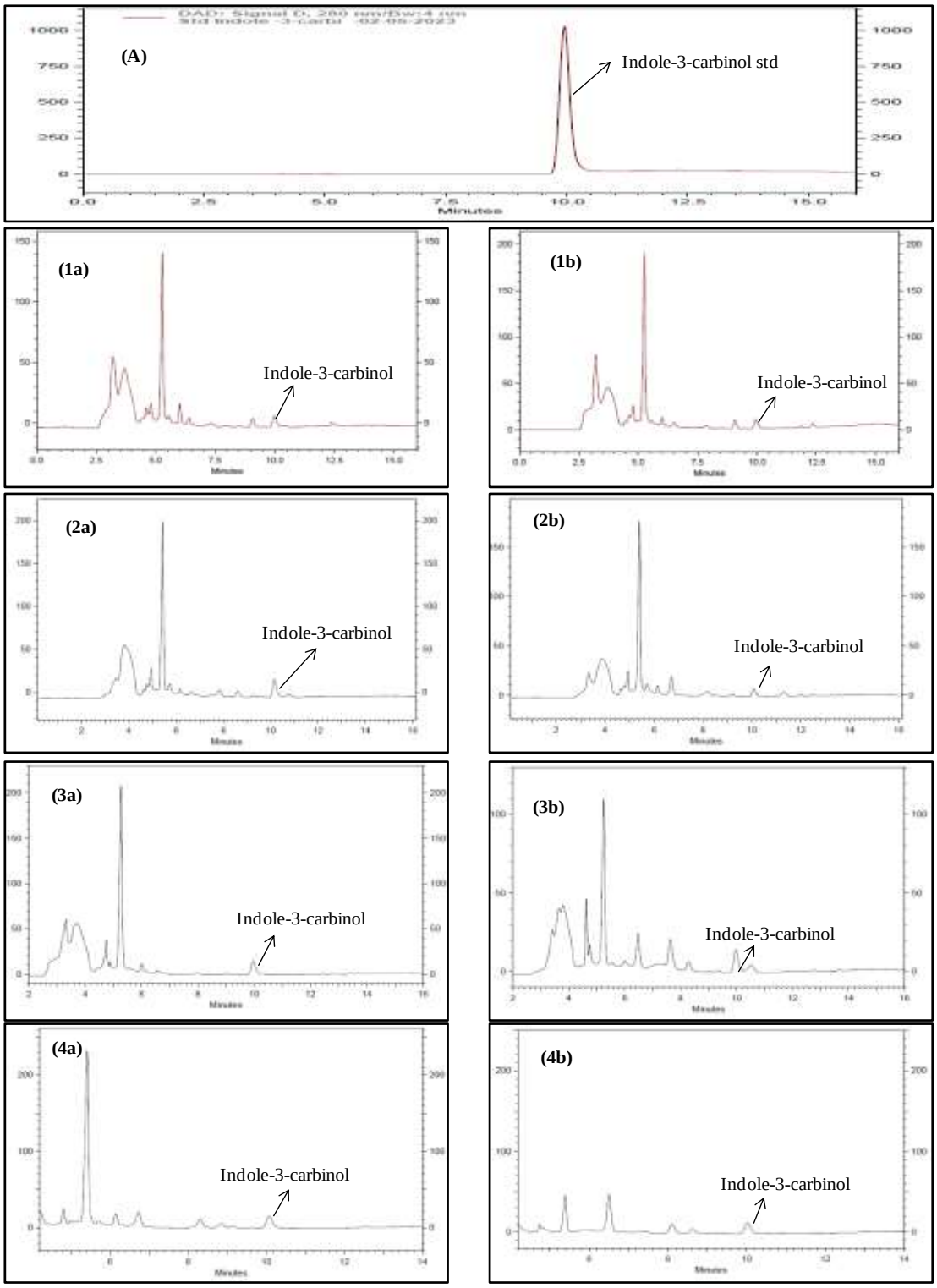

Figure 4.6 RP-HPLC chromatogram of Brassicaceae vegetables (A) Standard peak of indole-3-carbinol (1a) cabbage: HA, (1b) cabbage: LA, (2a) cauliflower: HA, (2b) cauliflower: LA, (3a) knol-khol: HA, (3b) knol-khol: LA, (4a) radish: HA, (4b) radish: LA. HA= High altitude and LA= Low altitude.

In addition to this, the vegetable samples were subjected to quantification of another very important signature compound of Brassicaceae vegetables, *i.e.* sulforaphane, which is an sulphur-containing secondary metabolite belonging to isothiocyanates group of compounds (Table 4.63 and Figure 4.7). Its concentration varied from 8.94±0.24 to 0.88±0.08 µg/mg of dry powder extract (DPE) in various test samples *viz.* cabbage: HA: 8.94±0.24 and LA: 4.16±0.05; radish: HA: 4.48±0.04 and LA: 3.08±0.11; cauliflower: HA: 4.11±0.02 and LA: 3.50±0.04; and knol-khol: HA: 3.24±0.06 and LA: 2.93±0.05 µg/mg. This trend was followed by T_2 (cabbage: HA: 3.06±0.06 and LA: 1.97±0.09; cauliflower: HA: 3.47±0.02 and LA: 2.98±0.13; radish: HA: 2.46±0.02 and LA: 1.99±0.09; and knol-khol: HA: 1.95±0.06 and LA: 1.78±0.06 µg/mg), T_1 (cabbage: HA: 2.47±0.05 and LA: 2.05±0.04; cauliflower: HA: 2.74±0.10 and LA: 2.23±0.12; radish: HA: 2.50±0.10 and LA: 1.99±0.07; and knol-khol: HA: 2.12±0.11 and LA: 1.72±0.03 µg/mg); and T_4 (cabbage: HA: 2.04±0.07 and LA: 1.00±0.07; cauliflower: HA: 1.62±0.07 and LA: 1.58±0.07; radish: HA: 1.61±0.03 and LA: 1.16±0.10; and knol-khol: HA: 1.43±0.23 and LA: 0.88±0.08 µg/mg), respectively. Among all the studied Brassicaceae vegetables, cabbage showed maximum accumulation of sulforaphane under test conditions. Rest all trends were similar to those obtained for indole-3-carbinol and kaempferol.

Table 4.63 Comparative effect of location and treatments on sulforaphane (µg/mg of DPE) content of cruciferous vegetables

ALT	TRE	Cabbage			Cauliflower			Knol-khol			Radish		
		1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**	1st year	2nd year	**Pooled**
HA	**T1**	2.45±0.05a	2.49±0.1b	**2.47±0.05^{bB***}**	2.73±0.16^{b}	2.75±0.16^{b}	**2.74±0.10^{bC**}**	2.15±0.2b	2.09±0.02b	**2.12±0.11^{bA**}**	2.55±0.09b	2.45±0.12b	**2.50±0.10^{bB**}**
	T2	3.10±0.13b	3.01±0.09c	**3.06±0.06^{cC***}**	3.48±0.03^{c}	3.46±0.02^{c}	**3.47±0.02^{cD**}**	1.91±0.21b	1.99±0.09b	**1.95±0.06^{bA*}**	2.43±0.05b	2.48±0.02b	**2.46±0.02^{bB***}**
	T3	8.73±0.6c	9.15±0.27d	**8.94±0.24^{dD***}**	4.10±0.12^{d}	4.11±0.15^{d}	**4.11±0.02^{dB***}**	3.25±0.09c	3.23±0.05c	**3.24±0.06^{cA**}**	4.48±0.11c	4.48±0.1c	**4.48±0.04^{cC***}**
	T4	2.10±0.09a	1.99±0.08a	**2.04±0.07^{aB***}**	1.64±0.09^{a}	1.61±0.06^{a}	**1.62±0.07^{aA}**	1.45±0.28a	1.41±0.19a	**1.43±0.23^{aA*}**	1.63±0.13a	1.58±0.06a	**1.61±0.03^{aA**}**
LA	**T1**	2.06±0.05b	2.03±0.03b	**2.05±0.04^{bB}**	2.27±0.15b	2.19±0.11b	**2.23±0.12^{bC}**	1.73±0.09b	1.71±0.09b	**1.72±0.03^{bA}**	1.97±0.14b	2.01±0.03b	**1.99±0.07^{bB}**
	T2	1.94±0.08b	2.00±0.1b	**1.97±0.09^{bB}**	3.02±0.24c	2.94±0.17c	**2.98±0.13^{cC}**	1.87±0.2b	1.90±0.04b	**1.78±0.06^{bA}**	1.96±0.16b	2.03±0.04b	**1.99±0.09^{bB}**
	T3	4.20±0.18c	4.12±0.1c	**4.16±0.05^{cD}**	3.50±0.14d	3.51±0.09d	**3.50±0.04^{dC}**	3.18±0.25c	3.15±0.29c	**2.93±0.05^{cA}**	3.06±0.17c	3.1±0.05c	**3.08±0.11^{cB}**
	T4	0.99±0.14a	1.00±0.05a	**1.00±0.07^{aA}**	1.60±0.07a	1.57±0.07a	**1.58±0.07^{aC}**	0.96±0.26a	1.08±0.1a	**0.88±0.08^{aA}**	1.11±0.17a	1.22±0.06a	**1.16±0.10^{aB}**
ALT		***			***			***			***		
TRE		***			***			***			***		
ALT×TRE		***			***			*			***		

HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1= FYM @150 q/ha, T_2= Azotobacter @ 8.6 kg/ha, T_3= FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T_4= Control. ALT×TRE - interaction of altitude and treatment.

Values in columns same letter (lowercase alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between treatments.

Value in row (pooled data), uppercase letters (large alphabet) indicate significantly different; P <0.05, Duncan's multiple range test between the crop.

*Mean values in each column (pooled data between groups) were significantly different via independent t-tests. Multivariate analysis of variance was utilized to illustrate the correlation among altitude and treatments. Significance levels: ***p≤0.001; **p≤0.01; *p≤0.05.*

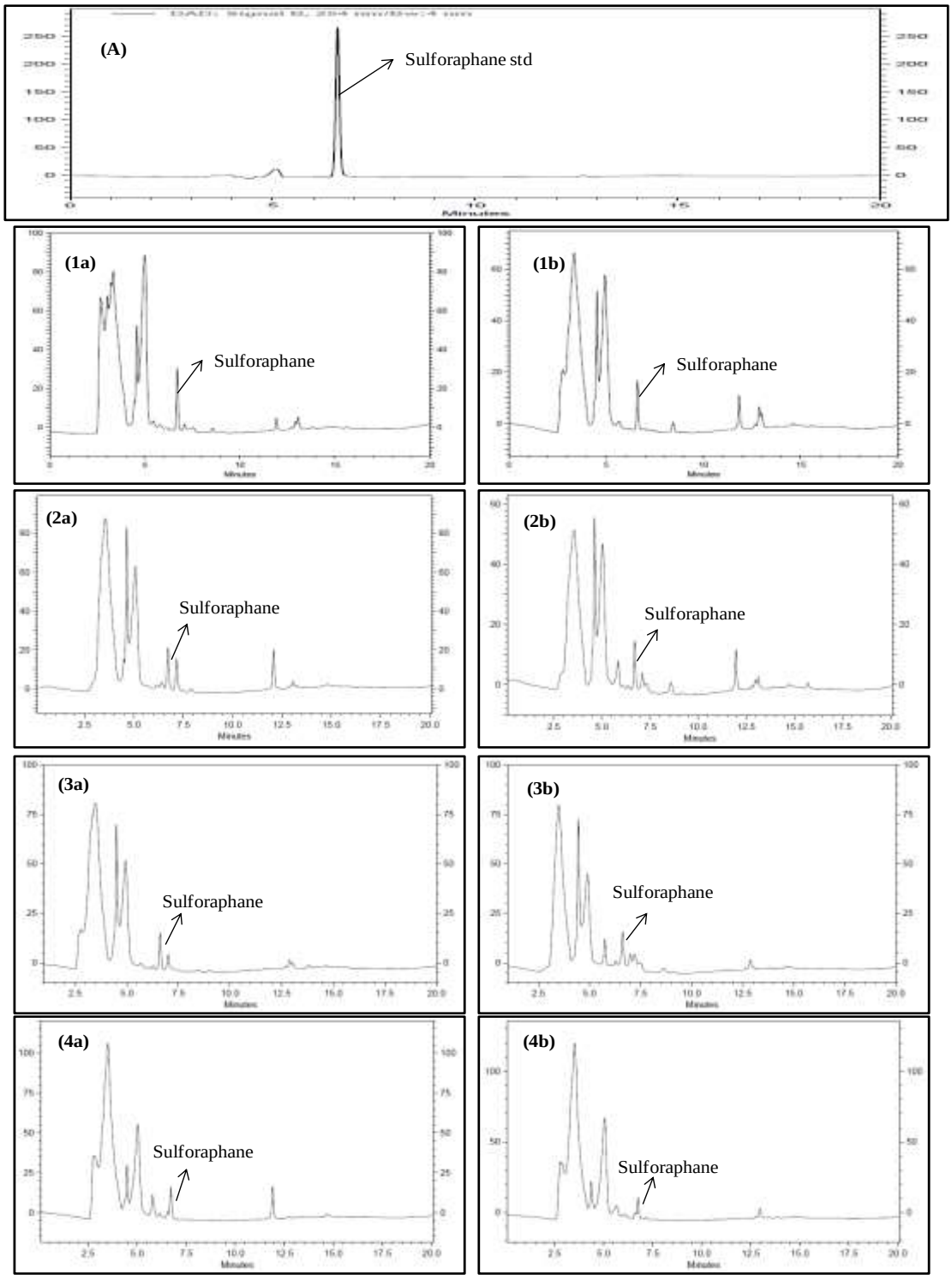

Figure 4.7. RP-HPLC chromatogram of Brassicaceaevegetables (A) Standard peak of sulforaphane, (1a) cabbage: HA, (1b) cabbage: LA, (2a) cauliflower: HA, (2b) cauliflower: LA, (3a) knol-khol: HA, (3b) knol-khol: LA, (4a) radish: HA, (4b) radish: LA. HA= High altitude and LA= Low altitude.

Overall, the application of treatment T_3 (*i.e.* co-treatment of FYM and *Azotobacter*) significantly increased the concentration of all the three tested glucosinolates (*i.e.* kaempferol, indole-3-carbinol and sulforaphane) at both the altitudinal locations. Although these compounds have been earlier reported in Brassicaceae vegetables, the novel finding of our study is that their accumulation is significantly boosted in the HA grown Brassicaceae vegetables (Ahmed *et al.,* 2014; Li *et al.,* 2017; Liang *et al.,* 2006). Though the plants synthesize these protective secondary metabolites as part of their defense mechanism under harsh environmental condition such as extreme temperature, drought, salt, and radiation *etc*, their dietary enrichment is highly recommended due to their disease preventing and health promoting activities in humans. These secondary metabolites are extremely effective in neutralizing reactive oxygen species, thus their regular consumption is linked with reduced incidences of oxidative damage and various inflammatory diseases, including coronary heart disease (Calderon-Montano *et al*., 2011). At higher elevations consumption of diet especially enriched in bioactive phytochemicals is highly recommended to offer protection against highly ionizing environmental conditions. Thus the present study could shed light on effective means to locally produce health promoting Brassicaceae vegetables at higher elevations using organic agri-techniques.

Chapter -5

CHAPTER-5
SUMMARY AND CONCLUSION

A field experiment was conducted at high altitude location, Defence Institute of High Altitude Research (DIHAR) - Defence Research and Development Organization (DRDO), HQ, Leh-Ladakh and at low altitude location, Defence Institute of High Altitude Research (DIHAR), Defence Research and Development Organization (DRDO), Base lab Chandigarh during the year of 2020-2021 and 2021-22 to determine the **"Comparative Study of FYM and *Azotobacter* on the Growth, Yield, Qualitative Traits and Phytochemical Aspects of Cruciferous Vegetables at Cold Desert Region and Plain Area**".

The experiment was set up using a Randomized Block Design (RBD) with two locations (high altitude and low altitude), three replications, and four treatments (T_1-FYM, T_2-*Azotobacter*, T_3-FYM+*Azotobacter*, and T_4-control (without any treatment). For research purposes, cruciferous vegetables such as cabbage cultivar Videshi, cauliflower cultivar WS909, knol-khol cultivar White Vienna, and radish cultivar Pusa Himani was taken for study purpose. Numerous observations on growth, yield, nutritional value, and phytochemical characteristics were carried out during the experiment.

This chapter presents an overview and highlights of the experimental results, culminating in a conclusion that demonstrates the research endeavour's outcomes. The summarized findings are as follows:

- This study revealed that both bio-organic treatment and altitude exhibit complex effects on plant growth, morphology, and the abiotic and biotic factors of the ecosystem. Moreover, significant variations were observed across all studied parameters.

- The findings of this study indicate that across both high-altitude (HA) and low-altitude (LA) locations, the highest growth attributes including plant height, leaf count, leaf dimensions (length and width), leaf area, plant spread, stem diameter, and chlorophyll and anthocyanin content of cruciferous vegetables

were consistently observed in T_3 treatment (FYM+*Azotobacter*) at all stages of growth, compared to the control group.

- During the harvesting stage, cabbage and cauliflower grown at HA exhibited increased plant height by 11.43% and 9.68% respectively compared to those grown at LA. Conversely, for knol-khol and radish, the maximum plant height observed (9.24% and 31.03% respectively) was in the LA samples compared to HA.

- At 90 DAT, the number of leaves in cabbage and cauliflower was 9.00% and 8.26% higher, respectively, in plants cultivated at LA compared to those at HA. No significant difference was observed in knol-khol. However, the maximum leaf count was recorded in radish plants grown at HA (19.34%) compared to those grown at LA.

- The leaf area showed a statistically significant increase ($p \leq 0.05$) of 54.25% in cabbage and 33.29% in cauliflower under the T_3 treatment at HA compared to LA grown crops. Conversely, knol-khol and radish exhibited the highest leaf area percentages at LA (54.60% and 49.90%, respectively) compared to HA.

- At 90 DAT, HA had the highest plant spread of cabbage (16.79%) and cauliflower (23.08%) as compared to LA-grown crops. Similar patterns were observed in the stem diameter of cabbage and cauliflower, which were 31.78% and 16.03% higher than the LA-cultivated crop, respectively.

- Later stage of plant development *i.e.*, 90 DAT and 60 DAS, T_3 treatment resulted in the highest leaf chlorophyll and anthocyanin content in HA grown cabbage (28.52% and 41.16%), cauliflower (28.97% and 17.48%), and radish (51.45% and 110.06%) compared to LA produced vegetables. However, there were no statistically significant differences observed in knol-khol for these traits between the altitudes.

- The current study demonstrated that treatment T_3 significantly enhanced cabbage and cauliflower, resulting in maximum head/curd diameter (15.86±0.43 and 14.31±0.15 cm), head/curd length (16.84±0.27 and 10.64±0.11 cm), head/curd weight per plant (1662.00±4.17 and 705.06±18.42

g), and yield (494.75±4.97 and 259.05±10.34 q/ha) at HA. Conversely, minimum head/curd diameter (13.04±0.06 and 14.11±0.19 cm), head/curd length (14.02±0.04 and 9.27±0.27 cm), head/curd weight per plant (834.76±25.66 and 466.6±13.47 g), and yield (302.06±11.31 and 209.05±0.72 q/ha) were recorded at LA cultivated crop.

- Regarding Knol-khol, samples produced in LA were substantially greater in yield (137.6 times) and knob weight per plant (59.8 times) than samples cultivated in HA. On the other hand, radish cultivated in HA had higher measured root diameter (35.24±0.79 mm), root weight per plant (200.20±6.41 g), and yield (390.64±4.65 q/ha) than grown in LA (31.9±0.52 mm, 155.59±4.72 g, and 308.13±8.53 q/ha, respectively).

- The Application of FYM+*Azotobacter* to HA soil led to notable enhancements in its chemical composition, exhibiting substantial increments in organic carbon (26.98%), nitrogen (19.12%), phosphorus (30.54%), potassium (4.52%), sulfur (37.89%), and manganese (46.72%) compared to LA soil. In contrast, LA soil displayed higher levels of zinc (86.21%), iron (55.86%), magnesium (51.16%), and copper (26.76%).

- The TSS content in knol-khol, cabbage, cauliflower and radish was found to be 9.15±0.07 $^{\circ}$B, 7.95±0.05 ^{0}B, 7.55±0.05 $^{\circ}$B and 5.30±0.10 $^{\circ}$B respectively which is higher in concentration at HA as compared to LA grown knol-khol (7.46±0.05 $^{\circ}$B), cabbage (7.75±0.05 $^{\circ}$B), cauliflower (6.58±0.08 $^{\circ}$B) and radish (4.10±0.00 $^{\circ}$B). Similarly, the maximum total carbohydrate content was observed in cabbage (73.52±0.27 µg/g), followed by knol-khol (67.39±1.10 µg/g), cauliflower (58.56±0.25 µg/g), and radish (38.33±0.25 µg/g), compared to LA grown cruciferous vegetable.

- Among the cruciferous vegetables, knol-khol displayed the highest percentages of titratable acidity at HA (0.37±0.02%), followed by cabbage (0.29±0.01%), cauliflower (0.26±0.01%), and radish (0.19±0.01%), compared to LA-grown sample. Similarly, the highest crude protein and dietary fiber content was observed in knol-khol (19.06±0.19 g/100g and 11.06±0.09%) compared to LA grown samples.

- At HA, the maximum percentages of crude fat were found in cauliflower (1.14±0.02%), cabbage (0.34±0.01%), knol-khol (0.26±0.02%) and radish (0.18±0.01%), respectively as compared to LA grown crops.

- This study is the first to compare the nutritional profiles of cruciferous vegetables cultivated organically in HA and LA in a systematic way. Nutrient profiling of cruciferous vegetablesfrom high and lower altitude experimental fields revealed significant variations. For instance, high altitude samples have greater N, K, Na, and Mn than LA samples. In contrast, Mg, Cu, Zn, and Fe was significantly high at lower altitude compared to HA grown Brassicaceae vegetables.

- In organic agri-treatments combine with FYM+*Azotobacter* exhibited significantly higher TPC, TFC and antioxidant (DPPH and FRAP) content in cabbage (9.56±0.15 µg GAE/mg of DPE, 14.48±0.41 µg RE/mg of DPE, 85.97±0.24% and 30.77±0.46 µg TE/mg of DPE followed by cauliflower, knol-khol and radish at HA grown crop as compared to LA cultivated samples.

- RP-HPLC analysis of phytocompound *i.e.* kaempferol was maximum found in HA grown cabbage (0.92±0.02 µg/mg) followed by cauliflower (0.81±0.01 µg/mg) and radish (0.73±0.01 µg/mg) as compared to LA cabbage (0.66±0.01 µg/mg), cauliflower (0.59±0.02 µg/mg) and radish (0.32±0.01 µg/mg) respectively. However, the maximum accumulation of indole-3-carbinol content at HA grown cauliflower (1.31±0.01 µg/mg), radish (1.01±0.03 µg/mg), knol-khol (0.91±0.02 µg/mg) and cabbage (0.65±0.02 µg/mg) than low altitude grown cauliflower (0.40±0.01 µg/mg), radish (0.85±0.02 µg/mg), knol-khol (0.74±0.01 µg/mg) and cabbage (0.52±0.00 µg/mg). Furthermore, higher sulforaphane content was found at HA grown cabbage (8.94±0.24 µg/mg), radish (4.48±0.04 µg/mg), cauliflower (4.11±0.02 µg/mg) and knol-khol (3.24±0.06 µg/mg) respectively as compared to LA cultivate Brassicaceae vegetables.

CONCLUSION

The results obtained from the present experiment entitled "**Comparative Study of FYM and *Azotobacter* on the Growth, Yield, Qualitative Traits and Phytochemical Aspects of Cruciferous Vegetables at Cold Desert Region and Plain Area**" following conclusion have been draw.

- The challenging environmental conditions of high mountainous regions, the application of FYM (150q/ha) + *Azotobacter* (8.6 Kg/ha) significantly impacts the growth and yield performance of cruciferous vegetables including cabbage, cauliflower, knol-khol and radish across various days after transplanting or sowing. At 90 days after transplanting (DAT) and 60 days after sowing (DAS), the results concluded that the positive influence of *Azotobacter* at high altitudes, enhancing morphological attributes such as plant height, leaf area, plant spread, leaf chlorophyll content, and leaf anthocyanin content. In addition, HA grown Brassicaceae vegetables such as cabbage, cauliflower and radish have been found to a higher head/curd/root diameter, head/curd/root weight per plant and increases in crop yields, in comparison to LA grown Brassicaceae a vegetable. Conversely, knol-khol demonstrated superior production at lower altitudes.

- The combination treatment of T_3 (FYM and *Azotobacter*) at HA could lead to improve the soil fertility and extensive enrichment of TSS, titratable acidity, total carbohydrate, total protein, fiber and macro nutrient in HA grown cruciferous vegetables were significantly higher than in LA grown vegetables.

- This study's key finding is that growing cruciferous vegetables at higher elevations while using the treatment T_3 (FYM+*Azotobacter*) may increase the amount of advantageous bioactive phytocompounds, which may have positive health effects for consumers. The cruciferous vegetables grown at higher altitudes shown an increase in their antioxidant activity, as determined by FRAP and DPPH assays, as well as higher levels of phenolic content and flavonoid content. Moreover, HPLC analysis showed that cruciferous vegetables cultivated at higher altitudes compared to those produced at lower altitudes had significantly higher quantities of three main flavonoids: kaempferol, indole-3-carbinol, and sulforaphane.

- Overall, this study concludes that combination of organic manure and biofertilizerto achieve enhanced soil fertility, productivity, nutritional and mineral composition as well as key bioactive phytocompounds of cruciferous vegetables at harsh environment of high altitudes region. Therefore, we suggested that growing cruciferous vegetables with increased nutritional value for local consumption at high altitudes using organic manure in conjunction with biofertilizers.

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Appendix

Appendix 1 Morphological parameters of cabbage cultivar Videshi

Source	DAT	Plant Height		Number of leaves		Leaf area		plant spread		Stem diamter		Leaf chlorophyll content		Leaf anthocyanin content	
		Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
ALT	30	329.89	636.73***	77.01	976.3***	89534.73	2393.22***	220.95	679.12***	104.33	879.68***	4.49	8.49**	66.63	2353.15***
	45	2.77	5.886*	12.27	169.06***	16259.38	96.96***	23.09	56.58***	0.26	1.99	989.45	607.45***	171.2	1353.18***
	60	2.59	6.58*	5.36	77.23***	54567.85	426.17***	60.8	87***	0.54	2.09	862.44	755.91***	55.66	446.45***
	75	19.55	48.68***	7.38	116.5***	161141.57	895.95***	73.64	87.88***	37.68	177.39***	1064	911.8***	165.64	1457.12***
	90	42.96	77.63***	16.97	365***	283278.8	2374.46***	142.74	262.11***	212.71	1276.53***	1019.99	965.84***	200.51	1733.69***
TRE	30	21.11	40.75***	6.98	88.45***	7652.95	204.56***	25.39	78.03***	12.43	104.82***	90.71	171.59***	5.78	204.15***
	45	50.74	107.93***	14.44	198.99***	41889.01	249.8***	55.83	136.82***	31.91	240.45***	175.69	107.86***	20.62	163.01***
	60	34.82	88.31***	11.51	165.83***	42912.13	335.14***	50.59	72.39***	25.88	99.62***	144.51	126.66***	22.93	183.91***
	75	37.93	94.45***	15.25	240.69***	46426.66	258.13***	49.88	59.53***	31.4	147.85***	169.11	144.92***	37.36	328.65***
	90	43.19	78.05***	16.91	363.69***	57467.54	481.7***	76.59	140.65***	39.77	238.66***	204.54	193.68***	56.37	487.42***
ALT×TRE	30	0.91	1.76	0.09	1.19	1323.45	35.38***	2.33	7.17**	0.37	3.09	1.53	2.89	0.32	11.25***
	45	5.51	11.72***	2.11	29.11***	5261.71	31.38***	1.34	3.29*	2.04	15.36***	14.42	8.85***	0.86	6.82**
	60	0.3	0.77	1.59	22.9***	1483.94	11.59***	0.64	0.91	0.57	2.18	2.65	2.32	1.05	8.42***
	75	0.5	1.24	1.24	19.5***	1581.51	8.79***	0.49	0.58	0.27	1.28	6.96	5.97**	2.85	25.08***
	90	0.95	1.72	0.82	17.6**	3102.48	26.01***	2.39	4.4*	0.07	0.44	1.36	1.29	5.17	44.7***
Error	30	0.52		0.08		37.41		0.33		0.12		0.53		0.03	
	45	0.47		0.07		167.69		0.41		0.13		1.63		0.13	
	60	0.39		0.07		128.04		0.7		0.26		1.14		0.12	
	75	0.4		0.06		179.86		0.84		0.21		1.17		0.11	
	90	0.55		0.05		119.3		0.54		0.17		1.06		0.12	
Total df	30	24		24		24		24		24		24		24	
	45	24		24		24		24		24		24		24	
	60	24		24		24		24		24		24		24	
	75	24		24		24		24		24		24		24	
	90	24		24		24		24		24		24		24	

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom
*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

Appendix 2 Morphological parameters of cauliflower cultivar WS909

Source	DAT	Plant Height		Number of leaves		Leaf area		plant spread		Stem diamter		Leaf chlorophyll content		Leaf anthocyanin content	
		Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
ALT	30	1415.42	2750.58***	136.09	1646.90***	127041.9	3702.24***	306.59	680.70***	106.01	1317.69***	1.22	1.54	35.21	1088.16***
	45	861.36	1071.96***	25.36	161.61***	143207.6	2624.67***	52.16	129.60***	52.3	535.78***	855.86	1102.15***	8.38	80.32***
	60	62.05	86.26***	13.52	136.24***	12039.55	127.51***	72.11	116.02***	16.93	124.8***	541.88	1067.96***	11.61	154.78***
	75	6.37	10.59**	11.08	69.08***	58.19	0.34	15.54	44.20***	1.99	7.32*	441.96	487.35***	13.55	157.65***
	90	68.01	82.28***	11.82	125.35***	302693.4	1451.71***	260.44	538.68***	96.92	411.22***	830.14	1087.42***	33.73	482.10***
TRE	30	39.67	77.10***	6.91	83.63***	8115.64	236.51***	23.71	52.63***	7.23	89.90***	86.97	109.19***	15.4	476.02***
	45	64.97	80.86***	17.2	109.59***	20929.19	383.59***	47.84	118.87***	18.96	194.25***	94.97	122.30***	16.45	157.74***
	60	65.61	91.21***	12.74	128.44***	35276.36	373.62***	68.27	109.85***	15.09	111.23***	105.23	207.40***	15.29	203.87***
	75	108.23	180.03***	22.37	139.44***	61585.53	362.93***	85.99	244.64***	37.91	139.56***	128.65	141.86***	13.91	161.92***
	90	189.57	229.37***	27.92	296.14***	119890.8	574.99***	167.86	347.20***	66.78	283.35***	174.76	228.92***	16.37	234.05***
ALT×TRE	30	9.04	17.57***	0.38	4.65*	5587.35	162.83***	7.25	16.09***	1.35	16.79***	4.34	5.45**	0.21	6.51**
	45	1.75	2.17	0.82	5.23**	3487.38	63.92***	1.83	4.54*	0.35	3.57*	1.23	1.58	1.38	13.19***
	60	5.04	7.00**	0.12	1.19	1661.64	17.60***	2.98	4.79*	1.16	8.55***	1.54	3.04	1.63	21.72***
	75	0.35	0.58	0.87	5.43**	1211.16	7.14**	12.86	36.59***	0.5	1.85	0.62	0.68	0.85	9.93***
	90	2.55	3.09	1.28	13.58***	973.3	4.67*	2.95	6.10**	0.33	1.4	2.04	2.67	0.34	4.9*
Error	30	0.51		0.08		34.31		0.45		0.08		0.8		0.03	
	45	0.8		0.16		54.56		0.4		0.1		0.78		0.1	
	60	0.72		0.1		94.42		0.62		0.14		0.51		0.07	
	75	0.6		0.16		169.69		0.35		0.27		0.91		0.09	
	90	0.83		0.09		208.51		0.48		0.24		0.76		0.07	
Total df	30	24		24		24		24		24		24		24	
	45	24		24		24		24		24		24		24	
	60	24		24		24		24		24		24		24	
	75	24		24		24		24		24		24		24	
	90	24		24		24		24		24		24		24	

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom
*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

Appendix 3 Morphological parameters of knol-khol cultivar White Vienna

Source	DAT	Plant Height		Number of leaves		Leaf area		plant spread		Leaf chlorophyll content		Leaf anthocyanin content	
		Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
ALT	30	762.53	1233.12***	111.24	415.80***	135165.1	4245.90***	713.41	1747.56***	708.29	1064.94***	0.07	1.37
	45	353.97	612.88***	58.56	340.12***	102400.9	2973.09***	198.84	386.75***	42.43	162.04***	7.07	156.83***
	60	85.77	105.49***	0.56	2.42	79793.37	1459.41***	44.09	42.30***	6.98	23.49***	15.71	152.43***
TRE	30	56.94	92.07***	10.39	38.85***	8838.42	277.64***	58.93	144.35***	63.28	95.14***	6.7	124.31***
	45	71.55	123.88***	17.55	101.95***	18016.09	523.08***	63.05	122.63***	64.07	244.69***	10.33	229.02***
	60	92.06	113.23***	33.16	144.01***	25537.79	467.08***	76.3	73.20***	123.74	416.65***	24.1	233.72***
ALT×TRE	30	3.2	5.17*	1.02	3.80*	2075.69	65.20***	1.5	3.67*	0.27	0.41	1.56	28.98***
	45	0.34	0.59	0.02	0.13	1346.2	39.09***	0.51	0.99	3.61	13.79***	0.37	8.24**
	60	1.08	1.33	0.5	2.15	319.23	5.84**	1.58	1.52	2.98	10.05***	1.29	12.51***
Error	30	0.62		0.27		31.83		0.41		0.67		0.05	
	45	0.58		0.17		34.44		0.51		0.26		0.05	
	60	0.81		0.23		54.68		1.04		0.3		0.1	
Total df	30	24		24		24		24		24		24	
	45	24		24		24		24		24		24	
	60	24		24		24		24		24		24	

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom

*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

Appendix 4 Morphological parameters of radish cultivar Pusa Himani

Source	DAT	Plant Height		Number of leaves		Leaf area		Leaf chlorophyll content		Leaf anthocyanin content	
		Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value	Mean Square	F Value
ALT	30	342.54	678.04***	0.91	7.55*	15428.01	4367.64***	711.23	628.64***	191.76	4882.52***
	45	389.7	703.26***	51.04	216.49***	15819.39	1044.11***	1809.26	2977.86***	443.16	4904.45***
	60	238.96	681.06***	39.6	123.37***	17750.72	1008.61***	1212.11	3315.37***	335.03	4986.18***
TRE	30	18.27	36.17***	11.74	97.56***	1512.37	428.15***	63.72	56.32***	6.23	158.67***
	45	63.66	114.87***	23.99	101.74***	6849.34	452.07***	108.24	178.15***	7.79	86.25***
	60	50.92	145.12***	25.92	80.74***	8655.96	491.84***	115.59	316.15***	14.55	216.49***
ALT×TRE	30	2.58	5.10*	0.14	1.16	352.06	99.67***	1.85	1.63	0.7	17.80***
	45	0.72	1.29	1.35	5.72**	412.33	27.21***	8.36	13.76***	0.57	6.35**
	60	4.21	11.99***	0.59	1.85	669.64	38.05***	6.35	17.38***	0.85	12.66***
Error	30	0.51		0.12		3.53		1.13		0.04	
	45	0.55		0.24		15.15		0.61		0.09	
	60	0.35		0.32		17.6		0.37		0.07	
Total df	30	24		24		24		24		24	
	45	24		24		24		24		24	
	60	24		24		24		24		24	

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom
*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

Appendix 5 Yield parameters of cabbage cultivar Videshi and cauliflower cultivar WS909

Cabbage							
		Number of inner leaf	**Head Diameter**	**Head Length**	**Head weight /plant**	**Compactness**	**Yield**
ALT	Mean Square	86.22	31.24	34.15	2138722.66	0.03	82386.91
	F Value	366.61***	354.3***	1030.39***	4456.93***	9.92**	1393.27***
TRE	Mean Square	209.59	8.08	8.5	322257.57	0.01	36589.71
	F Value	891.15***	91.65***	256.45***	671.56***	3.16	618.78***
ALT×TRE	Mean Square	0.29	0.24	0.41	67916	0.01	7084.09
	F Value	1.25	2.68	12.26***	141.53***	3.38*	119.8***
Error		0.24	0.09	0.03	479.86	0.00	59.13
Total df		24	24	24	24	24	24
Cauliflower							
		Curd Diameter	**Curd Length**	**Curd weight /plant**	**Compactness**	**Yield**	
ALT	Mean Square	3.61	19.15	244468.61	0.98	3804.7	
	F Value	82.78***	246.22***	1521.60***	181.83***	94.28***	
TRE	Mean Square	21.85	7.06	99650.44	0.29	13473	
	F Value	500.88***	90.75***	620.24***	53.72***	333.87***	
ALT×TRE	Mean Square	1.28	0.12	1161.18	0.11	457.27	
	F Value	29.33***	1.5	7.23**	19.98***	11.33***	
Error		0.04	0.08	160.67	0.01	40.35	
Total df		24	24	24	24	24	

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom

*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

Appendix 6 Yield parameters of knol-khol cultivar White Vienna and radish cultivar Pusa Himani

Radish					
		Diameter of root	**Length of root**	**Fresh weight of root**	**Yield**
ALT	Mean Square	27.86	0.69	7643.01	33957.32
	F Value	56.96***	1.24	413.32***	715.02***
TRE	Mean Square	78.76	40.92	7280.3	29398.52
	F Value	161.01***	73.90***	393.70***	619.03***
ALT×TRE	Mean Square	1.79	0.62	93.92	105.47
	F Value	3.67*	1.12	5.08*	2.22
Error		0.49	0.55	18.49	47.49
Total df		24	24	24	24
Knol-Khol					
		Knob equatorial diameter	**Knob polar diameter**	**Knob weight /plant**	**Yield**
ALT	Mean Square	0.36	0.07	3418.66	47050.16
	F Value	0.16	0.03	49.43***	517.49***
TRE	Mean Square	629.07	740.21	45758.27	57730.85
	F Value	281.87***	317.17***	661.62***	634.96***
ALT×TRE	Mean Square	23.61	11.83	2036.19	4757.78
	F Value	10.58***	5.07*	29.44***	52.33***
Error		2.23	2.33	69.16	90.92
Total df		24	24	24	24

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom

*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

Details of cost of cultivation of cruciferous vegetable grown at HA vs. LA

Appendix 7 (A) Common cost

S.No.	Particular	Quantity	Rate(Rs.)	Cabbage: Total(Rs.)	Cauliflower: Total(Rs.)	Knol-khol: Total(Rs.)	Radish: Total(Rs.)
1. Field preparation							
a.	Pre- irrigation	10 hour	100/ hour	1000	1000	1000	1000
b.	Labour for irrigation	3 labour	450/labour	1350	1350	1350	1350
c.	Ploughing by disc plough	1 time	1200/ha.	1200	1200	1200	1200
d.	Ploughing by cultivator	2 time	1000/ha.	2000	2000	2000	2000
e.	Planking	2 time	500/ha.	1000	1000	1000	1000
2. Layout and transplanting							
a.	Seed (cabbage)	400 g	20/g	8000	-	-	-
	Cauliflower	450 g	40/g	-	18000	-	-
	Knol-khol	1200 g	20/g	-	-	24000	-
	Radish	8000 g	5/g	-	-	40000	-
b.	Labour for sowing of nursery	10 labour	450/labour	4500	4500	4500	
c.	Labour for layout	20 labour	450/labour	9000	9000	9000	9000
d.	Labour for transplanting of seedlings	25 labour	450/labour	11250	11250	11250	11250
3. Cultural practices							
a.	Labour for three weeding	60 labour	450/labour	27000	27000	27000	27000
b.	Irrigation by tube well	100 hour	100/ hour	10000	10000	10000	10000
c.	Labour for irrigation	10 labour	450/labour	4500	4500	4500	4500
4. Plant protection							
a.	Spraying of Neem oil 4 times	20 Liter	500/Liter	10000	10000	10000	10000
b.	Labour for spraying	4 labour	450/labour	1800	1800	1800	1800
5. Harvesting							
a.	Labour for Harvesting	15 labour	450/labour	6750	6750	6750	6750
6	Sub total	-	-	**99350**	**109350**	**155350**	**86850**
7	Interest on cultivation cost @ 4 %	-	-	3974	4374	6214	3474
8	Total	-	-	103324	113724	161564	90324
9	Marginal risk @ 10 %	2 months	10%	10332.4	11372.4	16156.4	9032.4
10	Land rent	4 months	5000/Months	20000	20000	20000	20000
11	Total cost of cultivation	-	-	**133656.4**	**145096.4**	**197720.4**	**119356.4**

Appendix 7 (B) Variable cost of cultivation

						Total cost of cultivation			
	Treatment	**Particulars**	**Input**	**Rate (Rs)**	**Total**	**Cabbage**	**Cauliflower**	**Knol-khol**	**Radish**
HA	**T1**	FYM	150q/ha	100/q	15000	148656.4	160096.4	212720.4	134356.4
	T2	Azotobacter	8.6 kg/ha	240/Kg	2064	135720.4	147160.4	199784.4	121420.4
	T3	FYM + Azotobacter	150q/ha + 8.6 kg/ha	100/q + 240/Kg	17064	150720.4	162160.4	214784.4	136420.4
	T4	Control	-	-	0.00	133656.4	145096.4	197720.4	119356.4
LA	**T1**	FYM	150q/ha	50/q	7500	141156.4	152596.4	205220.4	126856.4
	T2	Azotobacter	8.6 kg/ha	240/Kg	2064	135720.4	147160.4	199784.4	121420.4
	T3	FYM + Azotobacter	150q/ha + 8.6 kg/ha	50/q + 240/Kg	9564	143220.4	154660.4	207284.4	128920.4
	T4	Control	-	-	0.00	133656.4	145096.4	197720.4	119356.4

Appendix 8 Economics of different treatments of cruciferous vegetable grown at HA vs. LA

Location	Treatments	Yield (q/ha)	Rate (Rs/q)	Gross return (Rs/ha)	Cost of treatment (Rs/ha)	Total cost of cultivation (Rs/ha)	Net return (Rs/ha)	B:C Ratio
Cabbage								
HA	**T1**	375.72	2200	826584	15000	148656.4	677927.6	4.56
	T2	368.41	2200	810502	2064	135720.4	674781.6	4.97
	T3	494.75	2200	1088450	17064	150720.4	937729.6	6.22
	T4	220.37	2200	484814	0.00	133656.4	351157.6	2.63
LA	**T1**	248.05	1500	372075	7500	141156.4	230918.6	1.64
	T2	245.99	1500	368985	2064	135720.4	233264.6	1.72
	T3	302.06	1500	453090	9564	143220.4	309869.6	2.16
	T4	194.45	1500	291675	0.00	133656.4	158018.6	1.18
Cauliflower								
Location	**Treatments**	**Yield (q/ha)**	**Rate (Rs/q)**	**Gross return (Rs/ha)**	**Cost of treatment (Rs/ha)**	**Total cost of cultivation (Rs/ha)**	**Net return (Rs/ha)**	**B:C Ratio**
HA	**T1**	191.36	3500	669760	15000	160096.4	509663.60	3.18
	T2	187.24	3500	655340	2064	147160.4	508179.60	3.45
	T3	259.05	3500	906675	17064	162160.4	744514.60	4.59
	T4	130.66	3500	457310	0.00	145096.4	312213.60	2.15
LA	**T1**	180.55	2500	451375	7500	152596.4	298778.60	1.96
	T2	171.6	2500	429000	2064	147160.4	281839.60	1.92
	T3	209.05	2500	522625	9564	154660.4	367964.60	2.38
	T4	106.38	2500	265950	0.00	145096.4	120853.60	0.83
Knol-Khol								
Location	**Treatments**	**Yield (q/ha)**	**Rate (Rs/q)**	**Gross return (Rs/ha)**	**Cost of treatment (Rs/ha)**	**Total cost of cultivation (Rs/ha)**	**Net return (Rs/ha)**	**B:C Ratio**
HA	**T1**	257.72	2000	515440	15000	212720.4	302719.60	1.42
	T2	250.21	2000	500420	2064	199784.4	300635.60	1.50
	T3	370.99	2000	741980	17064	214784.4	527195.60	2.45
	T4	196.71	2000	393420	0.00	197720.4	195699.60	0.99
LA	**T1**	360.49	1500	540735	7500	205220.4	335514.60	1.63
	T2	356.58	1500	534870	2064	199784.4	335085.60	1.68
	T3	508.64	1500	762960	9564	207284.4	555675.60	2.68
	T4	204.11	1500	306165	0.00	197720.4	108444.60	0.55
Radish								
Location	**Treatments**	**Yield (q/ha)**	**Rate (Rs/q)**	**Gross return (Rs/ha)**	**Cost of treatment (Rs/ha)**	**Total cost of cultivation (Rs/ha)**	**Net return (Rs/ha)**	**B:C Ratio**
HA	**T1**	332.61	1500	498915	15000	134356.4	364558.60	2.71
	T2	328.09	1500	492135	2064	121420.4	370714.60	3.05
	T3	390.64	1500	585960	17064	136420.4	449539.60	3.30
	T4	214.1	1500	321150	0.00	119356.4	201793.60	1.69
LA	**T1**	258.85	1000	258850	7500	126856.4	131993.60	1.04
	T2	247.43	1000	247430	2064	121420.4	126009.60	1.04
	T3	308.13	1000	308130	9564	128920.4	179209.60	1.39
	T4	150.11	1000	150110	0.00	119356.4	30753.60	0.26

Appendix 9 Nutritional parameters of cruciferous vegetables

Cabbage											
		TSS	**Acidity**	**Total protein**	**Nitrate**	**Phosphate**	**Sulphate**	**Carbohydrate**	**Crude fat**	**Dietary fiber**	**Ash**
ALT	Mean Square	0.45	0	8.77	30.2	94452.56	4272.8	16.01	0.00	0.05	2.25
	F Value	82.19***	14.52**	137.26***	1.03	677.35***	71.18***	174.67***	19***	2.09	1737.06***
TRE	Mean Square	1.59	0.02	18.21	60261.77	55114.88	12470.22	128.62	0.05	2.29	0.28
	F Value	288.35***	76.52***	284.89***	2061.20***	395.25***	207.73***	1403.56***	689.04***	96.77***	218.92***
ALT×TRE	Mean Square	0.00	0.00	0.6	182.31	346.18	899.8	1.08	0.00	0.24	0.13
	F Value	0.73	2.17	9.46***	6.24**	2.48*	14.99***	11.82***	2.72	10.06***	100.08***
Error		0.01	0	0.06	29.24	139.44	60.03	0.09	0	0.02	0
Total df		24	24	24	24	24	24	24	24	24	24
Cauliflower											
		TSS	**Acidity**	**Total protein**	**Nitrate**	**Phosphate**	**Sulphate**	**Carbohydrate**	**Crude fat**	**Dietary fiber**	**Ash**
ALT	Mean Square	6.77	0.00	1.08	1819	5053.83	362395.2	68.01	0.03	0.01	0.03
	F Value	903.13***	9.00***	38.67***	81.43***	22.75***	20676.65***	3155.15***	100.94***	0.65	17.37***
TRE	Mean Square	1.12	0.02	10.14	7559.75	92682.08	6606.42	59.22	0.36	2.63	0.72
	F Value	149.90***	294.92***	361.85***	338.43***	417.28***	376.93***	2747.68***	1324.36***	162.97***	376.65***
ALT×TRE	Mean Square	0.01	0.00	0.06	98.71	706.9	982.66	0.56	0.00	0.16	0.08
	F Value	1.94	6.67**	2.28	4.42*	3.18*	56.07***	26.20***	16.67***	9.87***	41.80***
Error		0.01	0.00	0.03	22.34	222.11	17.53	0.02	0.00	0.02	0.00
Total df		24	24	24	24	24	24	24	24	24	24
Knol-Khol											
		TSS	**Acidity**	**Total protein**	**Nitrate**	**Phosphate**	**Sulphate**	**Carbohydrate**	**Crude fat**	**Dietary fiber**	**Ash**
ALT	Mean Square	8.82	0.02	74.17	21035.54	27417.21	30317.75	28.02	0	3.23	3.74
	F Value	846.81***	161.48***	1506.17***	175.22***	140.46***	783.97***	865.33***	5.50*	281.40***	3816.21***
TRE	Mean Square	2.23	0.02	21.58	108210	104971.3	16034.31	357.32	0.01	2.13	0.62
	F Value	213.8***	170.94***	438.16***	901.37***	537.76***	414.62***	11036.87***	99.32***	185.94***	634.78***

ALT×TRE	Mean Square	0.1	0	0.61	1542.06	613.06	1121.45	0.16	0	0.18	0.02
	F Value	9.72***	0.92	12.39***	12.85***	3.14	29***	4.9*	6.83**	15.38***	23.94***
Error		0.01	0	0.05	120.05	195.2	38.67	0.03	0	0.01	0
Total df		24	24	24	24	24	24	24	24	24	24
Radish											
		TSS	**Acidity**	**Total protein**	**Nitrate**	**Phosphate**	**Sulphate**	**Carbohydrate**	**Fat**	**Dietary fiber**	**Ash**
ALT	Mean Square	7.59	0	95.52	227253.9	54537.34	2506.15	2.34	0.07	2.31	2.39
	F Value	1401.92***	3.9	1866.09***	1898.65***	144.77***	115.81***	45.99***	734.78***	330.67***	688.76***
TRE	Mean Square	0.66	0.01	6.38	46442.66	214320.1	3142.99	64.57	0	1.61	1.43
	F Value	121.69***	63.60***	124.70***	388.02***	568.93***	145.24***	1270.32***	26.09***	231.22***	412.57***
ALT×TRE	Mean Square	0.01	0	0.06	3996.57	1358.8	103.51	0.88	0	0.04	0.04
	F Value	1.36	1.92	1.09	33.39***	3.61*	4.78*	17.24***	0.52	5.14*	11.23***
Error		0.01	0	0.05	119.69	376.71	21.64	0.05	0	0.01	0
Total df		24	24	24	24	24	24	24	24	24	24

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom
*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

Appendix 10 Macro and micro mineral content of cruciferous vegetables

Cabbage									
		N	**Mg**	**Zn**	**Cu**	**Fe**	**Mn**	**Na**	**K**
ALT	Mean Square	224578	60509.08	3.17	8.86	1057.62	33.87	92265.92	318000.6
	F Value	136.65***	674.85***	137.28***	3049.88***	7246.04***	4335.04***	4537.33***	110.56***
TRE	Mean Square	466498.6	6486.31	5.75	0.32	18.22	0.95	10133.9	418728.8
	F Value	283.86***	72.34***	249.04***	110.13***	124.81***	122.11***	498.35***	145.59***
ALT×TRE	Mean Square	15581.99	1063.6	1.41	0.12	2.89	0.07	1350.97	41089.27
	F Value	9.48***	11.86***	60.89***	40.25***	19.78***	8.44***	66.44***	14.29***
Error		1643.4	89.66	0.02	0	0.15	0.01	20.33	2876.16
Total df		24	24	24	24	24	24	24	24
Cauliflower									
		N	**Mg**	**Zn**	**Cu**	**Fe**	**Mn**	**Na**	**K**
ALT	Mean Square	27579.68	53045.14	1.27	11.93	941.13	62.82	57947.85	421445.4
	F Value	38.48***	913.10***	47.65***	13377.87***	2415.73***	12680.98***	710.91***	196.86***
TRE	Mean Square	259569.8	5378.29	9.78	0.25	90.23	1.82	5260.37	529363
	F Value	362.17***	92.58***	368.38***	275.98***	231.61***	367.54***	64.53***	247.26***
ALT×TRE	Mean Square	1633.35	302.51	0.04	0.12	5.01	0.13	724.35	74656.01
	F Value	2.28	5.21*	1.67	129.83***	12.86***	26.43***	8.89***	34.87***
Error		716.7	58.09	0.03	0	0.39	0	81.51	2140.89
Total df		24	24	24	24	24	24	24	24
Knol-Khol									
		N	**Mg**	**Zn**	**Cu**	**Fe**	**Mn**	**Na**	**K**
ALT	Mean Square	1899129	31875.53	0.16	16.06	9.08	77.69	48726.08	417956.9
	F Value	1512.54***	646.12***	5.86*	9467.74***	47.12***	6640.00***	965.36***	100.76***
TRE	Mean Square	551823	4743.36	8.38	0.17	81.69	2.68	5078.33	1472112

	F Value	439.49***	96.15***	306.50***	97.64***	424.06***	228.94***	100.61***	354.89***
ALT×TRE	Mean Square	15596.92	281.14	1.56	0.08	0.31	0.08	584.39	201015
	F Value	12.42***	5.7**	57.26***	48.51***	1.6	6.42**	11.58***	48.46***
Error		1255.59	49.33	0.03	0	0.19	0.01	50.47	4148.09
Total df		24	24	24	24	24	24	24	24
Radish									
		N	**Mg**	**Zn**	**Cu**	**Fe**	**Mn**	**Na**	**K**
ALT	Mean Square	2444095	315.3	1.22	20.55	164.01	56.7	184193	4031678
	F Value	1852.81***	8.19*	39.69***	11886.36***	1164.66***	4657.33***	903.36***	558.74***
TRE	Mean Square	163480.1	4341.32	7.13	0.32	17.55	1.06	34682.55	2208192
	F Value	123.93***	112.72***	232.18***	187.94***	124.64***	86.77***	170.10***	306.03***
ALT×TRE	Mean Square	1385.41	965.16	0.72	0.03	0.37	0.43	1903.93	787023.9
	F Value	1.05	25.06***	23.49***	16.34***	2.6	35.20***	9.34***	109.07***
Error		1319.13	38.52	0.03	0	0.14	0.01	203.9	7215.72
Total df		24	24	24	24	24	24	24	24

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom
*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

Appendix 11 Phytochemical content of cruciferous vegetables

Cabbage								
		TPC	**TFC**	**FRAP**	**DPPH**	**kaempferol**	**Indole-3-carbinol**	**sulforaphane**
ALT	Mean Square	1.45	41.76	111.2	40.82	0.03	0.1	20.13
	F Value	95.18***	1171.11***	739.45***	159.74***	407.58***	715.76***	1838.36***
TRE	Mean Square	11.6	15.45	87.66	509.53	0.43	0.16	30.79
	F Value	763.63***	433.28***	582.93***	1993.84***	5402.46***	1132.27***	2812.25***
ALT×TRE	Mean Square	0.14	1.06	1.45	0.26	0.03	0	5.93
	F Value	9.39***	29.73***	9.65***	1.04	315.93***	4.47*	541.70***
Error		0.02	0.04	0.15	0.26	0	0	0.01
Total df		24	24	24	24	24	24	24
Cauliflower								
		TPC	**TFC**	**FRAP**	**DPPH**	**kaempferol**	**Indole-3-carbinol**	**sulforaphane**
ALT	Mean Square	1.38	8.82	108.55	1001.04	0.05	2.45	1.01
	F Value	110.91***	4118.73***	3596.69***	5698.12***	1100***	8170.68***	152.91***
TRE	Mean Square	6.23	7.3	54.28	198.78	0.31	0.51	5.46
	F Value	501.77***	3410.26***	1798.69***	1131.48***	6842.91***	1714.16***	828.41***
ALT×TRE	Mean Square	0.02	0.4	0.78	32.95	0.01	0.21	0.1
	F Value	1.56	189.07***	25.76***	187.55***	282.79***	694.35***	14.40***
Error		0.01	0	0.03	0.18	0	0	0.01
Total df		24	24	24	24	24	24	24
Knol-khol								
		TPC	**TFC**	**FRAP**	**DPPH**	**Indole-3-carbinol**	**sulforaphane**	
ALT	Mean Square	13.94	2.88	22.25	243.97	0.09	0.76	
	F Value	702.34***	422.60***	133.06***	1817.57***	642.86***	73.52***	ND
TRE	Mean Square	5.98	6.88	33.63	85.7	0.43	3.83	
	F Value	301.55***	1008.35***	201.10***	638.48***	2935.05***	370.64***	

ALT×TRE	Mean Square	0.06	0.01	0.02	0.87	0	0.04	
	F Value	3.27*	0.88	0.09	6.51**	21.52***	3.74*	
Error		0.02	0.01	0.17	0.13	0	0.01	
Total df		24	24	24	24	24	24	
Radish								
		TPC	**TFC**	**FRAP**	**DPPH**	**kaempferol**	**Indole-3-carbinol**	**sulforaphane**
ALT	Mean Square	27.95	2.25	96.84	1717.72	0.34	0.04	3
	F Value	3517.62***	379.91***	1819.34***	17402.69***	6292***	75.98***	512.30***
TRE	Mean Square	6.04	5.46	37.39	482.54	0.09	0.42	5.97
	F Value	760.44***	922.31***	702.43***	4888.73***	1610.46***	782.27***	1017.92***
ALT×TRE	Mean Square	0.17	0.03	1.51	52.05	0.02	0.01	0.32
	F Value	21.64***	5.7**	28.41***	527.34***	432.92***	14.54***	55.30***
Error		0.01	0.01	0.05	0.1	0	0	0.01
Total df		24	24	24	24	24	24	24

ALT: Altitude, TRE: Treatment, ALT×TRE- interaction of altitude and treatment. df: Degree of freedom
*Level of significance: ***p≤0.001; **p≤0.01 and *p≤0.05.*

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Effect of cold arid high-altitude environment on bioactive phytochemical compounds of organically grown Brassicaceae vegetables for nutri-health security in mountainous regions

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High-altitude (HA) environment presents immense physiological adversities for humans that have been overcome by supplementing bio-active phytochemicals from functional foods that support and accelerate acclimatization under these extreme environmental conditions. Several agricultural interventions have been investigated to enhance the phytochemical content in vegetables however; these studies have been limited to low-altitude (LA) regions only. In view of an existing knowledge gap, current work is designed to compare the phytochemical compositions of HA and LA-grown Brassicaceae vegetables (cabbage, cauliflower, knol-khol, and radish) using organic treatments via farm yard manure (FYM) and *Azotobacter*. The open field study was conducted as a two-factorial randomized block design. The first factor was treatment (T_1-FYM, T_2-*Azotobacter*, T_3-FYM + *Azotobacter*, and T_4-control) while the second was locations (HA and LA). Among all these treatments, the application of treatment T_3 in HA-grown cabbage showed the highest total phenolic content (TPC; 9.56 µg/mg), total flavonoids content (TFC; 14.48 µg/mg), and antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl (DPPH; 85.97%) and ferric reducing antioxidant power (FRAP; 30.77 µg/mg) compared to LA grown samples. Reverse Phase high performance liquid chromatography (RP-HPLC) analysis showed that treatment T_3 at HA led to significantly high kaempferol (0.92 µg/mg) and sulforaphane (8.94 µg/mg) contents in cabbage whereas, indole-3-carbinol (1.31 µg/mg) was higher in HA grown cauliflower. The present study provides scientific evidence for the enrichment of health-promoting phytochemical compounds in Brassicaceae vegetables grown with T_3 treatment specifically at HA.

Keywords Organic farming, Brassicaceae, Phytochemical compounds, Antioxidant, Secondary metabolites

Abbreviations

DPE	Dry powder extract
TPC	Total phenolic content
TFC	Total flavonoids content
FRAP	Ferric reducing antioxidant power
TPTZ	2,4,6-Tripyridyl-*s*-triazine

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DPPH	2,2-Diphenyl-1-picrylhydrazyl
Trolox	6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
GAE	Gallic acid equivalent
RE	Rutin trihydrate equivalent
TE	Trolox equivalent
RT	Retention times
q	Quintal
ANOVA	Analysis of variance
HA	High altitude
LA	Low altitude
MSL	Mean sea level
RP-HPLC	Reverse-phase high performance liquid chromatography

The exposure to high altitude regions such as that of the union territory of Ladakh in India, is well-known for acclimatization adversities faced by sojourners due to multi-factorial physiological challenges[1,2]. The most immediate and damaging impact of the hypobaric hypoxic environment of high altitude is oxidative stress due to increased levels of reactive oxygen species (ROS)[3]. Although an inherent anti-oxidant system combats the oxidative damage sometimes it may not suffice to dampen the damage caused by the overwhelming oxidative stress, thus resulting in the development of high-altitude illnesses of varying degrees such as acute mountain sickness (AMS), high altitude cerebral edema (HACE), and high altitude pulmonary edema (HAPE), etc.[1,3]. Under such situations, supplementation of potent anti-oxidant compounds supports the body's defense system against the damages caused by ROS. However, serious ramifications along with limited bio-absorption of synthetic anti-oxidants has led to the recently increased exploration of natural and food based anti-oxidant sources[4,5].

Brassicaceae is a diverse plant family covering about 3500 species and categorized among the most widely consumed vegetables globally encompassing bokchoy, broccoli, brussels sprouts, cabbage, cauliflower, and many more[6,7]. The Brassicaceae plants are naturally rich in bioactive compounds with numerous health benefits including anti-oxidant efficacy[7]. The importance of such health-promoting compounds increases manifolds under adverse climatic conditions such as that of HA regions, making it all the more important to consume bioactive phytochemical compounds rich foods under such situations[8]. Unfortunately, due to the adverse climatic conditions and shorter cultivation periods at HA, most food supplies are met via imports from far-flung low-altitudinal regions, leading to a loss of nutritional quality during long-distance transport[8]. At the same time, excessive usage of chemical fertilizers for enhancing yield and nutritional quality of food crops at HA puts highly vulnerable mountain ecosystems under threat and also affects soil and human health adversely[9]. Thus, there is an urgent need to investigate eco-friendly agricultural interventions to grow nutritionally rich food crops in HA regions to ensure nutri-health security under extreme environmental conditions of HA.

Organic farming has become increasingly popular in the past few decades as it ensures food safety and soil health[10]. Organic manure such as FYM and biofertilizer (*Azotobacter*) not only decreases the need for chemical fertilizers but also provides all the required nutrients to the plants[11,12]. The rhizosphere of plants is covered by a variety of microorganisms, including bacteria and cyanobacteria, which, when applied to the seeds, plant surface, and soil, aids in the conversion of essential nutrients like nitrogen, potassium, and phosphorus from non-absorbable to absorbable forms, which is necessary for the plant's growth[12,13]. While substantial research has examined the impact of organic farming on the enrichment of phytochemical composition of low-altitude (LA) grown Brassicaceae vegetables, very limited attention has been given to the HA environment where these phytochemicals may play a preventive and therapeutic role against physiological disturbances under extreme environmental conditions. Hence, the present study delves into a comparative analysis of the impact of organic practices on phytochemical composition and anti-oxidant efficacy of Brassicaceae vegetables cultivated in HA vs. LA regions.

Materials and methods

Plant sample

Two consecutive year (2020–2022) field trials were conducted in the open-experimental fields at HA location (Agriculture Research Unit, Defence Institute of High Altitude Research (Leh), India, 3340 mean sea level (msl), 34° 08′ 2″ N; 77° 34′ 3″ E) and LA location (Defence Institute of High Altitude Research, base lab Chandigarh, India, 321 msl, 30° 41′ 31″ N and 76° 47′ 10″ E). Studies were carried out using cruciferous vegetable *i.e.*, cabbage (*Brassica oleracea* L. var. *capitata*) cultivar Videshi, cauliflower (*Brassica oleracea* L. var. *botrytis*) cultivar WS909, khol-khol (*Brassica oleracea* L. var. *gongylodes*) cultivar White Vienna and radish (*Raphanus sativus* L.) cultivar Pusa Himani at both HA and LA field locations. Crop seeds were procured from Beejsheetal Research Pvt. Ltd., Mantha Road, Jalna, Maharashtra. The field trials had 12 plots of each vegetable by following a two-factorial randomized block design (2FRBD) with four treatments [(T_1- FYM @ 150 quintals per hectare (q/ha); T_2- *Azotobacter* @ 8.6 kg/ha; T_3- FYM @ 150 q/ha + *Azotobacter* @ 8.6 kg/ha; T_4- control (without fertilizer)] replicated thrice. For the experiments, a recommended dose of FYM and *Azotobacter* (procured from International Panaacea Ltd.) was used *i.e.* 150 q/ha and 8.6 kg/ha respectively[14]. The area of each plot was 1.62 m^2 (1.35 m length × 1.20 m width) and a distance of 0.5 m was maintained between adjacent blocks as well as experimental plots. The transplantation of seedlings was done at 2–3 true leaf stage or 15–18 cm height. Plant spacing was maintained for cabbage and cauliflower (60 cm × 45 cm), knol-khol (30 cm × 20 cm), and radish (30 cm × 10 cm) amongst plant-to-plant and line-to-line in all the experimental plots. FYM and *Azotobacter* were applied in each plot before transplanting the seedlings. The field was irrigated by flooding at an interval of 3 days at HA and 6–7 days interval at LA during an early stage of plant establishment, followed by one-week interval (HA)

and 2 weeks interval (LA) at later stages. At both locations, there was no use of synthetic fertilizers, pesticides, or herbicides. Weeds were removed manually two to three times during the growing period. The edible portion of cruciferous vegetables was randomly harvested at the maturity stage from each plot. Five kilograms of fresh samples were taken from each treatment and location, shade-dried, well-mixed, and grinded into powder. The powder was then stored at 4 °C in airtight ziplock bags for until further analysis.

Chemicals

HPLC grade methanol, acetonitrile, acetone, sodium nitrite, sodium hydroxide, and gallic acid were procured from Merck (India). DPPH (1,1-diphenyl-2-picrilhydrazyl), potassium persulfate (PPS), Folin–Ciocalteu (FC) reagent, aluminum chloride, Trolox, quercitin, kaempferol, indole-3-carbinol, sulforaphane, and anion multi-element standards were purchased from the Sigma Aldrich Pvt. Ltd (Switzerland). Sodium bicarbonate, sodium chloride, boric acid, rutintrihydrate, and sodium carbonate were purchased from Himedia (India). The deionized water from the water purification instrument [Merck Millipore Academic, United States of America (USA)] was used for various analyses. All other chemicals were of analytical grade and purchased from Rankem, LobaChemie, and Qualigens Fisher Scientific.

Sample extraction

The extraction method, duration, temperature, solvent type, and moisture content all play an important role in isolating the essential chemical compounds from plant materials. As a result, a standardized extraction procedure is required for effective yield of desired phytocompounds[15]. In the current research study, 30 g of pulverized sample was extracted thrice via maceration for 24 h at room temperature under dark conditions using 100 ml (each time) of solvent (80% methanol and 20% distilled water). The extracts were filtered to Whatman filter paper grade 1. Further, rotavapor (Buchi R-215, Switzerland) was used to concentrate the filtered extract at a temperature of 40 °C, followed by lyophilization (Esquire biotech Freeze dryer 18N, India) at − 80 °C and 0.050 mbar pressures. These lyophilized extracts were stored in an air-tight container at − 20 °C for further analyses.

Evaluation of total phenolic content

The total phenolic content (TPC) of sample extracts was determined using the Folin-Ciocalteu (FC) reagent with minor modifications[8]. 70 µL of standard solution (Gallic acid; 2.000–0.332 µg/mL)/extracts (10 mg/mL) were combined in 630 µL of deionized water, followed by the addition of FC reagent (70 µL) and incubation at room temperature for 5 min. In addition, 140 µL of Na_2CO_3 solution (20%) was put into each reaction mixture and incubated in the dark conditions for 60 min at room temperature. Following incubation, the absorbance of the samples and standard was measured spectrophotometrically at 750 nm. The results were expressed in µg of Gallic acid equivalent (GAE)/mg of dry powder extract (DPE).

Evaluation of total flavonoids content (TFC)

TFC was evaluated by the aluminum chloride method with minor modifications[15,16]. 170 µL of standard solution (Rutintrihydrate; 1.46–3.00 µg/mL)/extracts (10 mg/mL) were mixed with 680 µL of deionized water, along with 51 µL of $NaNO_2$ (0.72 M) and incubated for 5 min. Subsequently, in each reaction mixture, 51 µL of $AlCl_3$ (0.75 M) was added and then incubated for 6 min. Further, 340 µL of NaOH (1.00 M) was added to each reaction mixture. The total reaction volume was made up to 1700 µL by the addition of 408 µL deionized water. Finally, the absorbance was recorded spectrophotometrically at 510 nm. The outcomes were presented in µg of rutin trihydrate equivalent (RE)/mg of DPE.

Antioxidant activity

Evaluation of ferric reducing antioxidant power (FRAP)

The FRAP assay was accomplished as per the technique suggested by Bhardwaj et al.[17] and Kumar et al.[8] with minor amendments. Acetate buffer (pH 3.6) 300 mM, TPTZ solution (20 mM in 40 mM HCl), and 20 mM $FeCl_3$ (dissolved in water) were mixed in the ratio of 10:1:1 to make FRAP solution, and this FRAP solution was reacted with methanol extract of samples/standard (10.000 mg/mL) in the ratio of 1:30 followed by incubation in the dark conditions (30 min at 37 °C). The blue-colored product (Ferrous tripyridyltriazine complex) was obtained and absorbance was recorded at 593 nm spectrophotometrically. Trolox (0.976—250.000 µg/mL) was used as an assay standard, and outcomes were indicated in µg of Trolox equivalent (TE)/mg of DPE.

Evaluation of antioxidant capacity (DPPH radical scavenging activity)

The DPPH radical scavenging activity of extracts was estimated by Zeljkovıc et al.[18] and Bhardwaj et al.[17] with minor modifications. DPPH reagent (0.135 mM) was prepared in methanol. The methanolic extracts of test samples (30 mg/mL) / standard (0.480—1.500 µg/mL) were mixed at a ratio of 1:15 with DPPH using a vortex mixer and left at room temperature for 30 min. After incubation, absorbance was measured at 517 nm using a spectrophotometer. Quercetin (QR) was used as a standard. The potential to scavenge radicals was determined by the given formula:

$$\text{Radical scavenging activity (\%)} = \frac{R_{sam} - R_{sas}}{R_{sam}} \times 100$$

R_{sam} = DPPH radical absorbance in methanol; R_{sas} = DPPH radical absorbance in sample/standard.

Reverse phase high-performance liquid chromatography (RP-HPLC) analysis

The determination of key phytochemical compounds viz. kaempferol, indole-3-carbinol and sulforaphane was done using RP-HPLC technique (Agilent, Infinity 1200 Series) with photodiode array detector (DAD) as explained by Ahmed et al.[19] and Kumar et al.[8] for Kaempferol, Li et al.[20] for Indole-3-carbinol and Liang et al.[21] for sulforaphane with some modifications, respectively. Sample peaks, using a sample injection volume of 10 µL, were separated on a Phenomenex C18 column (5 µm, 100 A, 250 × 4.6 mm) maintained at 25 °C temperature with a flow rate of 0.6 mL/min. Before being employed for analysis, all the HPLC quality grade solvents were filtered using a 0.45 µm filter. For kaempferol determination, an isocratic solvent system was deputed using 50% formic acid (0.1%, v/v) and 50% acetonitrile for 18 min with absorbance at 254 nm. For indole-3-carbinol estimation, a gradient elution system was employed by using acetonitrile as mobile phase A and water-formic acid (99.9:0.1, v/v) as mobile phase B with absorbance at 280 nm. The details of the gradient method used were as follows: from 0 to 4 min, 30% mobile phase A; from 4 to 10 min, 50% mobile phase A; from 10 to 12 min, 30% mobile phase A; from 12 to 16 min, 30% mobile phase A. For the determination of sulforaphane, the following mobile phase gradient was used: mobile phase A: acetonitrile; mobile phase B: water-formic acid (99.9: 0.1, v/v) with absorbance at 254 nm. The gradient method used was as follows: from 0 to 4 min, 40% A; from 4 to 10 min, 70% A; from 10 to 12 min, 70% A; from 12 to 20 min, 40% A. Kaempferol, indole-3-carbinol, and sulforaphane standards were used for identification and quantification by making a comparison between RT (retention times) of unspecified peaks with specified standard, and outcomes were presented as µg/mg of DPE.

Statistical analysis

All analytical assays were repeated thrice and results were compiled as mean ± standard deviation (SD). The data across both consecutive years of the study were pooled (combined) to calculate the average. For determining the significance of the data, viz. results of various phytochemical parameters of Brassicaceae vegetable sample collected from HA and LA experimental fields, an independent t-test and two-way ANOVA were employed at a significance level of *** $p \leq 0.001$; ** $p \leq 0.01$ and * $p \leq 0.05$ and one-way ANOVA analysis with Duncan's multiple range tests ($p < 0.05$) was employed in SPSS 16.0 (SPSS Corporation, Chicago, IL)[8].

Ethical approval

There is no need of any ethics approval as this investigation was not related with any animal or human subject.

Plant guideline statement

Experimental research and field studies on plants cultivated, including the collection of plant material, complies with relevant institutional, national, and international guidelines and legislation.

Consent for publication

All authors have approved the manuscript and agree with its submission to Journal of *Scientific Reports*.

Result and discussion

Total phenolic content

Foods derived from plants are rich in polyphenolic compounds, which are effective antioxidants with a plethora of established health benefits, such as anti-inflammatory, anti-mutagenic, and free radical scavenging properties, etc.[8,22]. In the present study, as has been previously reported by Heimler et al.[23], presence of significant quantities of polyphenolic compounds was demonstrated in all the tested cruciferous vegetables samples grown under different conditions (Table 1). A noteworthy observation of the current investigation was the impact of different organic treatments (FYM and *Azotobacter* alone or in combination) and distinct altitudinal conditions (HA *vs* LA) on the phenolic content of cruciferous vegetables, namely cabbage, cauliflower, knol-khol, and radish. TPC varied from 4.18 to 9.56 µg of GAE mg/DPE. One-way ANOVA analysis indicated that treatment T_3 showed the highest response in all the different types of test vegetables (cabbage, cauliflower, knol-khol, and radish,). Similar trends were followed by T_2, T_1, and T_4, respectively. Notably, cabbage exhibited significantly higher TPC content in T_3 treatment at both locations. Furthermore, an independent t-test analysis for TPC content between the HA and LA locations demonstrated a significantly higher content in the HA compared to the LA region. Furthermore, a significant effect of interaction between altitude and treatments (ALT × TRE) was found in the TPC values of cabbage, knol-khol, and radish. The findings of the current study revealed that the T_3 treatment could maximally boost the TPC values of Brassicaceae vegetables grown at both locations. The higher content of TPC in the T_3 treatment is most likely due to the cooperative effect of organic manure and plant growth stimulating rhizobacteria (*Azotobacter*) in the biosynthesis that activates the acetate shikimate pathway, resulting in greater phenolics production. These findings are consistent with previous findings of higher TPC levels in organically grown cabbage[24], broccoli[25], and cauliflower[26]. Similarly, in another study carried out by Dutta et al.[27], the phenolic content in turmeric rhizomes was found to be increased when inoculated with rhizobacteria.

However, it is further noteworthy that despite similar treatments, HA-grown Brassicaceae vegetable samples showed a significantly higher boost in the TPC content than LA-grown vegetables. Plants at higher elevations are exposed to abiotic stresses like overwhelmingly intense UV-B radiation, which has a wide range of effects on plant growth, morphology, and physiology especially triggering different defensive mechanisms which also includes production of polyphenolic secondary metabolites[28,29]. There are few reports such as by Kumar et al.[8] where it found that extract of *Eruca sativa* samples from high altitude had more phenolic content as compared to low altitude samples. Thus, co-stimulation of plants with abiotic stresses along with organic practices might have lead to the observed rise of polyphenolic secondary metabolite composition. Similarly, Naguib et al.[25] have

ALT	TRE	Cabbage	Cauliflower	Knol-khol	Radish
HA	T_1	$7.61 \pm 0.08^{bC***}$	$7.19 \pm 0.04^{bB**}$	$6.87 \pm 0.14^{bA***}$	$8.05 \pm 0.09^{bD***}$
	T_2	8.32 ± 0.19^{cC}	$7.44 \pm 0.11^{bB**}$	$7.03 \pm 0.23^{bA***}$	$8.34 \pm 0.09^{cC***}$
	T_3	$9.56 \pm 0.15^{dC**}$	$8.68 \pm 0.20^{cB**}$	$7.97 \pm 0.27^{cA***}$	$8.96 \pm 0.16^{dB***}$
	T_4	$6.27 \pm 0.15^{aC**}$	$6.06 \pm 0.15^{aB*}$	$5.48 \pm 0.05^{aA***}$	$6.62 \pm 0.01^{aD***}$
LA	T_1	6.88 ± 0.13^{bD}	6.69 ± 0.11^{bC}	5.11 ± 0.04^{bA}	5.71 ± 0.03^{bB}
	T_2	8.27 ± 0.07^{cD}	6.96 ± 0.04^{cC}	5.42 ± 0.08^{cA}	5.81 ± 0.11^{bB}
	T_3	8.91 ± 0.03^{dD}	8.07 ± 0.11^{dC}	6.55 ± 0.01^{dA}	7.18 ± 0.07^{cB}
	T_4	5.73 ± 0.09^{aC}	5.73 ± 0.02^{aC}	4.18 ± 0.08^{aA}	4.63 ± 0.05^{aB}
ALT		***	***	***	***
TRE		***	***	***	***
ALT × TRE		***	NS	*	***

Table 1. Comparative effect of location and treatments on total phenolic content (µg GAE /mg of DPE) of Brassicaceae vegetables grown at HA versus LA. HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1 = FYM @ 150 q/ha, T_2 = *Azotobacter* @ 8.6 kg/ha, T_3 = FYM @ 150 q/ha + *Azotobacter* @ 8.6 kg/ha and T_4 = Control. ALT x TRE—interaction of altitude and treatment. TPC, Total polyphenolic content; GAE, Gallic acid equivalent; DPE, Dry powder extract. Values in columns different lowercase letters (small alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between treatments. Value in row, different uppercase letters (large alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between the crop. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments. Level of significance: *** $p \leq 0.001$; ** $p \leq 0.01$ and * $p \leq 0.05$, NS = not significant.

also reported that higher abiotic stress in organic farming increased the TPC content in organically grown *Brassica olaracea*, var. *italica*.

Total flavonoids content

Flavonoids are a sub-category of polyphenols that are highly advised in the nutritionist recommended health promoting diets due to their high efficiency as natural antioxidants as well as preventive and therapeutic properties[30]. The current investigation outlines the impact of different organic treatments on the flavonoid content of Brassicaceae vegetables, namely cabbage, cauliflower, knol-khol, and radish, cultivated at different altitudes. TFC varied from 6.96 to 14.48 µg of rutin trihydrate (RE) per milligram of dry powder extract (DPE) in the current study (Table 2). One-way ANOVA analysis revealed that the treatment T_3 maximally boosted the flavonoid contents also as it could increase the TPC levels in all the tested Brassicaceae vegetables (cabbage, cauliflower, knol-khol and radish). This trend was also followed by T_2, T_1, and T_4 treatment groups, respectively. Out of these, cabbage exhibited the highest increase in the TFC level in T_3 treatment at both locations. Overall, cultivation at HA regions supported significantly higher enrichment of TFC, as proved by an independent t-test analysis for TFC content between the HA and LA. A significant interaction between altitude and treatments (ALT × TRE) was found in the TFC of cabbage, cauliflower, and radish ($p < 0.05$). Similar to the TPC levels, the observed higher content of TFC in the T_3 treatment can be explained by cooperative effect of FYM and *Azotobacter* treatments in the activation of acetate shikimate biosynthetic pathway[25,27]. These findings are consistent with observations made by earlier researchers where TPC and TFC levels were found to increase with the supplementation of bio-organic fertilizer to cultivated *Brassica oleracea* var. *capitata*[23], *Brassica oleracea* var. *italica*[25] and *Brassica oleracea* var. *botrytis*[26].

However, as discussed earlier in the manuscript, the key findings of the study demonstrate that HA samples possess significantly higher TFC values than LA samples. Since these secondary metabolites function as part of a plant's defense mechanisms against abiotic stressors like UV radiations, their raised levels in HA-grown plants are well justified[31]. Our findings are in accordance with the earlier research conducted over a flora of Brassicaceae family (*E. sativa*), Where higher secondary metabolites content was found at HA in comparison to LA[8] This strategy to boost TFC levels in organically grown vegetables may prove to be a boon to growing anti-oxidant-rich vegetables at HA for local consumption under extreme altitudes that possess a tremendous threat to human health.

Antioxidant activity

The antioxidant activity of naturally occurring bioactive phytochemicals has been attributed to numerous mechanisms of action, including hydrogen atom transfer, single electron transfer, and their ability to bind transition metals[8,32]. The dietary resource provides an enrichment of a variety of phytochemicals with distinct phenolic groups acting through their unique modes of action in synergistically enhancing the free radical scavengers, crucial in reducing ROS load of the human body[33]. In order to assess the anti-oxidant potentials of LA and HA-grown Brassicaceae vegetables, a combination of two different assays were deployed, *i.e.* DPPH and FRAP,

ALT	TRE	Cabbage	Cauliflower	Knol-khol	Radish
HA	T_1	$11.95 \pm 0.12^{bD***}$	$10.37 \pm 0.04^{bC***}$	$9.10 \pm 0.07^{bB***}$	$8.68 \pm 0.02^{bA***}$
	T_2	$12.55 \pm 0.12^{cD***}$	$10.94 \pm 0.02^{cC***}$	$9.42 \pm 0.02^{cB***}$	$9.15 \pm 0.02^{cA***}$
	T_3	$14.48 \pm 0.41^{dD***}$	$12.34 \pm 0.10^{dC***}$	$10.65 \pm 0.05^{dB***}$	$9.88 \pm 0.17^{dA**}$
	T_4	$9.56 \pm 0.19^{aD***}$	$9.06 \pm 0.03^{aC***}$	$7.99 \pm 0.07^{aB***}$	$7.48 \pm 0.07^{aA***}$
LA	T_1	9.41 ± 0.15^{bC}	9.35 ± 0.04^{bC}	8.43 ± 0.12^{bB}	8.23 ± 0.05^{bA}
	T_2	9.74 ± 0.03^{cD}	9.54 ± 0.04^{cC}	8.77 ± 0.09^{cB}	8.40 ± 0.04^{cA}
	T_3	10.85 ± 0.03^{dD}	10.52 ± 0.03^{dC}	9.86 ± 0.13^{dB}	9.14 ± 0.05^{dA}
	T_4	7.98 ± 0.16^{aD}	8.45 ± 0.02^{aC}	7.32 ± 0.05^{aB}	6.96 ± 0.09^{aA}
ALT		***	***	***	***
TRE		***	***	***	***
ALT × TRE		***	***	NS	**

Table 2. Comparative effect of location and treatments on total flavonoid content (µg RE/mg of DPE) of Brassicaceae vegetables grown at HA versus, LA. HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1 = FYM @ 150 q/ha, T_2 = *Azotobacter* @ 8.6 kg/ha, T_3 = FYM @ 150 q/ha + *Azotobacter* @ 8.6 kg/ha and T_4 = Control. ALT x TRE—interaction of altitude and treatment. DPE, Dry powder extract; TFC, Total flavonoid content; RE, Rutin trihydrate equivalent. Values in columns different lowercase letters (small alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between treatments. Value in row, different uppercase letters (large alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between the crop. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments. Level of significance: *** $p \leq 0.001$; ** $p \leq 0.01$ and * $p \leq 0.05$, NS = not significant.

since the full antioxidant potential of a sample cannot be determined by a single experiment due to different mechanisms of actions of different anti-oxidant compounds[34].

The DPPH assay detects the presence of anti-oxidant compounds which reduce the ROS burden via the mechanism of electron transfer[35]. Thus, DPPH assay was deployed to assess the effect of organic treatments on the free radical scavenging efficacy of various Brassicaceae vegetable samples at different altitudes (Table 3). In the present study, the DPPH scavenging activity varied from 24.74 to 85.97%. As per expectation, higher TPC and TFC levels of T_3 correspond to the highest DPPH assay based anti-oxidant activity among all the treatments of vegetable samples. The observation of higher anti-oxidant activities in T_3 plants despite similar growth conditions as T_1, T_2, and T_4 treated plants hints towards a synergistic effect of FYM and *Azotobacter* on secondary metabolites synthesis and their agglomeration. Further, T_3 treatment of HA demonstrated higher antioxidant

ALT	TRE	Cabbage	Cauliflower	Knol-khol	Radish
HA	T_1	$81.06 \pm 0.62^{bD**}$	$79.52 \pm 0.34^{bC***}$	$65.99 \pm 0.38^{bB***}$	$53.80 \pm 0.34^{bA***}$
	T_2	$82.80 \pm 0.22^{cD*}$	$80.95 \pm 0.30^{cC***}$	$67.48 \pm 0.65^{cB***}$	$55.00 \pm 0.20^{cA***}$
	T_3	$85.97 \pm 0.24^{dD***}$	$85.49 \pm 0.20^{dC***}$	$71.61 \pm 0.26^{dB***}$	$59.68 \pm 0.24^{dA***}$
	T_4	$65.35 \pm 0.25^{aC***}$	$67.18 \pm 0.24^{aD***}$	$61.62 \pm 0.23^{aB***}$	$32.90 \pm 0.22^{aA***}$
LA	T_1	78.77 ± 0.58^{bD}	64.89 ± 0.27^{bC}	60.10 ± 0.25^{bB}	34.64 ± 0.06^{bA}
	T_2	80.56 ± 0.85^{cD}	65.55 ± 0.80^{bC}	61.13 ± 0.23^{cB}	35.60 ± 0.51^{cA}
	T_3	82.70 ± 0.50^{dD}	70.25 ± 0.60^{cC}	64.15 ± 0.47^{dB}	38.71 ± 0.39^{dA}
	T_4	62.23 ± 0.45^{aD}	61.26 ± 0.15^{aC}	55.82 ± 0.24^{aB}	24.74 ± 0.33^{aA}
ALT		***	***	***	***
TRE		***	***	***	***
ALT × TRE		NS	***	**	***

Table 3. Comparative effect of location and treatments on DPPH content (% inhibition) of Brassicaceae vegetables grown at HA versus LA. HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1 = FYM @150 q/ha, T_2 = *Azotobacter* @ 8.6 kg/ha, T_3 = FYM @ 150 q/ha + *Azotobacter* @ 8.6 kg/ha and T_4 = Control. ALT x TRE—interaction of altitude and treatment. DPE: Dry powder extract; DPPH: 2, 2-diphenyl-1-picrylhydrazyl assay. Values in columns different lowercase letters (small alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between treatments. Value in row, different uppercase letters (large alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between the crop. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments. Level of significance: *** $p \leq 0.001$; ** $p \leq 0.01$ and * $p \leq 0.05$, NS = not significant.

ALT	TRE	Cabbage	Cauliflower	Knol-khol	Radish
HA	T_1	$25.41 \pm 0.24^{bD***}$	$22.44 \pm 0.21^{bC***}$	$17.98 \pm 0.32^{bB***}$	$15.19 \pm 0.08^{bA***}$
	T_2	$26.85 \pm 0.34^{cD***}$	$24.16 \pm 0.16^{cC***}$	$19.02 \pm 0.59^{cB*}$	$16.13 \pm 0.35^{cA***}$
	T_3	$30.77 \pm 0.46^{dD***}$	$27.34 \pm 0.14^{dC***}$	$20.58 \pm 0.19^{dB*}$	$18.12 \pm 0.13^{dA***}$
	T_4	$20.67 \pm 0.52^{aD***}$	$19.57 \pm 0.06^{aC***}$	$15.03 \pm 0.07^{aB***}$	$11.12 \pm 0.23^{aA***}$
LA	T_1	21.82 ± 0.13^{bD}	18.20 ± 0.16^{bC}	15.95 ± 0.06^{bB}	10.72 ± 0.22^{bA}
	T_2	22.90 ± 0.65^{cD}	19.13 ± 0.18^{cC}	17.08 ± 0.47^{cB}	11.54 ± 0.32^{cA}
	T_3	25.01 ± 0.28^{dD}	22.91 ± 0.25^{dC}	18.79 ± 0.70^{dB}	13.62 ± 0.25^{dA}
	T_4	16.75 ± 0.19^{aD}	16.27 ± 0.17^{aC}	13.09 ± 0.38^{aB}	8.61 ± 0.12^{aA}
ALT		***	***	***	***
TRE		***	***	***	***
ALT × TRE		***	***	NS	***

Table 4. Comparative effect of location and treatments on FRAP (µg TE/mg of DPE) content of Brassicaceae vegetables grown at HA versus LA. HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1 = FYM @ 150 q/ha, T_2 = *Azotobacter* @ 8.6 kg/ha, T_3 = FYM @ 150 q/ha + *Azotobacter* @ 8.6 kg/ha and T_4 = Control. ALT x TRE—interaction of altitude and treatment. DPE: Dry powder extract; TE: Trolox equivalent; FRAP: Ferric reducing antioxidant power assay. Values in columns different lowercase letters (small alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between treatments. Value in row, different uppercase letters (large alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between the crop. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments. Level of significance: *** $p \leq 0.001$; ** $p \leq 0.01$ and * $p \leq 0.05$, NS = not significant.

	TPC	TFC	FRAP	DPPH
High-altitude				
TPC	1	.617*	0.56	0.275
TFC		1	.971**	.869**
FRAP			1	.943**
DPPH				1
Low-altitude				
TPC	1	.782**	.761**	.661*
TFC		1	.987**	.967**
FRAP			1	.976**
DPPH				1

Table 5. Correlation between TPC, TFC, FRAP, and DPPH. TPC, Total polyphenolic content; TFC, Total flavonoid content; FRAP, Ferric reducing antioxidant power assay; DPPH, 2, 2-diphenyl-1-picrylhydrazyl assay. **Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed).

activity in comparison to LA which may be due to the higher accumulation of secondary metabolites under abiotic stresses of HA.

Amongst all these Brassicaceae vegetables, cabbage exhibited a significantly higher DPPH response in T_3 treatment at HA which also justify the positive correlation of antioxidant activity with TPC and TFC (Table 5). Similar correlation was found in *T. foliolosum* and *E. sativa* between secondary metabolites and their antioxidant activity[8,36]. Plant growth-promoting rhizobacteria (PGPR) are responsible for inducing wide spectrum of systemic resistance via triggering the expression of a battery of genes and pathways to upregulate the accumulation of diverse bioactive molecules[37]. These findings are consistent with observations made by authors where the application of PGPR enhanced the antioxidant capacity of *B. olaracea* L. var. *italica*[24] and *Glycine max*[38].

Further, the FRAP test was another anti-oxidant assay deployed to determine specific antioxidants that could reduce Fe^{3+}-TPTZ (ferric tripyridyltriazine) into Fe^{2+}-TPTZ (ferrous tripyridyltriazine)[17]. The production of the ferrous complex (Fe^{2+}-TPTZ) is estimated as the development of the blue-colored complex after reaction incubation[17,39]. Plant extracts with a higher reducing capacity are interpreted as having a higher concentration of antioxidant component[40]. The effect of organic treatments and altitudinal conditions on FRAP assay of various Brassicaceae vegetable samples is shown in Table 4. FRAP assay results were found to vary from 8.61 to 30.77 µg of TE/mg of DPE. On performing a one-way ANOVA analysis, it was found that the T_3 treatment showed a significantly higher response with respect to all other treatments. Further, an independent t-test analysis for

FRAP content between the HA and LA locations demonstrated a significantly higher content in the HA region compared to the LA region. Additionally, cabbage exhibited significantly higher FRAP content in T_3 treatment at both the locations. A significant interaction between altitude and treatments (ALT × TRE) was found in the FRAP content of cabbage, cauliflower, and radish ($p < 0.001$). All the above results and correlation analysis (Table 5) indicate that phenolic compounds are as strong contributors for ferric ion chelating activity as they were to DPPH scavenging activity. The study is in strong agreement with the results reported on *E. sativa* and *Onosma riedliana* where a similar relation was found[8,18].

Effect of different treatments on signature phytochemical compounds

RP-HPLC, which is a reliable and popular chromatographic method for quantifying secondary metabolites in plants[8], was deployed to develop a comparative profile of secondary metabolites from Brassicaceae plants grown at different altitudes (HA *vs.* LA). The linear regression equations: $y = 79691x - 28{,}706$, $R^2 = 0.99$, $y = 32887x + 65{,}956$, $R^2 = 0.99$ and $y = 4105x + 27{,}823$, $R^2 = 0.99$ were used to calculate the concentration of signature phyto-compounds in Brassicaceae vegetable extracts, for kaempferol (0.122–1000 µg/mL), indole-3-carbinol (0.244–1000 µg/mL) and sulforaphane (7.81–1000 µg/mL) respectively (Table 6,7 & 8).

Kaempferol is an important signature compound of Brassicaceae family that is known for its anti-cancerous, anti-arthritis, and anti-diabetic properties[19]. The variations in its levels following various organic treatments and also altitudinal conditions were assessed in the present study (Table 6 and Fig. 1). Its levels were found to vary from 0.18 to 0.92 µg/mg of DPE among various test samples. Since kaempferol is a natural flavonol, *i.e.* a type of flavonoid, changes in its levels following different treatments showed trends similar to that of TFC levels. Cabbage exhibited the highest kaempferol content in T_3 treatment at both locations. A statistically significant correlation was observed between HA and a boost in kaempferol content in Brassicaceae vegetables. Also, altitude and treatments (ALT × TRE) was found to positively interact with kaempferol contents of cabbage, cauliflower, and radish ($p < 0.001$).

Similarly, the content of another signature compound of Brassicaceae, *i.e.* indole-3-carbinol, was assessed with respect to various organic treatments and altitudinal conditions (Table 7 and Fig. 2). The indole-3-carbinol concentration was found to vary from 0.11 to 1.31 µg/mg of DPE. The treatment T_3 resulted in maximum accumulation of indole-3-carbinol content in all the Brassicaceae vegetables showing significantly higher contents at HA. Cauliflower showed maximum accumulation of this phytochemical compound in comparison to other tested vegetables. With respect to altitude and interactions with different bio-organic treatments (ALT × TRE) showed similar trends like kaempferol ($p < 0.05$ and $p < 0.001$).

In addition to this, the vegetable samples were subjected to quantification of another very important signature compound of Brassicaceae vegetables, *i.e.* sulforaphane, which is a sulfur-containing secondary metabolite belonging to isothiocyanates group known for lowering blood pressure, reducing cholesterol levels, and enhancing blood vessel function[21]. Its concentration varied from 0.88 to 8.94 µg/mg of DPE in various test samples (Table 8 and Fig. 3). Among all the studied Brassicaceae vegetables, cabbage showed maximum accumulation of sulforaphane under test conditions. Rest all trends were similar to those obtained for indole-3-carbinol and kaempferol.

Overall, the application of treatment T_3 (*i.e.* co-treatment of FYM and *Azotobacter*) significantly increased the concentration of all the three tested glucosinolates (*i.e.* kaempferol, indole-3-carbinol and sulforaphane) at

ALT	TRE	Cabbage	Cauliflower	Radish	Knol-khol
HA	T_1	0.26 ± 0.01^{bA}	$0.26 \pm 0.00^{bA**}$	$0.47 \pm 0.01^{bB***}$	ND
	T_2	$0.35 \pm 0.01^{cA*}$	$0.34 \pm 0.01^{cA***}$	$0.46 \pm 0.01^{bB***}$	
	T_3	$0.92 \pm 0.02^{dC*}$	$0.81 \pm 0.01^{dB***}$	$0.73 \pm 0.01^{cA***}$	
	T_4	$0.21 \pm 0.00^{aC*}$	$0.22 \pm 0.00^{aB***}$	$0.29 \pm 0.01^{aA***}$	
LA	T_1	0.25 ± 0.01^{bB}	0.24 ± 0.00^{bA}	0.25 ± 0.00^{bB}	ND
	T_2	0.33 ± 0.01^{cA}	0.27 ± 0.01^{cB}	0.24 ± 0.01^{bC}	
	T_3	0.66 ± 0.01^{dA}	0.59 ± 0.02^{dB}	0.32 ± 0.01^{cC}	
	T_4	0.19 ± 0.01^{aA}	0.18 ± 0.01^{aA}	0.18 ± 0.01^{aA}	
ALT		***	***	***	
TRE		***	***	***	
ALT × TRE		***	***	***	

Table 6. Comparative effect of location and treatments on kaempferol content (µg/mg of DPE) of Brassicaceae vegetables grown at HA versus LA. HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1 = FYM @ 150 q/ha, T_2 = *Azotobacter* @ 8.6 kg/ha, T_3 = FYM @ 150 q/ha + *Azotobacter* @ 8.6 kg/ha and T_4 = Control. ALT x TRE—interaction of altitude and treatment. DPE: Dry powder extract. Values in columns different lowercase letters (small alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between treatments. Value in row, different uppercase letters (large alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between the crop. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments. Level of significance: *** $p \leq 0.001$; ** $p \leq 0.01$ and * $p \leq 0.05$, ND = not detect.

ALT	TRE	Cabbage	Cauliflower	Knol-khol	Radish
HA	T_1	$0.44 \pm 0.01^{bA***}$	$1.03 \pm 0.02^{bC***}$	$0.42 \pm 0.01^{bA***}$	$0.56 \pm 0.02^{bB*}$
	T_2	$0.45 \pm 0.02^{bA**}$	$1.08 \pm 0.04^{cC***}$	$0.64 \pm 0.02^{cB***}$	$0.69 \pm 0.04^{cB**}$
	T_3	$0.65 \pm 0.02^{cA***}$	$1.31 \pm 0.01^{dD***}$	$0.91 \pm 0.02^{dB***}$	$1.01 \pm 0.03^{dC***}$
	T_4	$0.26 \pm 0.01^{aB***}$	$0.22 \pm 0.02^{aA**}$	$0.24 \pm 0.00^{aAB***}$	$0.36 \pm 0.02^{aC***}$
LA	T_1	0.31 ± 0.01^{bB}	0.22 ± 0.01^{bA}	0.30 ± 0.01^{bB}	0.50 ± 0.02^{bC}
	T_2	0.34 ± 0.02^{cA}	0.34 ± 0.01^{cA}	0.50 ± 0.02^{cB}	0.61 ± 0.02^{cC}
	T_3	0.52 ± 0.00^{dB}	0.40 ± 0.01^{dA}	0.74 ± 0.01^{dC}	0.85 ± 0.02^{dD}
	T_4	0.11 ± 0.01^{aA}	0.13 ± 0.01^{aB}	0.18 ± 0.01^{aC}	0.24 ± 0.01^{aD}
ALT		***	***	***	***
TRE		***	***	***	***
ALT × TRE		*	***	***	***

Table 7. Comparative effect of location and treatments on indole-3-carbinol (µg/mg of DPE) content of Brassicaceae vegetables grown at HA versus LA. HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1 = FYM @ 150 q/ha, T_2 = *Azotobacter* @ 8.6 kg/ha, T_3 = FYM @ 150 q/ha + *Azotobacter* @ 8.6 kg/ha and T_4 = Control. ALT x TRE—interaction of altitude and treatment. DPE: Dry powder extract. Values in columns different lowercase letters (small alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between treatments. Value in row, different uppercase letters (large alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between the crop. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments. Level of significance: *** $p \leq 0.001$; ** $p \leq 0.01$ and * $p \leq 0.05$.

ALT	TRE	Cabbage	Cauliflower	Knol-khol	Radish
HA	T_1	$2.47 \pm 0.05^{bB***}$	$2.74 \pm 0.10^{bC**}$	$2.12 \pm 0.11^{bA**}$	$2.50 \pm 0.10^{bB**}$
	T_2	$3.06 \pm 0.06^{cC***}$	$3.47 \pm 0.02^{cD**}$	$1.95 \pm 0.06^{bA*}$	$2.46 \pm 0.02^{bB***}$
	T_3	$8.94 \pm 0.24^{dD***}$	$4.11 \pm 0.02^{dB***}$	$3.24 \pm 0.06^{cA**}$	$4.48 \pm 0.04^{cC***}$
	T_4	$2.04 \pm 0.07^{aB***}$	1.62 ± 0.07^{aA}	$1.43 \pm 0.23^{aA*}$	$1.61 \pm 0.03^{aA**}$
LA	T_1	2.05 ± 0.04^{bB}	2.23 ± 0.12^{bC}	1.72 ± 0.03^{bA}	1.99 ± 0.07^{bB}
	T_2	1.97 ± 0.09^{bB}	2.98 ± 0.13^{cC}	1.78 ± 0.06^{bA}	1.99 ± 0.09^{bB}
	T_3	4.16 ± 0.05^{cD}	3.50 ± 0.04^{dC}	2.93 ± 0.05^{cA}	3.08 ± 0.11^{cB}
	T_4	1.00 ± 0.07^{aA}	1.58 ± 0.07^{aC}	0.88 ± 0.08^{aA}	1.16 ± 0.10^{aB}
ALT		***	***	***	***
TRE		***	***	***	***
ALT × TRE		***	***	*	***

Table 8. Comparative effect of location and treatments on sulforaphane (µg/mg of DPE) content of Brassicaceae vegetables grown at HA versus LA. HA- high altitude and LA- low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1 = FYM @ 150 q/ha, T_2 = *Azotobacter* @ 8.6 kg/ha, T_3 = FYM @ 150 q/ha + *Azotobacter* @ 8.6 kg/ha and T_4 = Control. ALT x TRE—interaction of altitude and treatment. DPE: Dry powder extract. Values in columns different lowercase letters (small alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between treatments. Value in row, different uppercase letters (large alphabet) indicate significantly different; $p < 0.05$, Duncan's multiple range test between the crop. Mean values in each column (between group) showed significantly different by independent t-test. Two-way ANOVA was applied to visualize the relationship between altitude and treatments. Level of significance: *** $p \leq 0.001$; ** $p \leq 0.01$ and * $p \leq 0.05$.

both the altitudinal locations. Although these compounds have been earlier reported in Brassicaceae vegetables, the novel finding of our study is that their accumulation is significantly boosted in the HA-grown Brassicaceae vegetables[19,21]. Though the plants synthesize these protective secondary metabolites as part of their defense mechanism under harsh environmental conditions such as extreme temperature, drought, salt, radiation, etc., their dietary enrichment is highly recommended due to their disease-preventing and health-promoting activities in humans. These secondary metabolites are extremely effective in neutralizing reactive oxygen species, thus their regular consumption is linked with reduced incidences of oxidative damage and various inflammatory diseases, including coronary heart disease[41]. At higher elevations, consumption of a diet especially enriched in bioactive phytochemicals is highly recommended to offer protection against highly ionizing environmental conditions. Thus the present study could shed light on effective means to locally produce health-promoting Brassicaceae vegetables at higher elevations using bio-organic techniques.

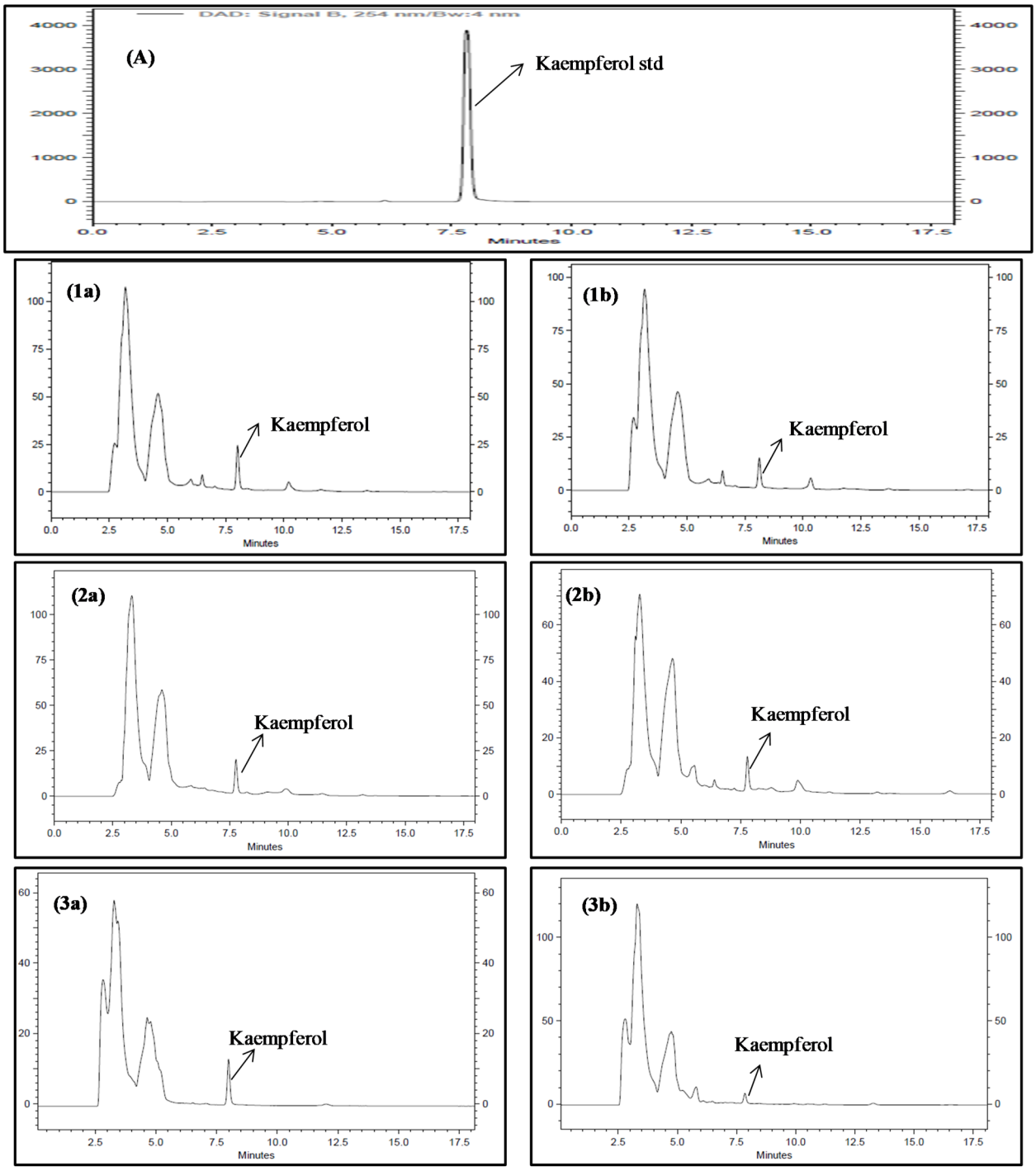

Figure 1. RP-HPLC chromatogram of Brassicaceae vegetables (**A**) Standard peak of kaempferol (**1a**) cabbage: HA, (**1b**) cabbage: LA, (**2a**) cauliflower: HA, (**2b**) cauliflower: LA, (**3a**) radish: HA, (**3b**) radish: LA. HA = High altitude and LA = Low altitude.

Conclusion

The potential of biofertilizers is currently being seriously explored globally as a strategy to reduce the usage of their chemical counterpart and develop an eco-friendly alternative to ensure the nutri-health security of the consumers. The current study has demonstrated that under extreme environmental condition of HA regions, the application of FYM and *Azotobacter* may have a significant impact on the bioactive phytochemical synthesis and accumulation in Brassicaceae vegetables viz. cauliflower, cabbage, knol-khol, and radish. The most important finding of the present study is the collaborative effect of FYM and *Azotobacter* (T_3 treatment) at HA which could

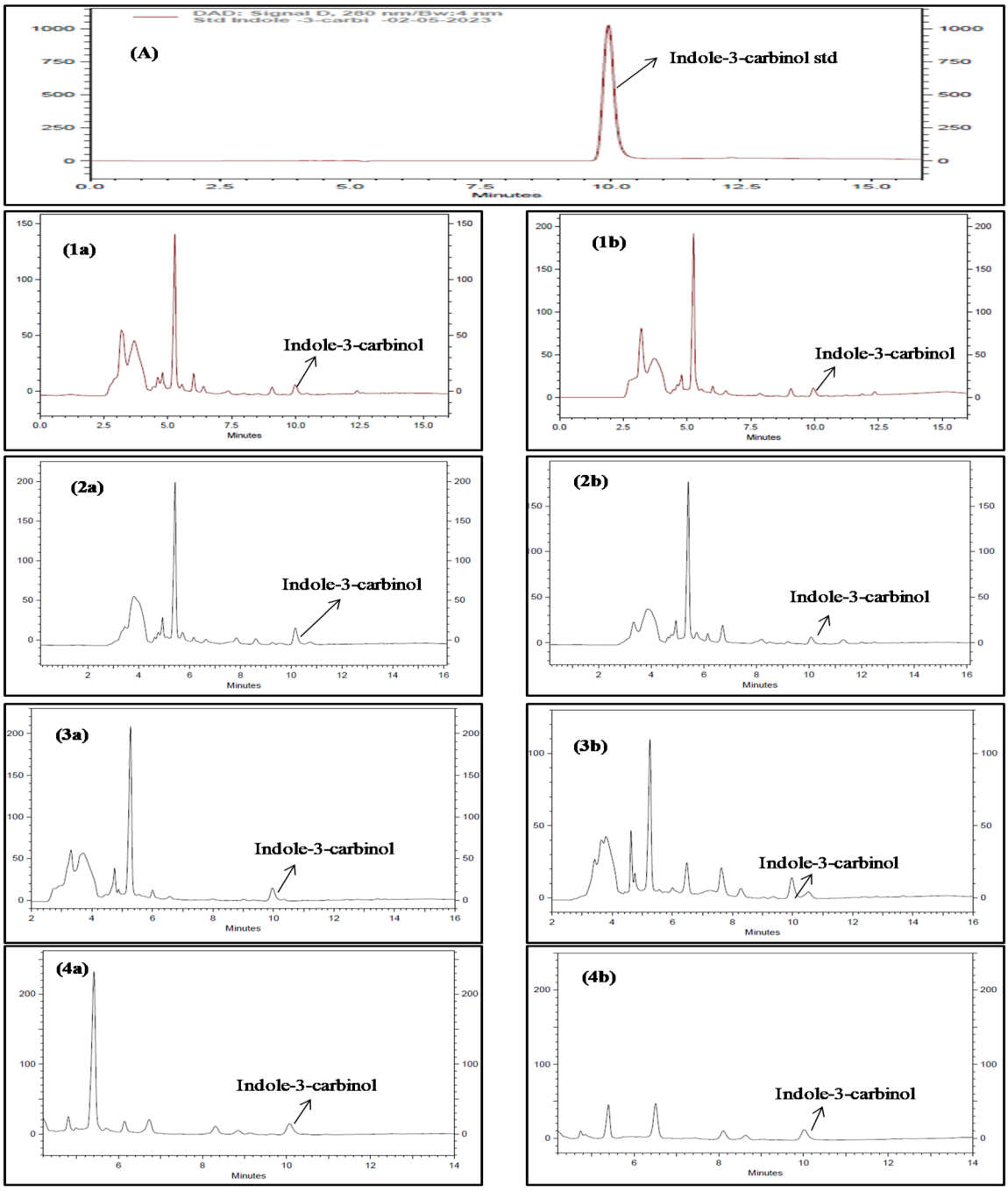

Figure 2. RP-HPLC chromatogram of Brassicaceae vegetables (**A**) Standard peak of indole-3-carbinol (**1a**) cabbage: HA, (**1b**) cabbage: LA, (**2a**) cauliflower: HA, (**2b**) cauliflower: LA, (**3a**) knol-khol: HA, (**3b**) knol-khol: LA, (**4a**) radish: HA, (**4b**) radish: LA. HA = High altitude and LA = Low altitude.

lead to the extensive enrichment of bioactive phytocompounds as demonstrated by the HPLC analysis where the quantified glucosinolates (kaempferol, indole-3-carbinol, and sulforaphane) were significantly higher in HA than in LA samples. Similarly, HA-grown Brassicaceae vegetables were found to have higher TPC and TFC values which corroborated with their higher antioxidant potential, in comparison to LA-grown vegetables. A significant correlation was found between TPC, TFC, DPPH, and FRAP assays. Therefore, by means of this study, organic manure combined with biofertilizer is being recommended to grow health promoting Brassicaceae vegetables enriched with specific glucosinolates and other anti-oxidant phytocompounds for local consumption at high altitudes. Further research could be conducted to study the effect of these bio-organic on phytocomponents profile of other families *i.e.*, Solanaceae, Cucurbitaceae and Fabaceae at high altitudes.

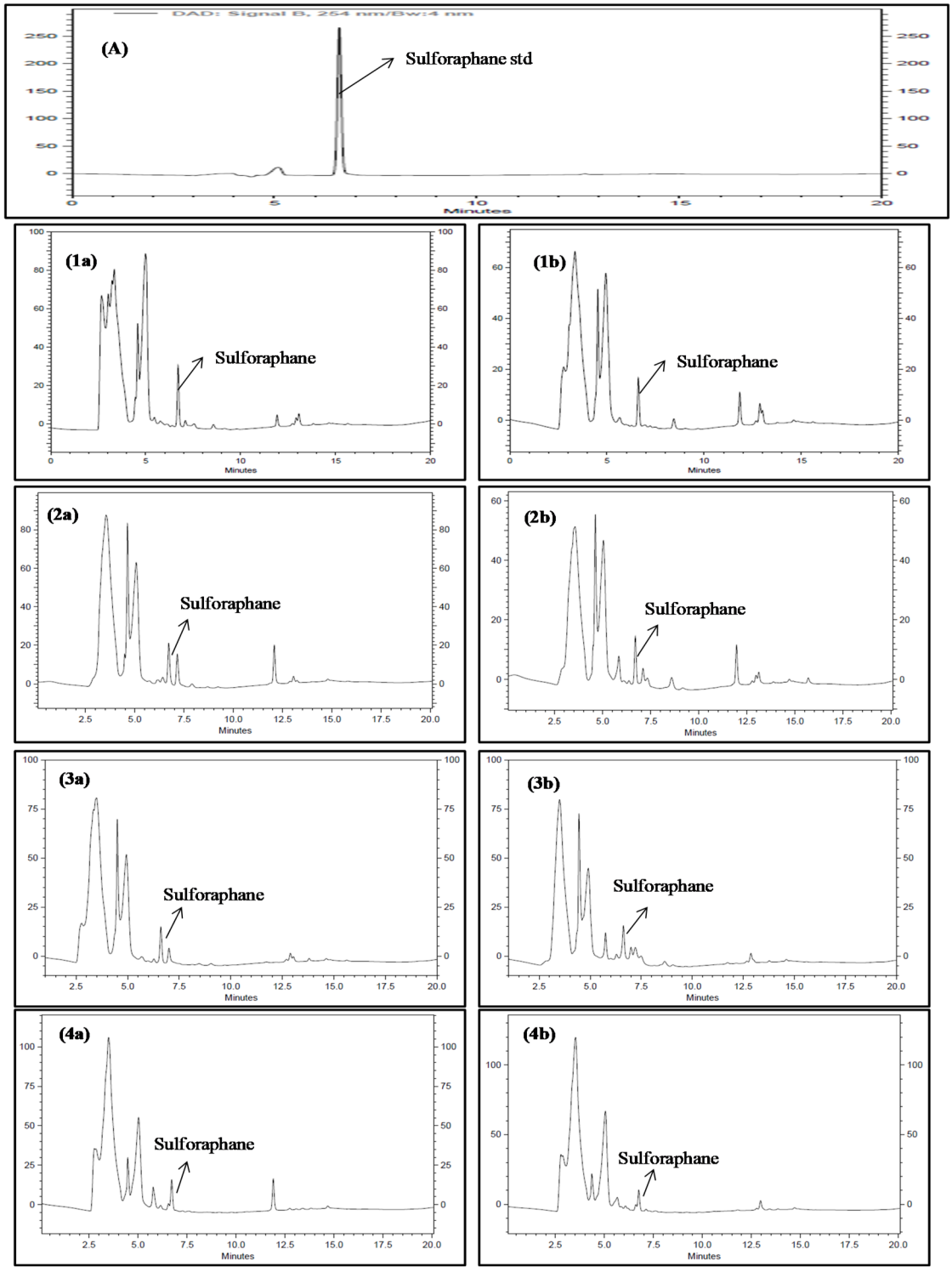

Figure 3. RP-HPLC chromatogram of Brassicaceae vegetables (**A**) Standard peak of sulforaphane, (**1a**) cabbage: HA, (**1b**) cabbage: LA, (**2a**) cauliflower: HA, (**2b**) cauliflower: LA, (**3a**) knol-khol: HA, (**3b**) knol-khol: LA, (**4a**) radish: HA, (**4b**) radish: LA. HA = High altitude and LA = Low altitude.

Data availability

All data supporting the findings of this study are available within the paper.

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Author contributions
S.S.: original draft preparation and carried out experiments, N.K., and P.B.: methodology, experimentation and data analysis, P.P.; Editing the Manuscript, M.K.P., & M.S.T.: help in collection of sample, R.K.; supervised in HPLC analysis, M.R.: supervised the work and edited the manuscript, S.S.: Study conceptualization and monitoring, overall supervision, guidance and manuscript correction and editing. All authors have seen the draft copy and approved the final version of manuscript.

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Competing interests
The authors declare no competing interests.

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Comparative Effect of FYM and *Azotobacter* on Morphology and Nutritional Quality of High and Low Altitude Grown Knol-khol (*Brassica oleracea* var. *Gongylodes* L.) Cultivar White Vienna

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ABSTRACT

High-Altitude (HA) environments pose unique challenges to crop cultivation due to extreme abiotic stresses. Organic agricultural treatments offer promising solutions to address these challenges and enhance crop performance and nutritional quality. Herein, we examine the comparative effects of Farm Yard Manure (FYM) and *Azotobacter* treatments on knol-khol at high *vs.* Lower Altitudes (LA), aiming to enhance resilience and nutritional value across varying altitudes. The field trial was conducted using a randomised block design as a two-factorial experiment. The first factor was treatments (T_1-FYM, T_2-*Azotobacter*, T_3-FYM+*Azotobacter*, and T_4-(control), and the second factor was cultivation locations (HA *vs.* LA). The findings revealed that the application of treatment T_3 at HA resulted in higher total soluble solids (1.38-fold), titratable acidity (0.06-fold), total carbohydrate (1.9-fold), crude protein (3.7-fold), crude fat (3-fold) and dietary fiber content (78-fold) whereas, yield (137.6-fold) and ash content (0.85-fold) content were found higher at LA. The current study emphasises the superior efficiency of the combination treatment of FYM and *Azotobacter* at HA to improve the nutritional quality of food crops compared to LA, with the added benefits of environmental sustainability and nutritional security.

Keywords: FYM; Azotobacter; Knol-Khol; Sustainable production; Nutritional Quality

1. INTRODUCTION

In recent decades, the world's population has increased exponentially, necessitating a more excellent supply of high-quality food. This requirement has been primarily met by using chemical fertilisers. Unfortunately, this practice led to a collapse in food nutritional value and a rise in various health problems[1]. Consequently, there is an increased focus on investigating agricultural practices to improve the nutritional profiles of food crops under different environmental conditions, such as high altitudes. Although, in general, research studies have established that the most efficient way to accomplish nutritional goals in food crops is through bio-organic farming, whether the same applies to high-altitude environments is a matter of intense investigation[2]. Plants that thrive in high-altitude regions are subject to various environmental factors, including temperature, humidity, daylight hours, UV radiation, wind, geology, and air pressure[3]. These abiotic stressful environmental variables majorly impact a plant's morphological and nutritional components[4]. In the Indian sub-continent, HA locations such as Leh-Ladakh have a huge unmet demand for fresh veggies due to the adversities of agriculture practices under a cold, arid climate, especially during long-harsh winters, which restrict the farming season for 4-5 months in a year. This region, which has limited resources, primarily relies on supplies from plain areas, particularly Chandigarh. However, these locations face many transportation-related challenges, which lead to inadequate nutritional quality in the food[5]. Hence, there is a need to establish a cultivation practice at HA that could achieve adequate production and nutritional composition compared to LA. Bio-organic inputs such as Farm Yard Manure (FYM) and biofertilisers (*Azotobacter)* are known to not only reduce dependency on chemical fertilisers but also provide plants with all of the nutrients needed for growth[6,7]. However, the suitability of its application in extreme environmental conditions such as high mountainous regions remains largely unexplored.

Knol-khol (*Brassica oleracea* var. *gongylodes* L.), also known as kohl rabi, belonging to the Brassicaceae family, is one of the most consumed vegetables in high altitude region of Ladakh because of its distinct flavor (mild, sweet, slightly peppery) and crunchy texture, similar to that of a broccoli stem or a radish[8]. It is

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cultivated for its edible swollen stem, although its leaves are consumed and used in various culinary preparations[9]. Nutritionally, cole crops and vegetables are an excellent source of nutritional value, as are dietary fiber, minerals, and vitamins, including A, B, and C, and low levels of calories and fat[10,11]. Due to its richness in fiber, nutritive value, and health benefits, knol-khol is one of the most popular and consumed vegetables in the HA region of Ladakh. Thus, it would be worth exploring and establishing suitable organic practices for growing nutritionally rich and chemical-free knol-khol crops at HA. This emphasises the necessity and importance of in-depth comparative research on the effect of FYM and *Azotobacter* at high and low altitude field locations, using knol-khol as a model crop.

Hence, the current study explores environment-friendly agricultural interventions to foster the growth of plentiful, nutritionally rich food crops in challenging environmental conditions of HA that could be comparable to LA cultivation. The study covers a detailed comparative analysis of the morphological and nutritional attributes of knol-khol cultivated in high-altitude Leh, India, versus low-altitude Chandigarh, India, with a specific focus on the effects of organic biofertilisers such as FYM and *Azotobacter*.

2 MATERIAL AND METHODS

2.1 Field Trial and Site

The field experiment was conducted over two consecutive years, namely 2020-2021 and 2021-22, in open fields at both high-altitude and low-altitude locations. The high altitude trials took place at the Agriculture Research Unit (ARU), Defence Institute of High Altitude Research (Leh), India, situated at an elevation of 3340 meters (34008.2'N; 77034.3'E). Meanwhile, the low altitude trials were carried out at the Defence Institute of High Altitude Research base lab in Chandigarh, India, located at 30º41′31″ N and 76º47′10″ E, at an altitude of 321 meters above Mean Sea Level (MSL). Temperature and relative humidity were recorded daily at both locations using a hygro-thermometer (445,702, Extech Instruments). The average mean maximum and minimum temperature at high altitudes was 25.74±2.03 and 4.40±1.07 °C. Whereas at low altitudes was 33.34±1.65 and 10.64±1.90 °C, respectively. The maximum relative humidity was (59.77±3.10 %) and (87.11±3.22%) at HA and LA, respectively, While minimum relative humidity was recorded (14.24±2.40 %) and (62.26±1.15 %), respectively. The fertility of the HA and LA soils is examined before transplanting (Table 1).

Table 1. Soil fertility status before Transplanting

Parameters	HA	LA
pH	7.76 ± 0.17	8.63 ± 0.49
EC (ms/cm)	0.76 ± 0.28***	0.37 ± 0.02
OC (%)	0.79 ± 0.01***	0.31 ± 0.01
N (Kg/ha)	35.24 ± 1.24***	22.28 ± 5.54
P (Kg/ha)	12.42± 0.13*	10.24 ± 0.71
K (Kg/ha)	180.32 ± 5.31	196.42 ± 3.07**
S (mg/Kg)	128.41 ± 6.81	126.34 ± 5.20
Zn (mg/Kg)	5.23 ± 0.84	8.53 ± 0.72***
Fe (mg/Kg)	3.12 ± 0.41	6.38 ± 1.06***
Cu (mg/Kg)	5.02 ± 0.07	7.23 ± 0.94***
Mn (mg/Kg)	3.48 ± 0.19***	1.53 ± 0.25

Mean values in each row (between group) showed significantly different by independent t-test, ****p≤0.001; **p≤0.01 and *p≤0.05*. EC: electrical conductivity, OC: organic carbon HA: high altitude and LA: low altitude

2.2 Experimental Design

This study used the knol-khol (*Brassica oleracea* L. var. *gongylodes*) cultivar White Vienna at both high and low-altitude field locations. Crop seed was procured from Beejsheetal Research Pvt. Ltd., Mantha Road, Jalna, Maharashtra. The field trial had 12 plots in total by following a two-factorial randomised block design (2FRBD) with four treatments [T_1= FYM @ 150 q/ha; T_2 = *Azotobacter* @ 8.6 kg/ha; T_3 = FYM @ 150 q/ha+ *Azotobacter* @ 8.6 kg/ha; T_4 = control (without treatment)] replicated thrice. Each plot was 1.62 m2 (1.35 m length × 1.20 m width), and a distance of 0.5 m was maintained between both two blocks and two plots. Seed seedlings have been transplanted at a 2 to 3 true leaf stage or 15 to 18 cm height. A consistent 30 cm × 20 cm plant spacing between individual plants and rows across all experimental plots has been maintained. FYM and biofertilser were applied in each plot before transplanting the seedlings. The field underwent flooding irrigation every three days at High Altitudes (HA) and 6-7 days intervals at Low Altitudes (LA) during the initial stage of plant establishment. This was followed by a one-week interval at HA and a two-week interval at LA during later stages. Notably, no synthetic fertilisers or pesticides were employed at either location. Weed control was carried out manually, with removal occurring two to three times throughout the growing period.

2.3 Morphological Attributes

Five representative plants were chosen randomly from each plot, and the plants were tagged for further measurement. Morphological traits were measured 30, 45, and 60 Days After Transplantation (DAT). The chlorophyll (leaf) and anthocyanin content were estimated via portable meter (CCM-200 plus and ACM-200 plus, ADC Bioscientific, UK). The knob was vertically sectioned at its central point to measure both polar and equatorial diameters. The digital vernier callipers were employed to measure the horizontal distance across the broadest part of the sectioned knob, extending from one side to the other[12]. The yield (q/ha) was computed by determining the knob weight per plot and then converting it to a per-hectare basis to obtain the total yield (q/ha). After harvesting, the dried kohlrabi samples were kept at -20 °C for later examination.

2.4 Estimation of Total Soluble Solids (TSS) and Titratable Acidity (TA)

Approximately 10 g of fresh knob was blended, and juice was extracted to estimate TSS using a hand refractometer (ATAGO, Tokyo). Titratable Acidity (TA) was calculated as a percentage of malic acid by titrating fresh knob juice with 0.1 N NaOH up to pH 8.2[13].

2.5 Estimation of Crude Fat

The Soxhlet system was used to calculate the crude fat content of dried samples[14]. The dried kohlrabi powder (3000 mg) was extracted in three soxhlet extractors using continuous petroleum ether at a flow rate of 2-3 drops per second, followed by sample drying at 95±4 °C. The crude fat percentages were calculated using the formula:

$$\text{Crude fat (\%)} = \frac{FF - FW}{S} \times 100 \quad \text{Eqn (1)}$$

FF= flask weight with fat, FW= flask weight without fat, S= sample weight

2.6 Estimation of Dietary Fiber

The dietary fiber of the knob was estimated according to method no. 978-10[15]. Moisture-free and defatted kohlrabi sample (2000 mg) was digested with 0.128 M (200 mL) of boiling H_2SO_4 for 35 minutes. The digested sample was filtered after the acid was discarded, and then it was washed with boiling distilled water to remove any remaining acid. To eliminate all base solubilised fractions, the sample was next subjected to a 35-minute treatment with 200 mL of boiling NaOH (0.313 M) solution. Once more, it was filtered and washed with hot distilled water. The residual remains were dried at 180°C for 95 minutes, weighed, and then ignited in a muffle furnace (Scientech laboratory equipment, India) at 560 ± 10°C for 2 hours. The dietary fiber percentage was calculated by using the following equation:

$$\text{Dietary fiber (\%)} = \frac{B - C}{A} \times 100 \quad \text{Eqn (2)}$$

Where: A= crude sample weight, B= sample weight before ignition, C= sample weight after ignition.

2.7 Estimation of Ash Content

Method No. 942-05 was used to determine the ash content in kohlrabi samples[15]. The 5000 mg sample was put in a crucible, heated to 560 ± 10 °C in a muffle furnace for six hours, until whitish grey residues were formed. The sample was cooled before being weighed. Ash content (%) was calculated by the following formula:

$$\text{Ash (\%)} = \frac{CA - BC}{S} \times 100 \quad \text{Eqn (3)}$$

CA= weight of crucible with ash, BC= blank crucible weight, S= sample weight

2.8 Estimation of Crude Protein

The crude protein content in knol-khol samples was analysed using a modified method with the Kjeldahl instrument (K-355, Buchi Labortechnik, Switzerland)[15]. For this, oven-dried kohlrabi sample (0.2 g) was digested through concentrated H_2SO_4 and digestion tablets until a light greenish color, which was obtained after two to three hours. Distillation was done with 32 % NaOH after digestion. The released ammonium gas was captured in a 4 % boric acid solution consisting of methyl red and bromo cresol green (indicator), generating ammonium borate that indicates nitrogen content. At last, the distillate was titrated with 0.25 M H_2SO_4 till a light pinkish color, and the volume consumed was noted. To estimate the protein content in the dried sample, nitrogen content was multiplied by the correction factor of 6.25[16].

2.9 Determination of Total Carbohydrate Content

The total carbohydrate content in the extracts was evaluated using the anthrone method with slight modifications[17]. In 100 mL of concentrated sulphuric acid, anthrone (200 mg) was dissolved and cooled by ice cooling. 400 µL of different concentrations of standard solution (Glucose; 3.9-1.000 µg/mL) and extracts were mixed with 2000 µL of anthrone reagent respectively, succeeded by placing in a water bath at 95 °C for 17 minute followed by cooling at room temperature. The absorbance of the standard and samples was calculated spectrophotometrically at 620. The findings were reported in micrograms of glucose equivalence per gram.

2.10 Statistical Analysis

The experimental data were replicated three times and are reported as mean ± standard deviation (SD). Statistical analyses were conducted to assess the significance of the morphological and nutritional parameters of kohlrabi data obtained from high and low altitudes. An independent t-test, one-way ANOVA, and post hoc analysis using Duncan's multiple range tests ($p \leq 0.05$) were carried out in SPSS 16.0 (SPSS Corporation, Chicago, IL). For morphological traits, a three-way ANOVA was employed to examine the relationship between altitude, treatment, and days after transplanting, including their interactions. In nutritional attributes, a two-way ANOVA was conducted to explore the interaction between altitude and treatments.

3. RESULTS AND DISCUSSION

3.1 Morphological Parameters

The growth parameters of kohlrabi, including plant height, number of leaves, leaf length with petiole, leaf width, leaf area, plant spread, leaf chlorophyll content, and leaf anthocyanin content, exhibited significant differences ($p \leq 0.05$) between the treatments and locations (Table 2).

Among the four treatments, the T_3 treatment outperformed all the other treatment groups at both locations, *i.e.*, HA and LA at 60 DAT. Various types of organic fertilisers (manures and biological nitrogen fixers, *etc.*), either alone or in combination, have improved vegetative growth compared

to untreated plants[18]. Possibly, free-living N-fixing bacteria like *Azotobacter* can assimilate atmospheric nitrogen yet they also produce specific phytohormones, such as GA3, IAA, and cytokinins. These phytohormones increase plant development and enhance nutrient accessibility to plant roots by increasing nutrient dissolution[19].

However, in current investigation, among the altitudes (HA & LA), the effect of T_3 treatment at LA resulted in increased plant height (3-fold), leaf width (1.4-fold), and leaf area (134.5-fold) as compared to HA-grown kohlrabi. The observed reduced height, leaf width, and area in HA-grown plants could be adaptive features in response to abiotic stressors like cold, frost, drought, low oxygen, high wind velocity, intense UV radiations, *etc*[20]. In contrast, no significant difference was observed concerning other parameters *viz.,* number of leaves, leaf length with petiole, plant spread, leaf chlorophyll content, and leaf anthocyanin content at both HA and LA-grown kohlrabi. The most probable underlying reason for this observation could be the extended photo-period at HA, which directly influences the colored pigment contents and photosynthetic rates. These findings are comparable with those of prior investigations at HA[3,21]. This is further corroborated by a significant interaction between altitude, treatment, and days after transplanting (ALT×TRE×DT) was found on the plant height, number of leaves, leaf width, leaf area, leaf chlorophyll content, and leaf anthocyanin content (Table 3).

3.2 Yield Parameters

Altitudinal conditions and treatments showed a significant effect on kohlrabi yield attributes (Table 4), including knob equatorial diameter, knob polar diameter, knob weight per plant, and yield (q/ha).

Based on our results, among all the treatments, T_3 treatment resulted in maximum yield parameters at both locations. However, among altitudes, T_3 treatment at the LA region resulted in higher knob weight per plant (59.8-fold) and yield (137.6-fold) as compared to HA-grown kohlrabi. There is no significant variation in knob equatorial diameter and polar diameter were observed in kohlrabi grown at both locations. It could be because of superior physical indices of knol-khol, which may be attributable to superior plant growth features in LA compared to producing kohlrabi[22]. In a two-way study, the effects of altitude and treatment (ALT×TRE) on knob equatorial and polar diameter, knob weight per plant, and yield (q/ha) were significantly different. These outcomes are consistent with the earlier findings of[1,23,24].

3.3 Nutritional Quality

3.3.1 TSS, Titratable Acidity and Total Carbohydrate Content

Carbohydrates are required for various biochemical reactions and enhance energy metabolism, which plays an important function in abiotic stress management[25]. The TSS content, titratable acidity, and total carbohydrate content of mature kohlrabi significantly differed ($p \leq 0.05$) among altitude and treatments (Table 5).

Based on our study, among all the treatments, T_3 treatment resulted in the highest content of TSS, titratable acidity, and total carbohydrate at both locations. However, among altitudes, T_3 treatment at the HA region resulted in higher content of TSS (1.38-fold), titratable acidity (0.06-fold), and total carbohydrate (1.9-fold) as compared to the LA-grown sample. The interactive effects between altitudes and treatments (ALT×TRE) were found on the TSS and total carbohydrate content except the titratable acidity content of the kohlrabi sample. As explained earlier, it might be due to prolonged photoperiod-driven higher photosynthetic rate and better soil nutrient availability at high altitudes. Rokaya[26] *et al.,* (2016) and Naryal[27] *et al.,* (2020) have also made similar observations on the impact of increasing elevation on enhancing the photosynthetic rate and total sugar content in mandarin and apricot fruit. Organic manuring influences general plant health, resulting in higher carbohydrate content and organic acids[28]. Mishra[29] *et al.,* (2014) have shown that a boost in the TSS was correlated with an increase in soil nutrient levels as well as bio-organic nutrient supplements.

3.3.2 Crude Protein Content

Proteins serve as the fundamental constituents of muscle tissue, drive nearly all biochemical processes within the body, regulate gene activity, and constitute the bulk of a cell's structural framework[30]. In the present study, T_3 treatment resulted in the highest content of crude protein at both locations (Table 5). However, among altitudes, T_3 treatment was found to have higher total protein (3.7-fold) at HA as compared to LA-grown knob. The interaction between altitudes and treatments (ALT×TRE) was also found to be significant *($p<0.05$)*. The increase in protein content with altitude could be due to N-rich compounds in the soil in highly mineralised form (NO_3^-), which boosts protein formation in plants[31]. Our findings are consistent with earlier studies on cabbage[32,33].

3.3.3 Crude Fat Content

Fats play an essential role in numerous physiological processes, including ensuring suitable energy intake and absorption of fat-soluble vitamins[34]. The crude fat content of kohlrabi differed significantly between location and treatments (Table 5). Based on our study, among all the treatments, T_3 treatment resulted in the highest content of crude fat at both locations. However, among altitudes, T_3 treatment at the HA region resulted in a higher content of crude fat (1.107-fold) as compared to the grown sample. It might be because high-altitude soil has higher levels of electrical conductivity and nitrogen content which amendment the largest concentrations of ions in the knob that encourage the synthesis of crude fat/fatty acid. Our findings correlate well with[35,36].

3.3.4 Ash Content

The ash content signifies the overall quantity of minerals in a food item. In contrast, the mineral content specifically denotes the concentration of individual inorganic components in the food, such as Ca, Na,

Table 2. Comparative effect of location and treatments on growth attributes of knol-khol cultivar white vienna

Parameters	Treatments	30 DAT		45 DAT		60 DAT	
		HA	LA	HA	LA	HA	LA
Plant height (cm)	T_1	16.04±0.53^{b}	28.89±0.75^{b***}	24.04±0.64^{b}	31.93±0.39^{c***}	29.11±0.95^{b}	32.51±1.08^{b*}
	T_2	17.08±0.60^{b}	28.61±1.31^{b***}	23.23±0.70^{b}	30.39±0.97^{b***}	27.91±0.60^{b}	31.58±0.96^{b**}
	T_3	20.54±0.94^{c***}	31.94±0.78^{c***}	26.30±0.71^{c}	34.53±1.09^{d***}	33.00±1.22^{c}	36.05±0.91^{c*}
	T_4	14.05±0.43^{a}	23.36±0.60^{a***}	18.47±0.74^{a}	25.90±0.64^{a***}	22.52±0.91^{a}	27.51±0.10^{a***}
Number of leaves	T_1	7.89±0.51^{b}	11.89±0.26^{b***}	11.78±0.19^{c}	14.94±0.10^{b***}	15.61±0.70^{b}	16.22±0.19^{b}
	T_2	7.50±0.44^{b}	11.78±1.08^{b**}	11.11±0.48^{b}	14.33±0.88^{b**}	15.33±0.60^{b}	15.89±0.79^{b}
	T_3	8.39±0.35^{c}	13.83±0.44^{c***}	13.17±0.00^{d}	16.33±0.44^{c***}	18.94±0.42^{c}	18.39±0.10^{c}
	T_4	6.17±0.34^{a}	9.67±0.17^{a***}	9.17±0.34^{a}	12.11±0.10^{a***}	12.61±0.19^{a}	13.22±0.35^{a}
Leaf length (cm)	T_1	13.94±0.42^{b}	27.27±0.73^{b***}	21.92±0.4^{b*}	30.17±0.19^{c**}	27.45±0.72^{b}	30.84±0.65^{c**}
	T_2	14.01±0.89^{b}	26.61±1.47^{b***}	20.92±0.92^{b}	28.67±1.13^{b***}	25.89±0.97^{b}	29.34±0.87^{b**}
	T_3	18.91±0.75^{c}	30.24±0.77^{c***}	24.65±0.89^{c}	32.56±0.89^{d***}	30.55±1.33^{c}	33.28±1.12^{d}
	T_4	11.68±0.61^{a}	21.98±0.45^{a***}	16.93±0.65^{a}	24.23±0.55^{a***}	20.85±0.79^{a}	25.41±0.21^{a***}
Leaf width (cm)	T_1	5.46±0.06^{c}	9.36±0.08^{b***}	9.42±0.07^{b}	11.1±0.24^{b***}	10.77±0.26^{b}	11.62±0.19^{b**}
	T_2	5.26±0.11^{b}	9.27±0.24^{b***}	9.63±0.26^{b}	10.84±0.15^{b**}	10.63±0.31^{b}	11.41±0.26^{b*}
	T_3	6.56±0.05^{d}	10.52±0.03^{c***}	11.37±0.08^{c}	12.17±0.10^{c***}	11.34±0.12^{c}	12.74±0.14^{c***}
	T_4	4.10±0.09^{a}	7.09±0.23^{a***}	7.45±0.11^{a}	8.40±0.20^{a**}	7.87±0.21^{a}	8.95±0.26^{a**}
Leaf area (cm^2)	T_1	54.13±2.84^{b}	218.20±9.26^{b***}	146.28±4.49^{b}	291.06±7.61^{c***}	197.14±4.76^{c}	311.18±10.59^{c***}
	T_2	54.77±2.00^{b}	211.61±9.66^{b***}	140.81±4.74^{b}	269.88±9.77^{b***}	181.17±6.82^{b}	294.78±7.04^{b***}
	T_3	83.61±3.90^{c}	266.33±5.66^{c***}	188.94±5.07^{c}	348.01±3.50^{d***}	246.46±10.75^{d}	381.02±6.91^{d***}
	T_4	33.08±1.63^{a}	129.82±3.68^{a***}	90.84±4.54^{a}	180.47±4.58^{a***}	106.04±4.69^{a}	205.11±4.62^{a***}
Plant spread (cm)	T_1	13.59±0.62^{b}	25.56±0.55^{b***}	22.22±0.89^{b}	28.82±0.82^{c***}	27.18±1.31^{b}	30.47±0.70^{b*}
	T_2	13.00±0.57^{b}	24.53±0.66^{b***}	21.58±0.74^{b}	27.28±0.71^{b***}	26.13±1.05^{b}	29.32±0.61^{b**}
	T_3	18.79±0.81^{c}	28.66±0.18^{c***}	26.07±0.28^{c}	31.52±0.9^{d***}	31.90±0.84^{c}	33.07±1.49^{c}
	T_4	11.04±0.55^{a}	21.30±0.91^{a***}	18.28±0.45^{a}	23.57±0.72^{a***}	22.23±0.76^{a}	25.42±1.07^{a***}
Leaf Chlorophyll content (cci)	T_1	16.18±0.67^{b}	26.64±1.44^{b***}	26.83±0.36^{b}	28.71±0.82^{b*}	30.85±0.65^{b}	32.13±0.37^{c*}
	T_2	15.35±0.78^{b}	26.05±0.78^{b***}	26.75±0.06^{b}	27.73±0.70^{b}	30.91±0.84^{b}	31.01±0.37^{b}
	T_3	18.35±0.65^{c}	29.80±0.33^{c***}	29.13±0.15^{c}	32.41±0.56^{c}	36.16±0.60^{c}	36.10±0.37^{d}
	T_4	10.83±0.44^{a}	21.68±0.93^{a***}	20.59±0.48^{a}	25.09±0.49^{a***}	23.56±0.67^{a}	26.55±0.16^{a**}
Leaf anthocyanin content (aci)	T_1	5.87±0.20^{b}	6.48±0.26^{b*}	7.48±0.19^{b}	8.65±0.09^{c***}	8.72±0.62^{b}	10.67±0.08^{b**}
	T_2	5.87±0.37^{b}	6.41±0.19^{b}	7.39±0.22^{b}	8.28±0.11^{b**}	8.20±0.17^{b}	10.47±0.23^{b***}
	T_3	6.52±0.29^{c}	7.22±0.09^{c*}	8.12±0.10^{c}	9.86±0.32^{d***}	11.68±0.51^{c}	11.92±0.15^{c}
	T_4	5.10±0.22^{a***}	3.68±0.12^{a}	5.59±0.36^{a}	6.15±0.14^{a}	5.91±0.26^{a}	7.91±0.07^{a***}

HA: high altitude and LA: low altitude, Values presented as means ± SD, T1: FYM @ 150 q/ha, T2: Azotobacter @ 8.6 kg/ha, T3: FYM @ 150 q/ha+ Azotobacter @ 8.6 kg/ha and T4: control. cci: chlorophyll content index, aci: anthocyanin content index, DAT: Days after transplanting.

Values in columns different lowercase letters (small alphabet) indicate significantly different; P≤0.05, Duncan's multiple range test between treatments.

Mean values in each row (between groups) showed significant differences by independent t-test (***p≤0.001; **p≤0.01 and *p≤0.05).

Table 3. Three-way ANOVA for location, treatment, and days after transplanting and their interactions on morphology parameters of knol khol cultivar white vienna

Source	df	*F*	Source	df	*F*
		Plant Height			**Plant spread**
ALT	1	1543.73***	ALT	1	1145.97***
TRE	3	322.809***	TRE	3	300.023***
DT	2	502.382***	DT	2	699.503
ALT×TRE×DT	6	3.065*	ALT×TRE×DT	6	0.64
		Number of leaves			**Leaf chlorophyll content**
ALT	1	535.83***	ALT	1	1045.31***
TRE	3	257.550***	TRE	3	598.453***
DT	2	1013.13***	DT	2	1597.95***
ALT×TRE×DT	6	3.191**	ALT×TRE×DT	6	3.716**
		Leaf length with petiole			**Leaf anthocyanin content**
ALT	1	1583.16***	ALT	1	235.257***
TRE	3	301.554***	TRE	3	560.814***
DT	2	484.419***	DT	2	1116.46***
ALT×TRE×DT	6	0.77	ALT×TRE×DT	6	18.459***
		Leaf width			
ALT	1	2147.36***			
TRE	3	1167.53***			
DT	2	2528.52***			
ALT×TRE×DT	6	7.845***			
		Leaf area			
ALT	1	7781.15***			
TRE	3	1243.83***			
DT	2	1854.06***			
ALT×TRE×DT	6	6.273***			

df: Degrees of freedom, *F*: F ratio, **p≤0.05*; ***p≤0.01*; ****p≤0.001*. ALT: Altitude, TRE: Treatment, DT: Days after transplanting

Tasble 4. Comparative effect of location and treatments on yield attributes of knol-khol cultivar white vienna

ALT	TRE	Knob equatorial diameter (mm)	Knob polar diameter (mm)	Knob weight / plant (g)	Yield (q/ha)
HA	T_1	81.76±2.37^{c**}	84.94±0.35^{c}	259.97±8.22^{b}	257.72±7.72^{b}
	T_2	73.78±0.73^{b***}	77.82±2.67^{b}	253.03±11.98^{b}	250.21±11.74^{b}
	T_3	90.64±1.89^{d}	89.35±1.24^{d}	365.03±9.62^{c}	370.99±8.90^{c}
	T_4	65.79±2.24^{a}	65.08±2.21^{a}	196.2±5.38^{a}	196.71±7.26^{a}
LA	T_1	77.24±1.13^{b}	82.34±0.56^{b}	290.6±6.12^{b***}	360.49±5.63^{b**}
	T_2	78.86±0.40^{b}	80.96±1.04^{b}	285.72±5.44^{b***}	356.58±4.33^{b***}
	T_3	89.89±0.08^{c}	90.68±1.28^{c}	424.86±11.83^{c***}	508.64±18.55^{c***}
	T_4	64.99±1.29^{a}	62.77±1.4^{a}	168.53±3.67^{a***}	204.11±1.86^{a}
ALT		NS	NS	***	***
TRE		***	***	***	***
ALT×TRE		***	*	***	***

HA: high altitude and LA: low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T_1: FYM @ 150 q/ha, T_2: *Azotobacter* @ 8.6 kg/ha, T_3: FYM @ 150 q/ha+ *Azotobacter* @ 8.6 kg/ha and T_4: control. ALT×TRE: interaction of altitude and treatment.

Values in columns different lowercase letters (small alphabet) indicate significantly different; *P≤0.05*, Duncan's multiple range test between treatments.

Mean values in each column (between groups) showed significant differences by independent t-test. Two-way ANOVA was applied to visualise the interaction between altitude and treatments (****p≤0.001*; ***p≤0.01* and **p≤0.05*).

Table 5. Comparative Effect of location and Treatments on Nutritional Attributes of Knol-Khol Cultivar White Vienna

ALT	TRE	TSS (°B)	TA (%)	Total Carbohydrate (µg/g)	Crude Protein (g/100g)	Crude Fat (%)	Dietary Fiber (%)	Ash (%)
HA	T_1	8.05±0.09^{b***}	0.23±0.01^{b**}	59.74±0.16^{b***}	16.05±0.31^{b***}	0.23±0.01^{b}	10.61±0.02^{b***}	11.55±0.02^{b***}
	T_2	8.05±0.05^{b***}	0.25±0.02^{b**}	60.12±0.17^{c***}	18.41±0.10^{c***}	0.27±0.01^{c}	10.57±0.09^{b***}	11.61±0.03^{c***}
	T_3	9.00±0.10^{c***}	0.33±0.01^{c***}	65.51±0.28^{d***}	19.40±0.12^{d***}	0.31±0.02^{d*}	11.09±0.17^{c**}	11.95±0.04^{d***}
	T_4	7.27±0.20^{a**}	0.18±0.01^{a**}	47.13±0.03^{a***}	14.72±0.20^{a***}	0.20±0.01^{a}	9.38±0.19^{a}	11.18±0.04^{a***}
LA	T_1	6.85±0.00^{c}	0.18±0.01^{b}	57.39±0.27^{b}	13.14±0.08^{b}	0.23±0.01^{b}	9.60±0.10^{b}	12.42±0.01^{c}
	T_2	6.63±0.06^{b}	0.18±0.00^{b}	57.61±0.15^{b}	14.12±0.34^{c}	0.25±0.01^{c}	9.64±0.07^{b}	12.21±0.03^{b}
	T_3	7.62±0.12^{d}	0.27±0.01^{c}	63.54±0.12^{c}	15.62±0.31^{d}	0.28±0.01^{d}	10.33±0.03^{c}	12.80±0.04^{d}
	T_4	6.42±0.08^{a}	0.12±0.02^{a}	45.32±0.13^{a}	11.63±0.14^{a}	0.21±0.01^{a}	9.14±0.04^{a}	12.01±0.03^{a}
ALT		***	***	***	***	*	***	***
TRE		***	***	***	***	***	***	***
ALT×TRE		***	NS	*	***	**	***	***

HA: high altitude and LA: low altitude, Values presented as means ± SD, ALT: Altitude, TRE: Treatment, T1: FYM @ 150q/ha, T2: Azotobacter @ 8.6 kg/ha, T3: FYM @ 150q/ha+ Azotobacter @ 8.6 kg/ha and T4: control. ALT×TRE: interaction of altitude and treatment. TSS: Total soluble solid, TA: Titratable acidity.

Values in columns different lowercase letters (small alphabet) indicate significantly different; P≤0.05, Duncan's multiple range test between treatments.

Mean values in each column (between groups) showed significant differences by independent t-test. Two-way ANOVA was applied to visualise the interaction between altitude and treatments (***p≤0.001; **p≤0.01 and *p≤0.05).

and K. In the present study, T_3 treatment resulted in the highest ash content at both locations (Table 5). However, among altitudes, T_3 treatment was found to result in higher ash (0.85-fold) at LA as compared to HA-grown knob. The interaction between altitudes and treatments (ALT×TRE) was also found to be significant ($p<0.05$). The knobs appear to absorb higher inorganic components (K, Ca, Cl, and Na) from the soil at LA, and These outcomes parallel the results reported by Kumar[32] *et al.,* (2015).

3.3.5 Dietary Fiber Content

Dietary fiber is the complex carbohydrate portion of a plant that is not readily digested, thus remains bound to the surface of the human colon, and is wholly or partially fermented in the large intestine[37]. Based on our study, among all the treatments, T_3 treatment resulted in the highest content of dietary fiber at both locations (Table 5). However, T_3 treatment at the HA region among altitudes resulted in higher dietary fiber content (78-fold) than the grown sample. This has a direct correlation with higher photosynthetic rates at HA, as explained earlier. These findings are consistent with earlier studies on butternut squash[38, 39].

4. CONCLUSION

The bio-organic potential is being investigated worldwide to minimise the consumption of chemical fertilisers and to develop eco-friendly, sustainable agricultural alternatives. In this direction, our study has elaborated that using FYM and *Azotobacter* alone or in combination significantly improves the morphology and nutritional composition of *B. oleracea* L. var. *gongylodes* vegetable cultivated at HA (Leh) *vs.* LA (Chandigarh). The study's key finding is that combining FYM and *Azotobacter* at high elevations can lead to an enrichment of knob yield, TSS, titratable acidity, crude protein, crude fat, dietary fiber, and total carbohydrate content in plants, as demonstrated in the T_3 treatment group. Therefore, organic manure and biofertiliser are being recommended for growers to produce high-quality knol-khol knobs at higher elevations to obtain maximum food and nutritional security advantages under challenging environmental conditions.

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प्रमाण-पत्र

प्रमाणित किया जाता है कि श्रीशारदुल्य शक्ला (LPU; DIHAR-DRDO) समान्य तुंगता तुलनात्मक अध्ययन में 17-19 नवम्बर, 2021 को आयोजित राष्ट्रीय वैज्ञानिक एवं तकनीकी राजभाषा संगोष्ठी-2021 में शोध पत्र-मौखिक/पोस्टर प्रस्तुत किया/ सक्रिय रूप से भाग लिया।

लेह लद्दाख
दिनांक: 19 नवम्बर, 2021

डॉ. नरेन्द्र सिंह
अपर निदेशक एवं संयोजक

डॉ. ओम प्रकाश चौरसिया
निदेशक एवं मुख्य संरक्षक

डी.आर.डी.ओ. चण्डीगढ़ क्लस्टर

आयोजक
रक्षा उच्च तुंगता अनुसंधान संस्थान, लेह लद्दाख

सह आयोजक
चरम प्राक्षेपिकी अनुसंधान प्रयोगशाला (टीबीआरएल) | रक्षा भू-सूचना विज्ञान अनुसंधान प्रतिष्ठान (डीजीआरई) | सी सी ई (अनु. एवं वि.) संपदा, उत्तर | क्षेत्रीय सैन्य उड़नयोग्यता केन्द्र (आरसीएमए)

Organizers

Certificate No. 2003 (SABCD-2022)

JAIPUR NATIONAL UNIVERSITY
A venture of the Seeding Group of Education Institutions
JAIPUR NATIONAL UNIVERSITY
JAIPUR, RAJASTHAN, INDIA

Shobhit
Institute of Engineering & Technology
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MEERUT, UP, INDIA

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JVC, BARAUT, UP, INDIA

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Co-Organizers

5th International Conference

(Advances in Smart Agriculture and Biodiversity Conservation for Sustainable Development) (SABCD - 2022)

Certificate

This is to certify that Prof./ Dr./ Mr./ Ms. Shardulya Shukla
of DIHAR - DRDO, Leh - Ladakh, L.P.U., Jalandhar, Punjab
actively participted as Keynote Speaker / Invited Speaker / Resource Person / Delegates / Research Scholar / Student
Delivered Lecture / Presented a Paper (Oral / Poster / Participation) entitled Comparative effect of FYM & Azobacter on the growth & yield attributes of cauliflower grown in cold desert (Leh) & low altitude (Chd.)
in the 5th International Conference on "Advances in Smart Agriculture and Biodiversity Conservation for Sustainable Development (SABCD - 2022)" held at Conference Hall, Jaipur National University, Jaipur, Rajasthan, India during March, 04 - 06, 2022.

Prof. Amar P. Garg
(Organizing Chairman)

Prof. R. L. Raina
(Vice Chancellor, JNU)
Jaipur, Rajasthan

Dr. Joginder Singh
(Organizing Secretary)

Certificate No (GNRSA-2020)..1952..2021

Co-Organized By Shobhit Deemed University — Education Empowers

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Collaboration with

4th International Conference

Global Approaches in Natural Resource Management for Climate Smart Agriculture (GNRSA-2020) during Pandemic Era of COVID-19

Certificate

This is to certify that Prof./ Dr./ Mr./ Ms. Shardulya Shukla

of DIHAR - DRDO, Leh-Ladakh, LPU Jalandhar, Punjab

actively participted as Keynote Speaker / Invited Speaker / Delegates / Research Scholar / Student

delivered lecture / presented a paper (~~Oral~~ / ~~Poster~~) entitled "Effect of organic manure ... Ladakh"

in the 4th International Conference on "Global Approaches in Natural Resource Management for Climate Smart Agriculture (GNRSA-2020) during Pandemic Era of COVID-19" held at Conference Hall, Shobhit Deemed University, Modipuram, Meerut, UP, India during 26-28 February, 2021

(Dr. Anant Kumar)	(Dr. Rashmi Nigam)	(Prof. A.P. Garg)	(Dr. Joginder Singh)
Managing Chairman	Organizing Convener	Organizing Chairman	Organizing Secretary