IMPACT OF BIOPRIMING, PUTRESCINE AND CALCIUM ON GROWTH, YIELD AND QUALITY OF GREEN GRAM (*Vigna radiata* L.)

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in

Agronomy

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DECLARATION

I, hereby declared that the presented work in the thesis entitled "Impact of Biopriming, Putrescine and Calcium on the growth, yield and quality of green gram (*Vigna radiata* L.)" in fulfilment of degree of **Doctor of Philosophy (Ph. D.)** is outcome of research work carried out by me under the supervision of Dr. Anaytullah Siddique, working as an Associate Professor, in the Department of Agronomy, School of Agriculture of Lovely Professional University, Punjab, 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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CERTIFICATE-I

This is to certify that the work reported in the Ph.D. thesis entitled "Impact of Biopriming, Putrescine and Calcium on the growth, yield and quality of green gram (*Vigna radiata* L.*")*. submitted in fulfillment of the requirement for the reward of degree of **Doctor of Philosophy (Ph.D.)** in the Department of Agronomy, School of Agriculture, is a research work carried out by Poonam Kumari, Registration no.: 12105302, is bonafide record of her original work carried out under my supervision and that no part of thesis has been submitted for any other degree, diploma or equivalent course.

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ABSTRACT

The present study entitled "Impact of Biopriming, Putrescine and Calcium on growth, yield and quality of green gram (*Vigna radiata* L.)" was conducted to check the effect of individual and in combination of Bio-priming with rhizobium and foliar application of Putrescine and Calcium on growth, biochemical, yield attributes, yield and seeds of green gram crop. Green gram is an important leguminous crop, essential for human nutrition and soil fertility because of its capacity to fix atmospheric nitrogen. But to improve its quality, production, and growth, new strategies like growth regulators and Bio-priming are needed. Rhizobium, a symbiotic nitrogen fixing bacteria, putrescine as a growth regulator and calcium as an essential nutrient are helpful. Parameters like growth attributes (plant height, number of branches, no. of leaves, leaf area, leaf area index, leaf area duration, root length, no. of nodules), biochemical attributes (SPAD index, chlorophyll content, total proteins, carbohydrates, PAL activity, flavanol, flavanoid, phenols) yield attributes (test weight, no of seed pod^{-1}, seed weight pod^{-1}, seed weight plant^{-1}, no. of pods plant^{-1}) quality parameters (nitrogen, phosphorus, potassium, arginine, protein), yield parameters are observed and the seed viability of these treatments were evaluated. The results from the study demonstrated that the treatment T_7 with the combined application of Bio-priming with rhizobium and foliar application of putrescine and calcium shows maximum results followed by the treatment T_5 with the combination of foliar application of calcium and putrescine in plant growth parameters. The foliar application of calcium and putrescine augmented the biochemical characteristics and yield parameters, resulting in elevated seed protein content, test weight, and total seed yield. The seed study indicated that the combined treatments were cost-effective, providing higher returns for yield and quality. The treatment T_3 which is the application of putrescine performs best as an individual application. In overall, the combination of Bio-priming with Rhizobium and foliar application of calcium and putrescine is beneficial as an effective agronomic approach for enhancing the growth, biochemical properties, and yield characteristics of green gram, thereby promoting sustainable agriculture and increased profitability.

Keywords: Calcium, Chlorophyll, Flavanol, Rhizobium, PAL, Putrescine.

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Place: Jalandhar

Date: ***Poonam Kumari***

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ABBREVATIONS

Abbreviation and symbol	Full form
%	Percent
&	And
µl	Microliter
µm	Micrometre
^{0}C	Degree Celsius
B	Blank
B:C	Benefit cost ratio
BNF	Biological nitrogen fixation
Ca	Calcium
Cd	Cadmium
Chl.	Chlorophyll
Cm	Centimetre
DAS	Days after sowing
EC	Electrical conductivity
et al.,	And others
FCR	Folin-Ciocalteau reagent
H_2O_2	Hydrogen peroxide
HCl	Hydrochloric acid
hr.	Hour
HPLC	High performance liquid chromatography
i.e.,	That is
IBA	Indole butyric acid
K	Potassium
kg/ha	Kilogram per hectare
LAD	Leaf area duration
LAI	Leaf area index
L	Litre
Mg	Milligram

Ml	Millilitre
Mm	Millimetre
Mm	Milimole
N	Nitrogen
Na_2CO_3	Sodium carbonate
NaOH	Sodium hydroxide
Nm	Nanometre
no.	Number
P	Phosphorus
PAL	Phenylalanine ammonia lyase
PAs.	Polyamine
PGPR	Plant growth promoting rhizobacteria
pH	Negative logarithm of hydrogen
Ppm	Parts per million
RBD	Randomised block design
RDF	Recommended dose of fertilizer
Rs.	Rupees
SA	Salicylic acid
SDS	Sodium dodecyl sulphate
SEM	Scanning electronic microscope
sp.	Species
SPAD	Soil plant analysis development
T	Treatment
TEM	Transmission electronic microscope
V	Volume
W	Weight
WP	Water potential
wt.	Weight
XRD	X-ray diffraction
Zn	Zinc

CHAPTER-1

INTRODUCTION

India is considered as the largest producer, consumer and exporter of pulses. Pulses are considered the most effective form of protein as they contain a high amount of protein (Nawaz *et al.,* 2021). Mung bean (*Vigna radiata*) is a legume highly significant in agriculture, nutrition, and human health. Mung bean farming, which originated in Asia, has a history spanning thousands of years, making it one of the earliest crops to be domesticated. This legume is well-known for its capacity to thrive in all climates and soil types, making it a desirable crop in both traditional and modern agricultural systems. In addition to its use in agriculture, mung bean is notable for its outstanding nutritional composition, characterized by elevated quantities of protein, fiber, vitamins, and minerals (Arif *et al.,* 2010).

Mung bean contains a high amount of protein, i.e. 25% protein, which is highest among pulses. High consumption of pulses in food helps to fight against malnutrition, which is widespread in the country. Mung bean (*Vigna radiata* L.) is a *Kharif* and spring season crop 22which comes from the family Fabaceae. It has its origin in the Indo-Burma region in Southeast Asia (Babarashi *et al.,* 2021). Mung bean contains high crude protein, fat, and starch that the cattle can utilize. Mung bean is classified as a short-duration crop, needing 60-90 days to complete its life cycle. It mainly grows in the March-May season. It is a self-pollinated crop requiring less water for growth and development. So, it is considered a drought-tolerant crop (Suchak & Pandya., 2020). As we know, pulse crops can fix nitrogen in the soil except for Phaseolus vulgaris, so mung bean also fixes N in the soil, which helps increase the crop yield and decrease the number of fertilizers used in the field. Mung bean is considered a cover crop. Its canopy helps maintain soil fertility and reduce the weed population in the soil. Mung bean is India's 3rd most crucial pulse after chickpea and pigeon pea. Green mung bean pods are fried as peas, and the sprouts are rich in vitamins and amino acids (Roy & Roy., 2022). Mung bean is extensively cultivated in several countries, including India, Pakistan, Bangladesh, Sri Lanka, Thailand, Laos, Cambodia, Vietnam, Indonesia, Malaysia, and southern China. India accounts for around 37% of the world's total land and 27% of its mung bean production. India cultivates 14.76 million ton of mung bean on 23.63 million hectares, with an average output of 506 kg per hectare. The principal producers of mung bean in India are Rajasthan (18.30 lakh ha), Maharashtra (3.28 lakh ha), Karnataka (2.69 lakh ha), Madhya Pradesh (1.82 lakh ha), Orissa (1.63 lakh ha), and Telangana (0.70 lakh ha). Apart from playing a vital role in the human diet and crop rotations, the area of the total pulse still has declined by 1.77% since 2018, while the area under mung bean also diminished by 9.19% in India (Nair & Schreinemachers., 2020).

Bio priming is a sustainable agriculture technique that treats seeds with helpful microbes to improve plant growth, increase output, and boost stress tolerance. Rhizobia species are prominent among the microorganisms used in Bio-priming due to their ability to establish symbiotic associations with leguminous plants, including soybeans, peas, and chickpeas (Kaur & Kumar., 2020). Rhizobium-based bio-priming can improve legume output by stimulating nitrogen fixation, improving nutrient absorption, and reducing abiotic and biotic stressors. This novel strategy aligns with sustainable agriculture principles, providing a practical alternative to traditional chemical seed treatments while minimizing environmental effects (Tyagi & Upadhyay., 2015).

Seed priming is a highly promising method for attaining optimal crop establishment, improving the biological nitrogen fixation (BNF) capability of legumes, and maximizing advantages from low-fertility soils. (Ramadhani *et al.,* 2020). Seed priming is a method of controlled hydration that involves immersing the seed in water or a solution with a low osmotic potential until it starts to undergo metabolic activity linked to germination, but without severe emergence. According to (Arif *et al.*, 2008), this method involves pre-soaking seeds and then inoculating them with beneficial bacteria. It is the combination of physiological soaking (seed hydration) phase and the biological activity come together. Beneficial microbes are applied directly or through seed inoculation to the soil and plant tissues. Soil application is recommended when there is a potential risk from inhibitors or antagonistic bacteria on plant tissues (Negi *et al.,* 2021). Seed priming, a process involving the hydration of seeds to stimulate metabolism without initiating germination, followed by drying enhances germination rates, stand establishment, and stress resilience in several crops. Bio-priming refers to seed priming using live bacterial inoculum, specifically the application of plant growth-promoting rhizobacteria. It enhances both the speed and uniformity of germination, guarantees quick, uniform, and robust crop establishment, hence improving harvest quality and production. Seed Bio-priming facilitates bacterial infiltration into seeds, enabling their acclimatization to prevailing circumstances. (Tyagi & Upadhyay, 2015).

Putrescine, a chemical belonging to the class of polyamines, is of great interest in biology, medicine, and agriculture because of its wide range of physiological functions and prospective uses (Babarashi *et al.,* 2021). Recent studies have revealed the role of putrescine in critical cellular functions such as cell growth, specialization, and programmed cell death, indicating its importance in maintaining good health and contributing to disease (Jadhav., 2019). Furthermore, putrescine is essential in plant growth and development, stress responses, and fruit ripening, highlighting its significance in agricultural production and food quality (Suchak & Pandya., 2020). Although putrescine is widely found and has biological importance, much remains to be discovered about how it is regulated and metabolized and its functional implications in many animals and physiological situations (Abd elbar., 2019). Polyamines such as putrescine, spermidine and spermine are low-molecular polyvalent cations that contain two or more amino groups (Sengupta & Raychaudhuri., 2017). They are ubiquitous compounds in myriad processes, including regulating protein and RNA synthesis and initiating and controlling cell division (Roy & Roy., 2022). Polyamines have been implicated as vital modulators in plants' various growth, physiological and developmental processes. They are not plant hormones because they are too abundant, but they might be considered plant growth regulators or merely one of several kinds of metabolites needed for specific developmental processes (Hussein *et al.,* 2023). Putrescine modulates various plant growth and development processes, including the formation and effectiveness of different nitrogen-fixing systems (Deotale *et al.,* 2019).

Applying calcium to the leaves of plants has become a popular agricultural technique for improving crop yield, quality, and ability to withstand environmental challenges.

Calcium is a crucial nutrient for the growth and development of plants, as it plays essential roles in the formation of cell walls, the integrity of membranes, the activation of enzymes, and the signaling pathways (Kazemi., 2013). Calcium insufficiency is prevalent in numerous crops, resulting in physiological problems, decreased yield, and poor post-harvest quality. Applying calcium directly to plants through foliar treatment is a very effective method that overcomes soil constraints and improves the efficiency of nutrient absorption (Mubeen *et al.,* 2020). Furthermore, applying calcium to the leaves has been demonstrated to alleviate abiotic conditions such as drought, heat, and salinity and reduce biotic challenges, including diseases and pests. Although foliar-applied calcium has potential benefits, there is still a lack of understanding regarding the mechanisms involved in its uptake, translocation, and physiological impacts (Rupali *et al.*, 2018). Calcium has been recognized as vital for plant growth and development for more than a century, and its significance has been extensively studied. Calcium is crucial for the development of growing plant cells, particularly in the development and integrity of cell walls. A strong and strengthened cell wall will exhibit more uniform growth and greater resistance to pest and disease assaults (Kazemi, 2013). Adequate calcium, in conjunction with other vital nutrients, promotes consistent growth of shoots, leaves, and flowers, resulting in superior fruit quality. Calcium not only contributes to cell wall construction but also activates many enzymes that facilitate cell division and reproduction. (Sharma & Dhanda., 2015).

So, there are a lot of scopes for improving pulse production by improving soil fertility and productivity by increasing soil organic carbon, increasing soil moisture storage capacity and using combined nutrient and pest management practices. Organic amendments like farm yard and poultry manure have improved soil fertility and health more effectively. At the same time, a foliar spray of different macro and micronutrients at the growing stage of the crop satisfies the nutrient demand and decreases water storage to some extent (Arif *et al.*, 2010). Mung bean helps in improving soil fertility due to the presence of nitrogen-fixing symbiotic rhizobium in root nodules. It enriches the soil and breaks the cycle of cereal–cereal rotations, ultimately improving soil health. Rhizobia is only known to fix nitrogen 50–100 kg/ha in combination with legumes. Rhizobium inoculation in summer mung bean is a sustainable and environmentally sound agro-technological activity (Negi *et al.,* 2021). The major limitation of pulse production is poor crop establishment. High yields can be attendant with early vigour. The primary causes of low productivity of pulses are also attributed to poor plant stand establishment and lower yield of pulses under harsh environmental conditions. However, the emergence of deep roots indicates quick seedling germination before the soil's upper layers are dried and crusty (Ramadhani *et al.,* 2020). Although mung bean has a long history and offers several advantages, there is still plenty of need for research to thoroughly investigate its potential in addressing global food security, boosting sustainable agriculture, and advancing human health and nutrition, which result from improved crop establishment and higher crop yield. This work helps enhance the productivity of green gram because putrescine is a good regulator which helps in the growth of plants. Calcium helps as a cementing agent for plants by preventing the breakage of pods and shattering loss. Rhizobium helps fix nitrogen (Arif *et al.,* 2008). Together, all of these components help to increase crop productivity and quality.

Objectives of the research are:

1. Effect of Rhizobium, Putrescine, & Calcium on morpho-physiological growth, yield and quality in *Vigna radiata*.

2. Impact of different treatments on biochemical responses in *Vigna radiata*.

3. Evaluation of different treatments on arginine analysis in seed.

4. Assessment of economics in *Vigna radiata*.

CHAPTER-2

REVIEW OF LITERATURE

Effect of Bio-priming with Rhizobium on *Vigna radiata*

Choudhury *et al.*, 2024 performed a research and showed that *Rhizobium leguminosarum* at a concentration of 20% worked best in seedling germination percentage (82.71%), shoot length (25.49 cm), fresh weight (2.77 g), dry weight (0.24 g), seedling vigour index I (34679.1%), and seedling vigour index II (19.85%) were seen in seeds bio-primed with Rhizobium leguminosarum at a 20% concentration.

Srivastava *et al.*, 2024 explains Bio-priming is a useful approach that involves the treatment of seeds with favourable biological agents. They demonstrate significant potential in enhancing the physiological functioning of seeds, thereby contributing critically to their uniform germination and vigour. Bio-priming-induced molecular and metabolic change promotes stress resilience to plants, promotes plant vitality, and boosts agricultural yield. Additionally, it is linked to the rehabilitation of damaged land and the enhancement of soil fertility, health, and nutrient cycling.

Saleem and Khan (2023) experimented with Rhizobium and Iron as nanoparticles to understand the morphological growth of green gram and the molecular anatomical structure of root nodules using XRD, SEM, and TEM. The impact of treating Rhizobium alone or in combination with Iron shows a synergistic effect on germination potential, dry biomass production, plant growth, pigment content, and protein in the seed of green gram.

Sutradhar *et al.*, 2023 conducted an experiment on seed priming with different concentrations of Rhizobium. The results showed that the treatment T_4 which is the application of Rhizobium leguminosarum seed treatment at a concentration of 20% shows better germination and outperformed all other treatments for all measured characteristics.

Ghosal and Sahu, 2022, performed a research trial on organic growth stimulants on mustard plants. Bio-priming growth stimulants show better results, such as good yield, and provide resistance from white flies compared to non-primed ones.

Ibrahim and El-Sawah., 2022 explained that the treatment with the application of *A. chroococcum* and Rhizobium to pre-soaked seeds may serve as a simple, economic, and effective inoculation method to enhance the growth, yield, nutrient absorption, and quality attributes of pea plants.

The experiment conducted by Annadurai *et al.,* 2021, revealed that the co-inoculation of Rhizobium sp. VRE1 and *C. tropicalis* VYW1 improved shoot and root length, chlorophyll content, and total biomass compared to when each treatment was inoculated individually.

Nawaz *et al.,* 2021 experimented on the efficiency of seed bio-priming techniques for healthy mungbean productivity. They found that bio-priming with *Pseudomonas fluorescens* + *Rhizobium phaseoli* increased mungbean yield and yield components (seeds/plant, 1000-seed weight, and harvest index). Bio-priming enhanced mungbean seed production by 8–12%.The study found that bio-priming as a dual seed treatment strategy may boost mungbean germination and terminal drought tolerance. Bio-priming increased seed yield among the treatments due to its robust antioxidant defence mechanism and higher nutrient uptake.

Pandey *et al.,* 2021 examine PGPRs which serve as a mechanism to improve nitrogen usage efficiency in diverse crops. The prolonged use of such bacteria may serve as a novel alternative to chemical fertilisers, mitigating their detrimental effects on soil and ecology while restoring the plant-soil ecosystem.

Sarkar *et al.,* 2021 explained that bio-priming acts as a practical technical solution for attaining the perfect combination of food-nutritional security, ecological responsibility, and agricultural profitability across various agro ecosystems.

Meena *et al.,* 2020 investigated that the treatment with the Bio-priming with BCFE proved impactful. They examine how Bio-priming might alleviate the effects of abiotic stress, such as drought and salt, by augmenting antioxidant enzyme activities and promoting root and shoot development in crops like maize and wheat.

The study performed by Choudhary *et al.,* 2019 found that applying 20 kg ha^{-1} of nitrogen (N) and sulphur (S), along with bio-priming using Rhizobium at a rate of 25 g kg^{-1} of seeds, on loamy sand soil significantly improved productivity, nutrient uptake, and soil nutrient availability in nitrogen and sulphur-deficient soils. This approach is beneficial for promoting sustainable food production of green gram.

Haldar and Darvhankar., 2019 experimented on mung bean to check the Influence of paclobutrazol and rhizobium on growth and yield. The experiment showed that a recommended dose of rhizobium + mycorrhiza will help increase the plant height, branch number, pod number and total soluble sugar. In combination with treated plants, Paclobutrazol + rhizobium + mycorrhiza helps increase the biological yield and total carbohydrates. When paclobutrazol + rhizobium is applied to the field, it helps increase protein content, chlorophyll content, and harvest index, whereas rhizobium-treated plants increase the pod yield.

Deshmukh *et al.,* 2017 findings demonstrated a substantial increase in seed germination and a significant decrease in Alternaria leaf spot, Anthracnose, and Macrophomina leaf blight as a result of seed bio-priming with *T. harzianum*, *T. viride,* and *P. aeruginosa* at a concentration of 10 grams of talc-based formulation per kilogramme of seeds.

Deshmukh *et al.,* 2016 conducted a study, and results showed that the average percentage of seed germination, plumule length, radical length, seedling fresh weight, dry weight, and seedling vigour index were significantly increased when mung bean seeds were treated with rhizobium priming.

Dhakal *et al.,* 2016 conducted a study to investigate the effects of integrated nutrition management on green gram. The results indicated that the treatment involving the combination of Rhizobium and other nutrients resulted in the highest number of nodules per plant, dry weight of nodules, seed production, harvest index, and nutrient content in mung bean.

Suman *et al.,* 2016 conducted a field experiment to investigate the effects of integrated nutrient management on the growth and yield of green gram. According to the findings of the experiment, it was observed that the application of rhizobium treatment resulted in the highest values for many growth parameters in *Vigna radiata*, including plant height, number of leaves per plant, number of branches per plant, number of pods per plant, and seed yield.

Tyagi and Upadhyay., 2015, tested rhizobium combinations in the field. The results showed that 100% RDF + 1.0 Vermicompost ha^{-1} + Rhizobium increased pods $plant^{-1}$, seeds pod^{-1}, test weight, seed yield, stover yield, biological yield, and protein yield. Applying 100% RDF + 1.0, Vermicompost ha^{-1} + Rhizobium increased seed production by 57.3% and RDF + Rhizobium by 10.2%.

Hussain *et al.,* 2014, investigated the growth, nodulation, and yield of mung bean plants under rhizobium and phosphorus treatments. The study noticed that the maximum number of nodules, pods, and plant height were significantly higher in the treatment applied with the rhizobium application.

Prasad *et al.,* 2014, conducted a study that concluded that rhizobium injection combined with the application of 60 kg P_2O_5 /ha substantially impacted the growth and yield characteristics of mungbean in an agri-horticultural system. The treatment with rhizobium resulted in a considerable increase in yield, with a production of 1011 kg/ha. The combined utilisation of Rhizobium inoculants and phosphorous fertiliser was deemed to be the optimal approach for enhancing mungbean output in agri-horticultural systems.

Kundu *et al.,* 2013 study showed that Rhizobium seed inoculation had a good and substantial effect on the growth parameters, nodulation, seed and stalk yield of green gram, and the bacterial population in the soil system. The application of Rhizobium inoculation, together with the recommended dose of NPK, was closely followed by the treatment combinations of Rhizobium inoculation paired with vermicompost.

Yadav *et al.,* 2013 conducted a research on three rhizosphere-competent bacteria strains on bioprimed seeds of chickpea and rajma. The findings indicated that bioprimed seeds exhibited a greater germination rate and superior plant development in both crops compared to non-bioprimed control plants. The simultaneous treatment of the microorganisms was found to improve seed germination and plant development more effectively than their solo application.

Hossain *et al.,* 2011, carried out a study on the effect of rhizobium and nutrients on the mung bean crop. Rhizobium inoculation, in combination with the use of recommended fertilizers, increased mung bean seed production. This may be attributed to the higher number of nodules per plant, pods per plant, seeds per pod, and the weight of 1000 seeds.

Raza *et al.,* 2004 experimented with two strains of rhizobium Q7 and Q14. Rhizobium significantly enhanced growth parameters like plant height, root length, number of nodules, and number of seeds per pod and yield parameters like number of pods per plant, 100-seed weight, and number of seeds per plant compared to Rhizobium alone. The Q7 strain stimulated vegetative development but resulted in a smaller seed size than other treatments. In contrast, the Q14 strain of rhizobium exhibited larger seed size and higher yield.

Effect of Calcium on *Vigna radiata*

El-beltagi *et al.,* 2022, treated their plant samples with two mM of salicylic acid alone and combined with a 2% calcium chloride solution. The samples showed reduced weight loss and lower levels of chlorophyll content, vitamin C, phenolic compounds, carotenoids, flavonoids, and glucosinolates compared to the control samples.

Heidaria *et al.*, 2022, experimented with the exogenous application of salicylic acid and calcium chloride to cotton plants. The results revealed that calcium chloride 1.5g/lt. provides good ball and seed yields.

Mahadevan. K (2022) experimented with different salicylic acid and calcium chloride doses on red bean plants. Their study showed the enhancement of growth parameters and levels of Chl and carotenoids, the reduction of sugars, and a considerable reduction in MDA and DPPH radicals in plants. The impact of SA and Ca was

attained with the administration of 0.75 mM SA and 50 mM CaCl2, which are suggested for enhancing the performance of red beans in saline environments.
Ashraf *et al.,* 2020 experimented with calcium chloride and zinc application on mung beans. Their findings demonstrated a notable impact of applying calcium (Ca), zinc (Zn), and their combination to the leaves on the levels of chlorophyll (Chl.), gas exchange (photosynthetic activity), nitrogen (N), phosphorus (P), potassium (K), and biomass characteristics in both saline and everyday environments.

Noroozi *et al.,* 2019, experimented with the foliar application of calcium chloride and selenium on wheat plants. The enhanced yield in plants treated with Calcium chloride is attributed to improved photosynthetic activity under the conditions. The application of Calcium chloride enhances the efficacy of photosystem II, hence enhancing the overall performance of photosynthesis.

Rupali *et al.,* (2018) researched calcium application and found that it increases the number of seeds per pod, seed weight, and seed yield in the black gram crop.

El-Hadidi *et al.,* 2017 conducted an experiment on different concentrations of Calcium and magnesium. The foliar application of calcium at 0.8% significantly increased tubers content at harvest and nitrogen, calcium and magnesium concentrations in leaves.

Siddique *et al.,* 2017 conducted an experiment on different doses of calcium and magnesium on cucumber crop. From the experiment it was revealed that the treatment with the application of 10 mM of calcium shows lesser no. of days to flowering, highest vine length, no. of branches and highest yield as compared to all other treatments.

Youssef et al., 2017 performed an experiment on different doses of calcium chloride and salicylic acid on romaine lettuce. The results from their study revealed that the treatment with 20 mM of calcium chloride and 1.5mM of salicylic acid increases the growth, yield and physiological parameters of lettuce crop.

Boro *et al.,* 2016 conducted an experiment on calcium and boron application on wheat crop. They revealed that different concentrations of calcium as compared to boron shows maximum plant height, flag leaf, number of grains and yield of the crop.

Ameeta and Shweta, (2015) performed a clinical trial on calcium and NaCl application. The results showed that the combined application of NaCl and $CaCl_2$ mitigated the effects of salt stress on the plants, promoting their development and inducing favourable changes in physiological parameters, such as an increase in Proline content under stress conditions and increased plant growth.

Costa *et al.,* 2014 investigated an experiment on foliar application of calcium and molybdenum on bean crop. The results from their study revealed that the treatment with the combination of calcium and molybdenum does not shows best results whereas the treatment with alone application of calcium increases overall yield and physiological potential.

Kazemi., 2014 conducted an experiment on foliar application of calcium chloride and humic acid alone and in combination of different concentrations. Results from the study shows that the treatment with the combination of calcium chloride and humic acid increases yield and prevent the crop from diseases.

Kazemi., 2013, experimented on the foliar application of calcium chloride and paclobutrazol on cucumber plants. The results show that treatment with low doses of calcium chloride increases cucumber yield.

Rab, A., & Haq, I. U. (2012) performed an experiment on foliar application of 0.35 and 0.6% calcium chloride and borax alone and in combination. The results from their research shows that the treatment with alone application of calcium chloride increases the plant height and no. of fruit per plant whereas decreases the incidence of blossom end rot in tomato plant. Overall the treatment with 0.6% of calcium chloride gives best results.

Effect of Putrescine on *Vigna radiata*

Kumar *et al.,* 2024 experimented on sorghum plants under cadmium toxicity. Putrescine and mycorrhiza are used to mitigate stress conditions. The plants exhibited a mitigating effect against high concentrations of Cd by the exogenous application of mycorrhiza and putrescine. The treatment with the combination of mycorrhiza and 5 mM putrescine exhibited a significant reduction in total free amino acid, ascorbic acid, catalase, APX, and peroxidase levels.

Hussein *et al.,* 2023, experimented on a wheat crop under different concentrations of putrescine. Applying putrescine as seed priming at a concentration of 1 mM resulted in the highest improvements in plant height, root length, number of leaves, and number of tillers, fresh weight, and dry weight. Putrescine also enhanced the levels of chlorophyll a, chlorophyll b, carotenoids, soluble sugars, amino acids, and phenols.

Babarashi *et al.,* 2021, experimented on mung bean plants, and their results show that treatment with putrescine mitigates the adverse effects of drought stress by enhancing proline and leaf chlorophyll content, improving membrane stability and ultimately boosting plant output in comparison to untreated control plants.

Suchak and Pandya (2020) studied priming putrescine and spermine at different concentrations. The treatment with putrescine priming at a 0.01 M concentration produced the highest seed germination, protein content, chlorophyll content, and first leaves.

Abd. Elbar *et al.,* 2019 performed a research experiment on the impact of applying putrescine to the leaves on the growth, biochemical composition, percentage of essential oil, and morpho-anatomical properties of *Thymus vulgaris* L. when treated with water deficiency conditions. Putrescine mitigates the effects of drought by affecting anatomical characteristics, maintaining chlorophyll levels, increasing the accumulation of total soluble phenolic compounds, and enhancing the activity of certain enzymes. As a result, putrescine enhances growth and oil production, helping thyme plants survive even under water-stress conditions.

Deotale *et al.,* 2019, experimented with different doses of putrescine and IBA on black gram's growth, yield, and biochemical parameters. Applying 75 ppm putrescine shows the best results in yield, development, and biochemical parameters like nitrogen, phosphorus, potassium, sugar, and protein.

Ghassemi *et al.,* 2018, experimented with the external application of polyamines, which resulted in enhanced ATP content, CCI, CSI, rubisco activity, Fv/Fm, endogenous polyamine content, MSI, antioxidant enzyme activity, nitrogen dioxide, potassium, calcium, magnesium dioxide, and seed yield. A reduction in ROS level and MDA content is also found. Putrescine had superior effects on mung bean plants' physiological, biochemical, and agronomical performance compared to other polyamines, regardless of the irrigation regimes.

Jadhav *et al.,* 2019 investigated the impact of different dosage growth regulators (putrescine and IBA) on biochemical and yield-related factors in black gram. Applying putrescine and IBA improved many biochemical parameters, including chlorophyll content, NPK content in leaves, and protein content in seeds. It also increased yield-related factors, including the number of seeds per pod, test weight, and seed yield per plant.

Jadhav *et al.,* 2018, performed a research experiment on different concentrations of putrescine and NAA. Their study's results show that applying putrescine alone and in combination significantly increases plant height, leaf area index, dry matter accumulation, and yield of pigeon peas.

Kumar *et al.,* 2019, experimented on a sorghum plant under cadmium toxicity. The external treatment of putrescine showed a mitigating effect by increasing the total reduced sugar content by 1.13%, 1.00%, and 0.90% on the recommended days after sowing (DAS). The mean overall reducing sugar content was considerably increased by 2.36%, 2.10%, and 1.89% when treated with a greater putrescine dosage than the control.

The experiment conducted by Sadeghipour, O. (2019) shows that treating mung bean seeds with polyamines (PAs), particularly a combination of Putrescine (Put), Spermidine (Spd.), and Spermine (Spm.), can improve the plant's ability to withstand drought. This is achieved by increasing the accumulation of osmo-protectants, enhancing water levels, chlorophyll content, and photosynthetic activity while reducing oxidative damage.

The research performed by Khan *et al.,* (2018) investigated the impact of externally applied salicylic acid (SA) and putrescine (Put.) on the process of phytoremediation of heavy metals, as well as on the growth parameters of chickpea plants cultivated in sandy soil. The SA and Put have been given individually and with plant growth-promoting rhizobacteria (PGPR). The results found that combining Salicylic acid and putrescine gives higher and better yield quality.

In their work, Kumar and Dwivedi (2018) did a pot experiment using maize variety. The aim was to investigate the impact of putrescine and glomus mycorrhiza on cadmium toxicity, specifically about sugar and protein levels. The study discovered that the treatment, which consisted of 0.15% Cd $(NO_3)_2$, 5mM Putrescine, and mycorrhiza, exhibited a notable rise in total sugar content by 4.22%, 5.03%, and 4.18%, which only included 0.15% Cd $(NO_3)_2$. As a result, it was concluded that the combination of Putrescine and mycorrhiza can be applied as a treatment for Cd-induced toxicity.

Naeem *et al.,* 2018 experimented with PGPR (salicylic acid and putrescine) on chickpea plants. Results showed synergistic effects of PGPR and PGR on chlorophyll, protein, and sugar levels. Applying salicylic acid and putrescine together successfully reduced lipid peroxidation and enhanced leaf area. It is believed that PGPR and PGR function together to improve plant development in sandy soil with moisture and nutrient deficiencies.

Yuan *et al.,* 2018, conducted a research trial on a foliar spray of putrescine (Put; 8 mM) on cucumber seedlings. Their study revealed that applying putrescine with 8 mM increases the size of the seedlings.

Ahmed *et al.,* 2016 performed a research on foliar application of 0.2 mM putrescine on wheat crop. The results from the study showed that putrescine application gives good results in growth, physiological and yield parameters of wheat crop.

Barzegar *et al.,* 2016 investigated in a research that applying HA and Put to the leaves of the plant greatly improved fruit production, relative water content (RWC), vitamin C and proline content, catalase and peroxidase activities, and water use efficiency (WUE). According to the findings, okra results best in the treatment with Hg at 300 mg l^{-1} and Put at 1.5 mM in terms of growth, yield, and fruit quality.

Nahar *et al.,* 2016 examine the physiological functions of putrescine, spermidine, and spermine in enhancing salt tolerance in mung bean seedlings subjected to a 48-hour exposure to 200 mM NaCl. Exogenous polyamines (PAs) decreased the amount of sodium in the cells. They regulated the balance of nutrients while influencing the quantities of naturally occurring PAs in mung bean seedlings under salt stress.

Amin *et al.,* 2011 conducted a research on foliar application of glutamine and putrescine on onion crop. The bulb yield and quality were enhanced by applying 100 mg l^{-1} of Put and 200 mg l^{-1} of glutamine through foliar application, either alone or in combination. Applying Put and glutamine to onions increases their yield-contributing characteristics and quality.

Rai et al., 2007 executed a field experiment to investigate the impact of varying amounts of Polyamine (Putrescine) on tomatoes. The 80 µM dose of putrescine exhibited optimal plant height, leaf count, leaf area, flower quantity, fruit production, harvest index, and total yield. Put also influences chlorophyll, carotenoids, ascorbic acid, total soluble sugars, and protein content in tomatoes. This indicates that Putrescine (80 µM) may serve as the optimal concentration for augmenting the growth and production of tomato.

Thavaprakash *et al.,* 2006 findings indicated that the application of putrescine and spermine at a concentration of 20 ppm on the leaves resulted in increased growth characteristics, such as plant height, number of trifoliate leaves per plant, leaf area index, and dry matter output. The yield attributing factors, such as the number of flowers per plant, the number of pods per plant, the fertility coefficient, the number of seeds per pod, and the percentage of filled seeds, increased. Applying putrescine and spermine increased seed weight percentage.

Krishnamurthy., 1991 conducted a pot experiment on diamine putrescine application on rice plants. From the research it was revealed that the exogenous application of diamine putrescine increases the fresh weight, dry weight and yield of rice plants.

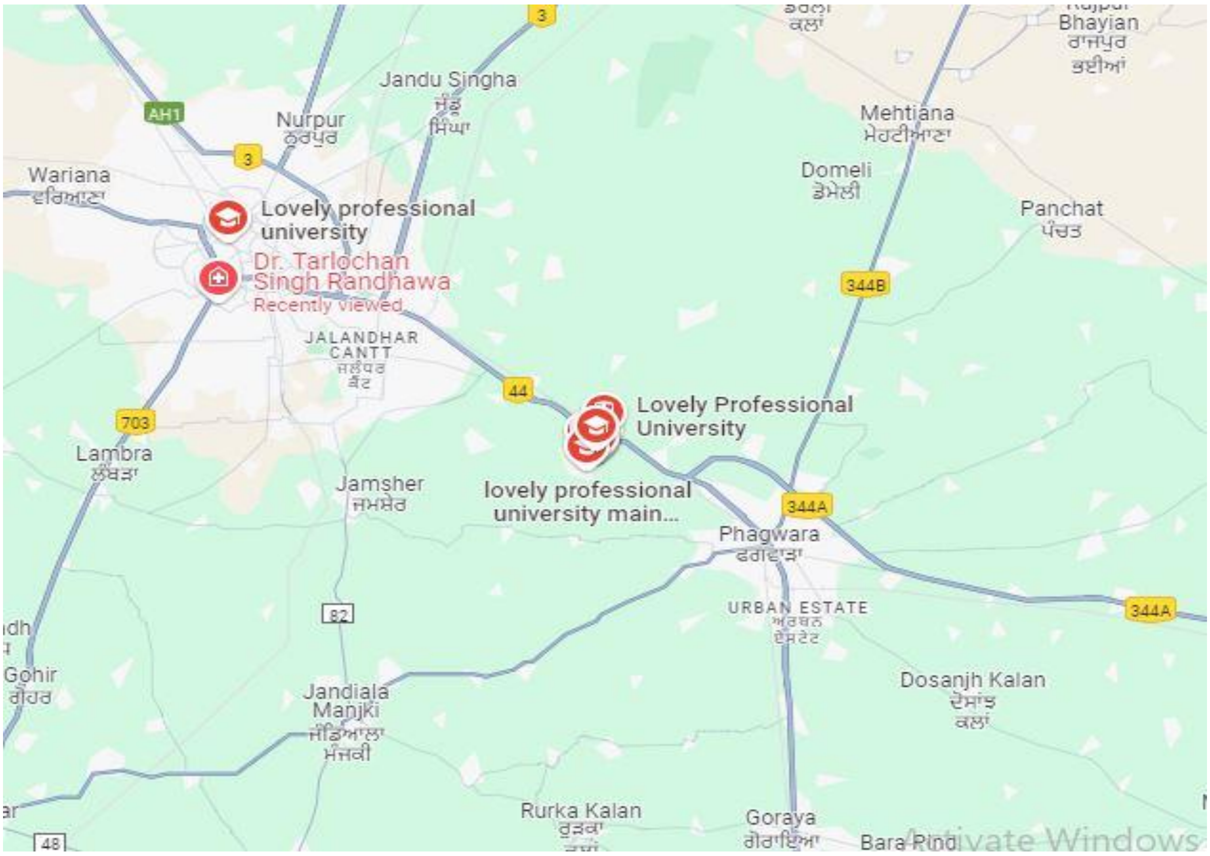
CHAPTER-3

MATERIAL AND METHODS

3.1 LOCATION OF TRIAL SITE

The current study was conducted in the agriculture research farm of Lovely Professional University, Punjab during the term from April 2022-June 2022 and July 2022-September 2022 in the summer season and April 2023-June 2023 and July 2023-September 2023 in the *Kharif* season to assess the “Impact of Bio-priming, Putrescine and Calcium on the growth, yield and quality of green gram (*Vigna radiata*. L)”. The farm is at a latitude of 31º22’31.81’’ North and a longitude of 75º23’03.02’’ East. It has an average elevation of 252m above sea level. The distance between the capital of India (Delhi) and Punjab is 350 km. Punjab is located in the sub-tropical area inside the central plain of the state's agro-climatic zone. This chapter provides a detailed explanation of the materials and procedures used during the current experiment.

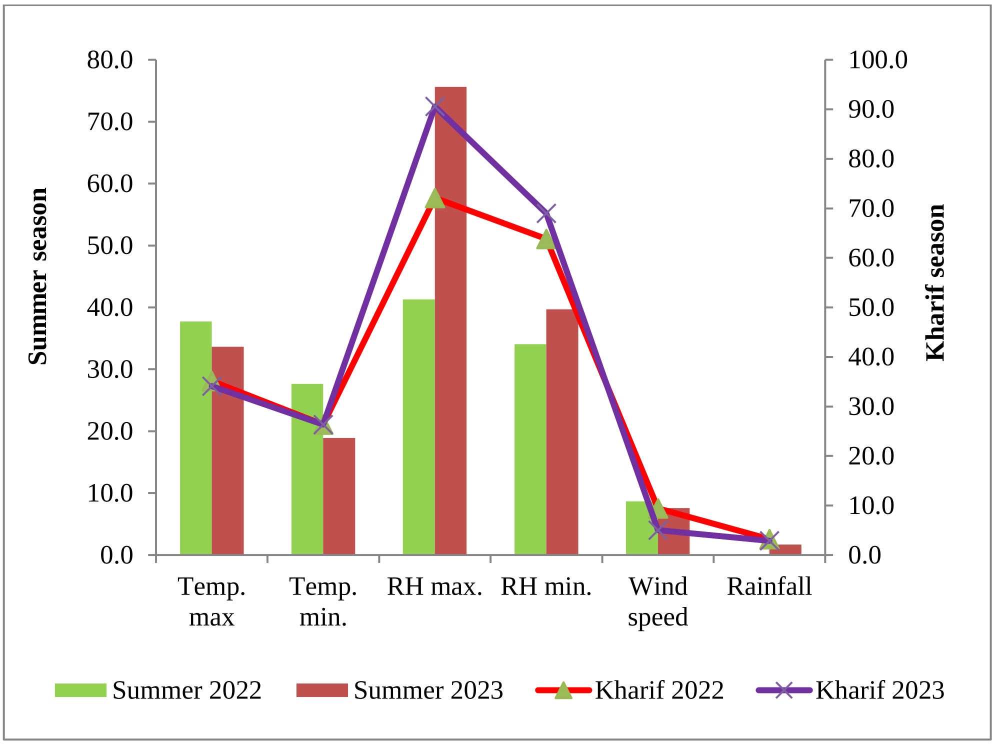
Fig. 3.1.1 Location of the field trial



3.2 WEATHER AND CLIMATIC CONDITIONS

The experimental location is located in a region within the subtropics. The temperature is cool and hot during the winter, and there is limited rainfall in July, August, and September. The primary source of rainfall in this region is the southwest monsoon. In the winter, the temperature consistently remains above freezing, particularly in December and January. The maximum temperature recorded throughout the summer months of April, May, and June was 47 degrees Celsius. The monsoon rainfall typically begins in the final week of June and continues until the end of September, barring any delays in the arrival of the southwest monsoon winds. The month of July experiences the most precipitation. The maximum temperature, minimum temperature, maximum relative humidity (RH), minimum relative humidity, wind speed and rainfall are measured. The time period for summer crop is from 20 March to 10 June and 1 July to 20 September in both the years 2022 and 2023 and the detailed meteorological data of year 2022 and 2023 is given in appendix-1 and fig.3.2.1.

Fig.3.2.1 Meteorological data of summer and kharif season 2022 and 2023



3.3 SOIL PARAMETERS

The study considered soil factors such as pH, electrical conductivity, NPK, and total organic carbon. These parameters were assessed for each replication's treatment plot using soil acquired prior to crop establishment and harvest. The discussion following pertains to the estimate of these parameters.

3.3.1 Soil pH

The soil pH was measured before planting the crop using a small pH meter called pH 97 WP Milwaukee. The suspension was created by combining soil and distilled water in a ratio of 1:5. A total of 5 grams of soil was placed in several beakers, each of which 25 millilitres of distilled water was added. The mixture was vigorously shaken for 5 minutes, after which it was allowed to settle. The pH meter was inserted into the soil water suspension to get a reading, and the measurement of the sample water was recorded accordingly (Sparks, 1996).

3.3.2 Electrical Conductivity (dSm^{-1})

The soil electrical conductivity (EC) was determined by using an EC meter. This required preparing a soil water suspension with a ratio of 1:5 and vigorously shaking it for 5 minutes, then allowing it to settle. Next, the conductivity anode is immersed into the suspension, and the collected samples' ECs (electrical conductivities) are recorded (Sparks, 1996).

3.3.3 Available Nitrogen

The Micro-Kjeldahl technique is often used to convert nitrate nitrogen in the soil into ammonium sulphate. For this estimation, 5 grams of soil were mixed with 25 ml of $KMnO_4$ and 25 ml of NaOH. The mixture was then slowly heated for one hour to facilitate digestion. A portion of the solution was transferred to a separate conical flask containing 25 ml of boric acid and a few drops of a mixed indicator. The solution was then titrated with 0.5 N NaOH until the green colour changed to pink (Subbiah and Asija, 1956).

$$\text{Nitrogen (\%)} = \frac{(S-B) \times 0.0014 \times 100}{5 \text{ (weight of the soil)}}$$

$$N\ (kg\ ha^{-1}) = X * 22400$$

3.3.4 Available Phosphorus

Phosphorus in soil exists in two forms: organic and inorganic. Plants absorb these nutrients through $HPO_4{}^{2+}$ and $HPO_4{}^{-}$ ions. To estimate the amount of phosphorus, 1 gram of soil was combined with a small amount of Darco-G and 20 ml of 0.5 N $NaHCO_3$. The mixture was then agitated in a mechanical shaker for 30 minutes and subsequently filtered using a Whatman filter paper number 1.5 ml of the aliquot was extracted and combined with 5 ml of ammonium molybdate and 1 ml of Stannous working solution. The estimation of the blue colour created in the sample was conducted using a spectrophotometer, as described by Olsen *et al.*, in 1954.

$$\text{Phosphorus } (kg\ ha^{-1}) = \frac{\text{Reading} \times 100}{\text{Graph Factor}} \times 2.24$$

3.3.5 Available Potassium

To measure the potassium content, 5 grams of soil were extracted from the sample and mixed with 25 millilitres of ammonium acetate. The mixture was then vigorously shaken for 5 minutes. After filtration, 5 ml of the suspension was extracted and transferred into a 25 ml volumetric flask. The remaining volume was adjusted by adding distilled water. The sample was placed into the flame photometer, and the measurement on the screen was carefully recorded for each sample (Jackson, 1973).

$K\ (Kg\ ha^{-1}) = \text{Reading} \times 2.24$

3.3.6 Organic Carbon

This procedure involves dissolving 1 gram of soil in a solution consisting of 10 ml of potassium dichromate and 20 ml of sulphuric acid (H_2SO_4) within a 250-ml conical flask. Subsequently, the mixture is cooled and added to 200 ml of distilled water, 10 ml of orthophosphoric acid, and four drops of diphenylamine. The solution is titrated with 0.5 N ammonium ferrous sulphate until the colour changes from dark green to ocean green (Walkey and Black, 1947).

$$\text{Organic Carbon (\%)} = \frac{(B-S) \times 0.0006 \times 100}{1 \text{ (Wt. of the soil sample)}}$$

Table 3.3.1 Soil analysis

S. No.	Particulars	Method used	Summer season		*Kharif* season	
			2022	2023	2022	2023
1.	Soil pH	Glass electrode	6.56	6.59	6.58	6.59
2.	Soil EC (dS m^{-1})	Electrical Conductivity	0.786	0.789	0.795	0.797
3.	Organic carbon (%)	Wet digestion method	0.47	0.50	0.51	0.52
4.	Available Nitrogen (Kg ha^{-1})	Alkaline potassium per magnate method	234.9	235.6	235.9	235.5
5.	Available Phosphorus (Kg ha^{-1})	Olsen's method	9.84	9.94	9.96	9.96
6.	Available Potassium (Kg ha^{-1})	Flame photometer method	145.67	146.5	147.53	147.65

3.4 EXPERIMENTAL DETAILS

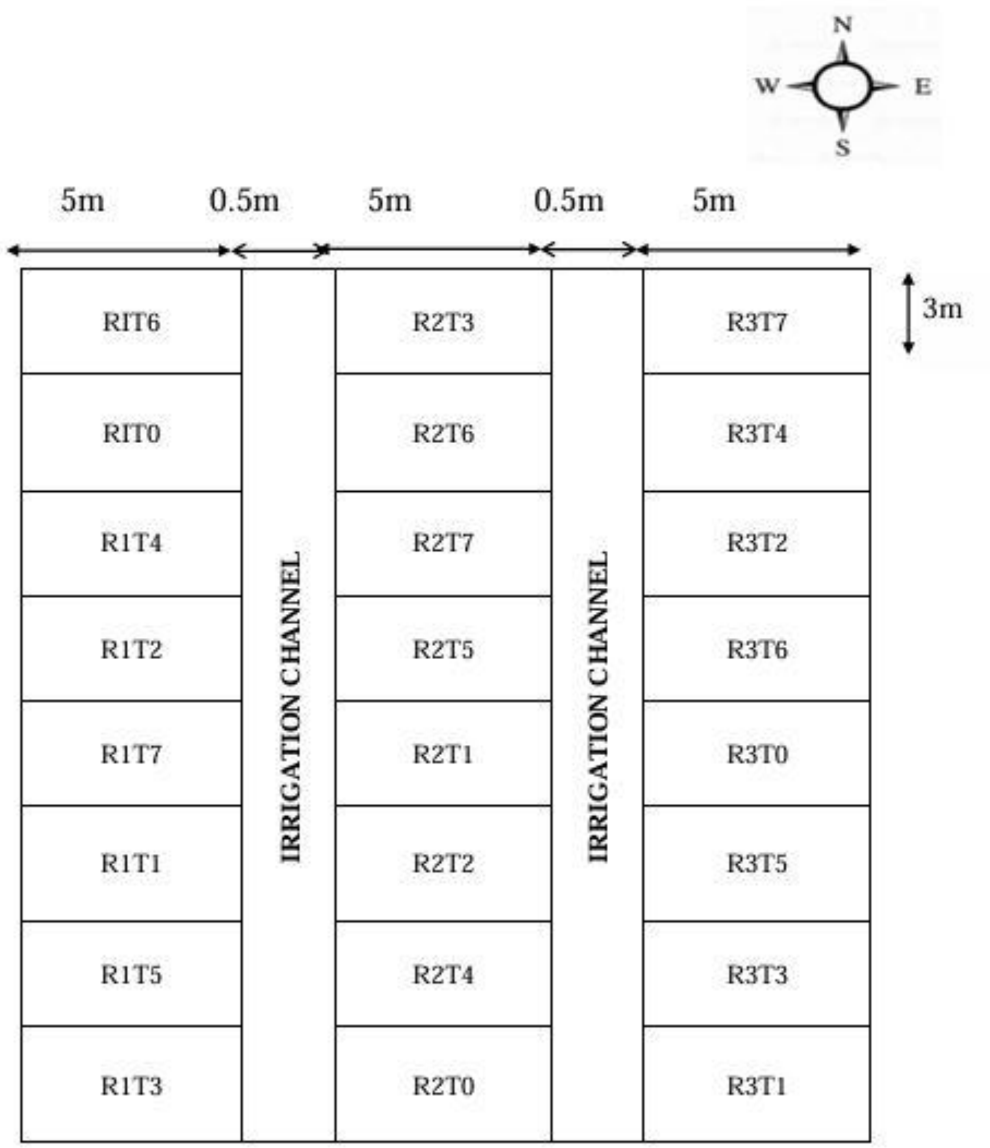
The experimental design was laid out during two consecutive seasons, i.e., the summer and the *Kharif* seasons in 2022 and 2023, using a Randomized Block Design having eight numbers of treatments and three numbers of replications. The number of treatments was allotted randomly in 24 plots for all the treatments to occur once in each replication. The replications were indicated as R1, R2 and R3 and treatments were named as T0, T1, T2, T3, T4, T5, T6 and T7, respectively. The details showing these three replications and eight treatments are given below:

3.4.1 Treatment details

Treatment no.	Treatment details
T_0	Control plot
T_1	Calcium (10mM)
T_2	Bio priming (rhizobium) (10ml for 1kg seed)
T_3	Putrescine (3mM)
T_4	Calcium (10mM) + Bio priming (rhizobium) (10ml)
T_5	Calcium (10mM) + Putrescine (3mM)
T_6	Bio priming (rhizobium) (10ml) + Putrescine (3mM)
T_7	Calcium (10mM) + Bio priming (rhizobium) (10ml)+ Putrescine (3mM)

Here, T represents treatments, there are 8 treatments ranging from (T_0 to T_7), T_0= control (only recommended dose of fertilizers are provided), T_1= calcium chloride (10mM), T_2= bio priming with rhizobium (10ml for 1kg seed), T_3= putrescine (3mM), T_4= calcium chloride (10Mm) + bio priming with rhizobium (10ml for 1kg seed), T_5= calcium chloride (10Mm) + putrescine (3mM), T_6= bio priming with rhizobium (10ml for 1kg seed) + putrescine(3mM), T_7= calcium chloride (10Mm) + bio priming with rhizobium (10ml for 1kg seed) + putrescine (3mM).

3.4.2 Layout design of the experiment



3.5 AGRONOMIC PRACTICES

The agronomic operation performed during the summer and *Kharif* seasons of 2022 and 2023 is given in table 3.5.

3.5.1 LAND PREPARATION

The field preparation was first done with a disc plough, followed by harrowing and planking to obtain a uniform and good tilth. The experimental design was then laid out after the field preparation was completed.

3.5.2 FERTILIZER APPLICATION

At the time of sowing, apply 5 kg of N (11 kg urea) and 16 kg P_2O_5 (100 kg of single superphosphate) per acre.

Table 3.5 Calendar showing the dates of the operation performed

Operations performed		Date at which operations performed			
		Summer 2022	**Summer 2023**	***Kharif* 2022**	***Kharif* 2023**
1.	Field preparation				
	a. Ploughing via disc plough	22 March	24 March	1 July	1 July
	b. Secondary tillage and planking via rotavator	23 March	24 March	1 July	3 July
	c. Seed bed preparation and sowing	25 March	27 March	2 July	4 July
	d. Fertilizer application	25 March	27 March	2 July	4 July
	e. Pre emergence herbicide application	26 March	28 March	4 July	5 July
	f. Thinning	9 April	10April	16 July	19 July
	g. Pesticide application	5 May	6 May	10 August	11 August
2.	Irrigation				
	a. First irrigation	25 March	27 March	2 July	4 July
	b. Second irrigation	18 April	19 April	27 July	29 July
	c. Third irrigation	3 May	4 May	11 August	14 August
	d. Fourth irrigation	18 May	19 May	26 August	26 August
3.	Weeding				
	a. First weeding	15 April	17 April	22 July	24 July

	b. Second weeding	30 April	2 May	6 August	8 August
4.	Treatment application				
	a. First application	10April	11April	17 July	19 July
	b. Second application	10 May	11 May	17 August	19 August
5.	Observation taken				
	a. First	25 April	26 April	2 August	4 August
	b. Second	25 May	26 May	3 September	5 September
5.	Harvesting	1 June	2 June	11 September	13 September
6.	Threshing and winnowing	6 June	7 June	17 September	18 September

3.5.3 SEED RATE AND SOWING

10 kg seed per acre for the *Kharif* season and 15kg per acre for the *Rabi* season were used. The seeds were sown with a spacing of 30*10 cm at a depth of about 4-6 cm as per the treatment. The variety selected for this study was SML-668.

3.5.4 IRRIGATION

The crop mostly requires four significant irrigations. The initial watering occurred one day post-sowing. The second irrigation occurred 25 days post-sowing. The third irrigation occurred at 40 days, while the last irrigation took place at 55 days post-sowing of the crop.

3.5.5 THINNING AND INTERCULTURAL OPERATION

Thinning was done after 15 days of sowing to remove the extra plants and maintain the desired population. The first weeding was done 20 days after sowing, and 2nd weeding was done manually 35 days after sowing by khurpi to avoid plant weed competition.

3.5.6. INSECT PEST MANAGEMENT

The crop was infested with aphids and caterpillars during the flowering stage. Chlorpyriphos of 2 ml per litre of water was sprayed to control the aphids and caterpillars.

3.5.7 HARVESTING AND THRESHING

The harvesting of crop was done at 80% of the pods matures by using a sickle for a meter square area and leaving the harvested crop in the field for about 3-4 days for complete drying of the pods. The crops were laid out on a floor, and threshing was done by beating the plant with a stick. Then, the threshed plants were cleaned, weighed, and recorded readings.

3.6 GROWTH ATTRIBUTES

3.6.1 Plant Height (cm)

Plant height was recorded at 30 and 60 days after sowing (DAS). The height of the tagged plant was measured from the ground to the apex of the uppermost leaf using a scale.

3.6.2 Total number of Branches

Total number of branches per plant was recorded manually, and the mean of each treatment was calculated.

3.6.3 Leaf number

After a few days of sowing, the plant's growth continued, and the number of leaves per plant was recorded. The total number of leaves in the whole plant was counted separately in each treatment in all three replicates. The mean of each treatment was calculated as several leaves per plant.

3.6.4 Leaf area

The laboratory calculated the Area covered by a particular plant leaf in its canopy after the leaf collection with the help of the Leaf area meter.

3.6.5 Leaf area index

LAI was a unit-less plant parameter that indicate an occupied canopy. It is obtained by dividing plant leaf area over plant land (Watson, 1947).

LAI= Total leaf area/ Total ground area

3.6.6 Leaf Area Duration (LAD)

LAD is the relationship between leaf area index, which is dignified against time. It was calculated with the help of the following formula:

LAD= $(L_1+L_2/2)*t_2-t_1$

Where LAI_1 is the leaf area index at time interval T_1

LAI_2 is the leaf area index at time interval T_2

T_1 and T_2 are time intervals

The final LAD was determined at absolute harvest by adding whole LAD values.

LAD is expressed in days

3.6.7 Days to 50% flowering

Observe flowering in the plant from the date of sowing. If 50% of the plant bears flowering, count the days to the 50% flowering.

3.6.8 Days to Physiological Maturity

From the date of sowing, observe maturity in the plant. If 80% of the pods turn brown in colour and dry, count the days to physiological maturity and harvest the crop.

3.6.9 Root length

Clean the roots of the plant with water. Place the roots on graph paper and measure them using a measuring scale.

3.6.10 Number of nodules

Manually count the total number of nodules at 30 and 60 DAS present in the root of the plant.

3.6.11 SPAD index (SPAD metre)

The SPAD index of the plants was taken using the SPAD meter. The selected and tagged plants were used to take the chlorophyll index. Readings were taken from the same leaf at different places. A minimum of 5 readings were taken, and the average was taken as a single reading. Likewise, readings were taken from different leaves of the tagged plants.

3.6.12 Chlorophyll content (mg g^{-1} fresh weight)

Arnon DI. (1949) proposed a method for estimating chlorophyll concentration.

Principle

Measure the absorbance at 645 nm and 663 nm after extracting chlorophyll with 80% acetone. The absorbance coefficient is used to determine the amount of chlorophyll.

Reagent

Acetone (80%, pre-chilled)

Procedure

Measure 20 millilitres of 80% acetone to extract chlorophyll from 100 milligrams of leaf material. After centrifugation at 5000 rpm for 10 minutes, the liquid at the top was transferred to a volumetric flask. The residue was subjected to repeated extractions until it lost its colour. A total of one hundred millilitres of 80% acetone was added to the flask. Spectrophotometer readings were taken at 645 and 663 nm about an 80% acetone blank to determine the extract's absorbance. The chlorophyll content was quantified using the methodology provided below.

Total chlorophyll (mg/g Fresh Weight) = 20.2(A645) +8.02(A663)*V1000*W

V = Final volume of the extract; W = Fresh weight of the leaves.

The value is denoted as mg/g of fresh weight.

3.6.13 Total soluble protein (Lowry, 1951):

Principle:

Folin-Ciocalteau reagent (FCR) and protein react to form a blue complex. The colour produced results from the protein's tyrosine and tryptophan amino acid reactions with the alkaline copper and the FCR's phosphomolybdic phosphotungstic components. At 660 nm, the colorimetric measurement of blue colour intensity is taken.

Reagents

1. Solution A: 0.2 per cent sodium hydroxide in 0.1 per cent sodium hydroxide.
2. For solution B, make a new solution of 1% sodium potassium tartrate and 0.5% copper sulphate ($CuSO_4.5H_2O$).
3. Mix 50 ml of solution A with 1 ml of solution B right before use to make Solution C, an alkaline copper solution.
4. When using Folin-Ciocalteau reagent (FCR), dilute it with water to the same volume as when you use it. A commonly used protein solution is Bovine serum albumin 50 milligrams in 50 millilitres of water.
5. For the working standard solution, 200ug protein/ml can be achieved by diluting 10 ml of the stock solution with 50 ml of water.

Procedure

Extraction of protein from the sample

1. Grind 0.5 g of the plant material in a mortar and pestle with an appropriate solvent solution (buffer or water).
2. Use the centrifuge to isolate the supernatant for protein quantification.

Protein Estimation

1. Utilize a pipette to extract 0.2, 0.4, 0.6, 0.8, and 1.0 ml of the working standard solution and put these volumes into a sequence of test tubes. Concurrently, transfer 0.1 ml and 0.2 ml of the sample extract into two separate test tubes.
2. Introduce water to increase the capacity in each tube to 1 ml. A tube carrying 1 cc of water serves as the blank.
3. Introduce 5ml of solution C, mix carefully, and permit incubation at ambient temperature for a period of 10 minutes.
4. Incorporate 0.5 ml of FCR, mix completely without delay, and allow it to incubate at room temperature in a dark setting for 30 minutes.
5. Assess the absorbance at 660 nm use the blank as a reference standard.
6. Construct a standard graph and ascertain the protein concentration in the sample.
7. Report the results as mg g^{-1}, mg/100g of the sample, or as a percentage.
8. A reducing agent, such as cysteine or NaCl, should be employed to prepare the extract if the sample has a high concentration of phenolic compounds or pigments.

Initially, precipitate the protein utilising trichloroacetic acid (TCA). Upon separation, dilute it in 2N NaOH and proceed with the protein quantification procedure.

3.6.14 Total protein and nitrogen content

Principle: Proteins consist of chains of amino acids and are essential components of food. Nitrogen is a crucial constituent of proteins. Every protein has its nitrogen composition. Measuring nitrogen levels is critical for determining protein content. The Kjeldahl Method is used to determine the concentration of nitrogen.

Requirements:

For digestion: Use Kjeldahl flasks with a volume ranging from 500 to 800 millilitres. The digestion process uses a heating apparatus set to raise the temperature of 250 ml of water from 25° C to a vigorous boiling point in about 5 minutes. Add 3 to 4 boiling chips or glass beads to avoid the risk of overheating.

For titration: Insert a rubber stopper into the flask, ensuring that the bottom end of a highly efficient scrubber trap or bulb is passed through it to avoid the mechanical transfer of alkali during the distillation process. Attach the upper part of the trap to a condenser using either rubber or glass tubing. Submerge the trap outlet of the condenser to ensure the complete absorption of ammonia distilled into acid within a 500-ml Erlenmeyer flask.

Reagents: 25 Concentrated Sulphuric acid, 10g sodium sulphate, 0.5g cupric sulphate.

Procedure:

Digestion:

- Measure about 1 gram of the sample. Then, add 0.5 grams of cupric sulphate and 10 grams of sodium sulphate to the sample.
- Place them inside a Kjeldahl flask. Dispense 25 ml of concentrated sulphuric acid.
- Place the flask on a heating apparatus for 2-3 hours to facilitate protein digestion.
- Subsequently, allow it to cool for 10 - 15 minutes.

Distillation:

- Initiate the protein assembly by adding distilled water to the Kjheldhal flask, thoroughly combining the contents, and placing the flask on the burner.
- Next, place the receiver on the other side, which contains H_2SO_4 and the indicator methylene red.
- To neutralise the sample, add a 50% NaOH (sodium hydroxide) solution until it turns black.
- Subsequently, five glass beads should be incorporated to prevent any unevenness, and a single drop of an anti-foaming agent should be included to avoid foam formation.
- Apply heat until the protein accumulates on the other side of the assembly, then collect the sample in a beaker with a volume of about 200 ml.

Titration:

- Fill the burette with a solution of NaOH with a normality of 0.1 and neutralise the sample.
- The sample turns yellow after neutralisation.
- Observe the reading at which the solution becomes neutral.

Fill the burette with sodium hydroxide (NaOH) solution for the blank. Add 50 ml of sulphuric acid (H_2SO_4) solution in a beaker at a concentration of 0.1N. Add the indicator methylene blue to the beaker. Titrate the solution until a yellowish colour appears.

Calculation:

$$\textbf{Protein content} = \frac{(B-S) \times N \times 1.4007}{W}$$

Where,

B= Blank, S= Sample, N = Normality, W = sample weight

3.6.15 Total carbohydrates (Tamboli *et al.*, 2020)

Total carbohydrates were measured with the Phenol sulphuric acid method

Requirements:

1. 2.5N HCl (25ml Conc. HCl +91ml of distilled water)

2. 5% phenol solution (5ml in 95ml of distilled water) stored in an amber colour bottle

3. 96% H_2SO_4 (96 m in 4ml of distilled water)

4. Na_2CO_3

Procedure:

1. Crush 0.1g of plant sample with little HCl.

2. Add 5ml of 2.5N HCl and place in boiling water bath for 3 hours and bring to room temperature

3. Add Na_2CO_3 pinch by pinch (up to bubble formation stops) and centrifuge at 5000rpm for 10 minutes

4. Pipette out 10ul of extract and add 90ul of distilled water, which is then mixed in a vortex mixture

5. Add 5% phenol (100ul of 5% phenol) and 500ul of H_2SO_4, place at room temperature for 30 minutes, and record the absorbance at 490nm.

3.6.16 PAL activity

Rao and Tower's (1970) method was used.

Principle

Spectrophotometrically measuring the rise of trans-cinnamic acid from phenylalanine at 650nm can be used to test the enzyme.

Reagents

1. Prepare 0.2M sodium borate buffer with a pH of 8.7
2. To make 0.01M L-phenylalanine (pH 8.7), sodium borate buffer is used.
3. 0.05M tris-HCl buffer having pH 8.8
4. 1N HCl
5. Ether with no peroxide
6. 0.05N NaOH: make fresh from 1N NaOH
7. Make standard cinnamic acid and mix it with borate buffer.
8. 0.8 ml/litre of mercaptoethanol

Procedure

3.0 grams of fresh leaf sample were mixed with 2.6 ml sodium borate with 0.8 ml/litre of 2-mercaptoethanol. At 2-4°C, this was centrifuged at 7000g for 10 minutes. The rest of the mixture was saved. To get the pH of the residue to 5.5, acetic acid (1M) was added. 1 ml of 0.05M Tris-HCl solution, 0.5 ml of 0.01M L-phenylalanine, and 0.4 ml of water were mixed and left at 30°C for 5 minutes. When 0.1 ml of the enzyme was added, the reaction began. It was then kept warm at 30°C for 60 minutes. A blank sample that did not contain phenylalanine was made. It stopped the process when 0.5 ml of 1N HCl was added. Again and again, 3.5 ml of ether was used to separate the mixture. The ether phage was removed, and the supernatant was separated and dried in air. The 3 ml of 0.05N NaOH was dissolved with the supernatant. Please keep it on rest for overnight. The mixture was centrifuged at 2000g for 15 minutes, and the supernatant was saved. One millilitre of the above supernatant (extract) was mixed with one millilitre of Folin-Ciocalteau solution. A wavelength of 650 nm was used to measure absorption after heating the mixture for one minute. The same steps were used to make a calibration curve of a known dilution of cinnamic acid as the sample. The amount of PAL in the sample was given as μ moles of cinnamic acid produced/min/mg protein.

3.6.17 Total Flavanol content

The flavanol content was determined using the methodology described by Akkol *et al.,* in 2008.

Principle: The fundamental concept behind the Aluminium chloride colorimetric approach is that Aluminium chloride creates stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavanol and flavonoids, which are resistant to acid.

Reagents: Methanol 80%, Sodium Acetate, Aluminium Chloride

Procedure: To estimate the amount of Flavanol, a plant sample weighing 0.05g was extracted using boiling 80% methanol for 3 hours. A mixture was prepared by combining 1 ml of methanol extract, 3 ml of sodium acetate, and 1 ml of aluminium chloride solution. The absorbance of the mixture was measured at a wavelength of 445 nm after 2.5 hours.

3.6.18 Total Flavanoid content

Reagents: Quercetin, 5% Sodium nitrite solution, 1% aqueous aluminium chloride, hydroxide solution.

Procedure:

1. Quercetin was kept as a standard for plotting the calibration curve to calculate the total flavonoid content.
2. 250ul of the sample was taken, and 10ul of 5% aqueous sodium nitrite solution was added.
3. After 5 minutes of incubation, 10ul of 10% aqueous aluminium chloride was added and incubated for 6 minutes at room temperature.
4. A UV spectrophotometer was added to 100ul of 1M aqueous solution hydroxide solution, and absorbance was measured at 510nm.
5. Total flavonoid content was calculated in mg quercetin in equivalent /gm dry weight.

3.6.19 Phenols

Phenols were calculated using the method given by Chattopadhyay and Samaddar (1980).

Reagents:

80% ethanol, Diethyl ether, 0.5M NaOH, 3% Sodium lauryl sulphate solution, FCR reagent.

Procedure:

100mg of plant sample was ground in mortar and pestle with 5ml of SDS solution. Centrifuge for 5 minutes at 2000g, then discard the supernatant. Wash the residue once with 5ml of SDS solution, twice with 5ml of ethanol and twice with 10ml of diethyl ether. The residue was dried and suspended in 3 ml of 0.5M NaOH. Keep it at room temperature overnight. Again, centrifuge the mixture at 2000g for 15 minutes to save the supernatant. Now, take 1ml of supernatant with 1ml of folinsciocalteau reagent and add to it. Heat the mixture for 1 minute and record the absorbance at 650nm.

3.6.20 Arginine content

The arginine content was analysed by HPLC using an Amnex HPX-87H column no. 18 BIST, column part no. TPB-46.180.30, column size of 4.6 *150mm, 5um with a mobile phase of MeC N/H_2O-70/30%. The buffer used is H_2SO_4-0.20% with a flow rate of 1.0ml/min and is detected under UV210nm.

3.6.21 Test weight

The weight of 1000 seeds is determined using an electric balance. A random sample of 1000 seeds was collected from each treatment plot, and their weight was recorded.

3.6.22 Number of seeds pod^{-1}

A total of 10 pods were randomly sampled from a more extensive collection of pods taken from 10 plants in each treatment plot to determine the number of pods per plant. The average number of seeds per pod was calculated better to understand the total number of seeds per pod.

3.6.23 Seed weight plant^{-1}

Harvest the crop and use a precise digital balance to weigh the seed weight per plant. Record the weight in grams.

3.6.24 Seed weight pod^{-1}

Harvest the crop and use a precise digital balance to weigh the seed weight per pod. Record the weight in grams.

3.6.25 Biological yield

The biological yield is obtained by adding the straw and seed yields.

3.6.26 Seed yield

The seed yield was determined by manually threshing half of the plot area for each experimental unit and recording the results in kilogrammes. Subsequently, it is transformed into kilogrammes per hectare (kg/ha).

3.6.27 Stover yield

The stover yield is calculated by subtracting the economical yield from the biological yield.

3.6.28 Harvest index

The harvest index is determined by calculating the economical yield (kg ha^{-1}) by the biological yield (kg ha^{-1}) and multiplying the result by 100.

$$\text{Harvest index (\%)} = \frac{\text{Economical yield (kg ha}^{-1}\text{)}}{\text{Biological Yield (kg ha}^{-1}\text{)}} \times 100$$

3.6.29 Economics

The cultivation costs and total income generated per hectare for each treatment were calculated using the prices of inputs and outputs that were in effect during the experimental period. The net returns per hectare were determined by subtracting the cultivation costs from the crop's overall monetary worth.

The benefit-cost ratio (B: C) was computed using the following formula

$$\text{B: C ratio} = \frac{\text{Gross return (Rs. ha}^{-1}\text{)}}{\text{Cost of cultivation (Rs. ha}^{-1}\text{)}}$$

PICTURES OF THE TRIAL

LAND PREPARATION

PREPARATION OF SEED BEDS

SOWING OF SEED

IRRIGATION

PRE-EMERGENCE HERBICIDE

APPLICATION OF HERBICIDE

PICTURES OF CROP

APPLICATION OF TREATMENT

FLOWERING

DATA RECORDING

PHYSIOLOGICAL MATURITY

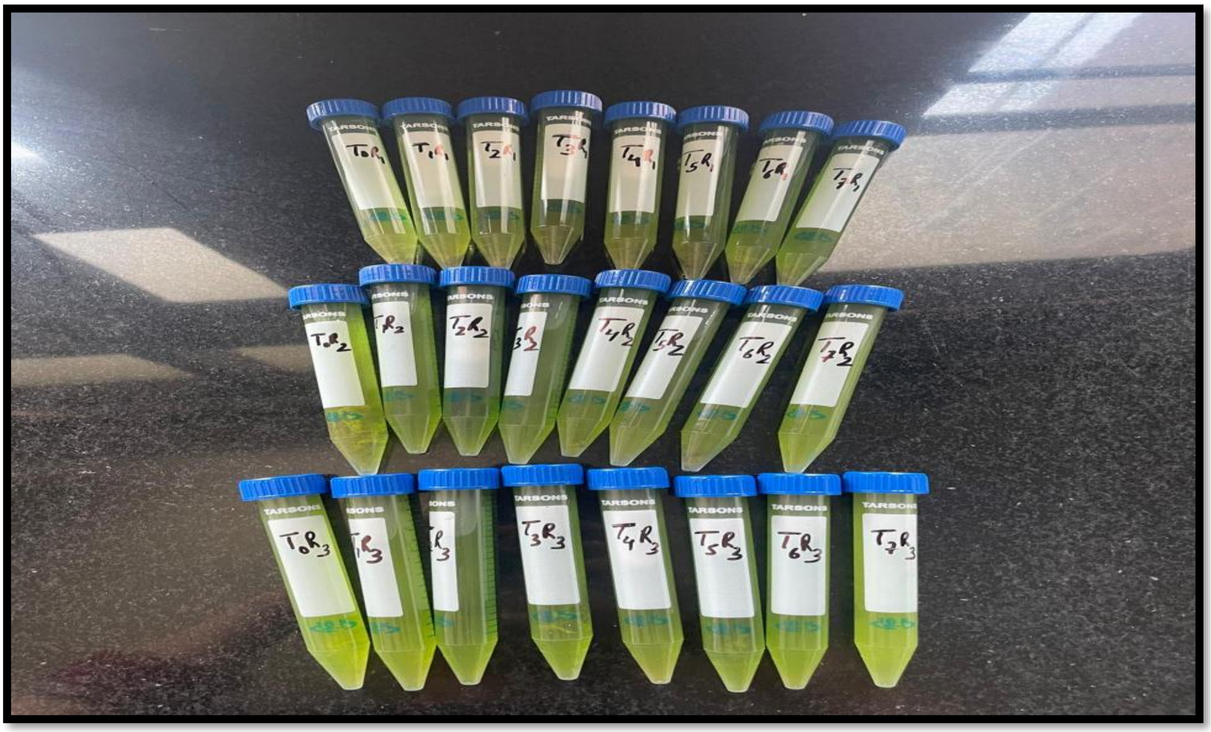
HARVESTING

AFTER HARVESTING

THREASHING

YIELD

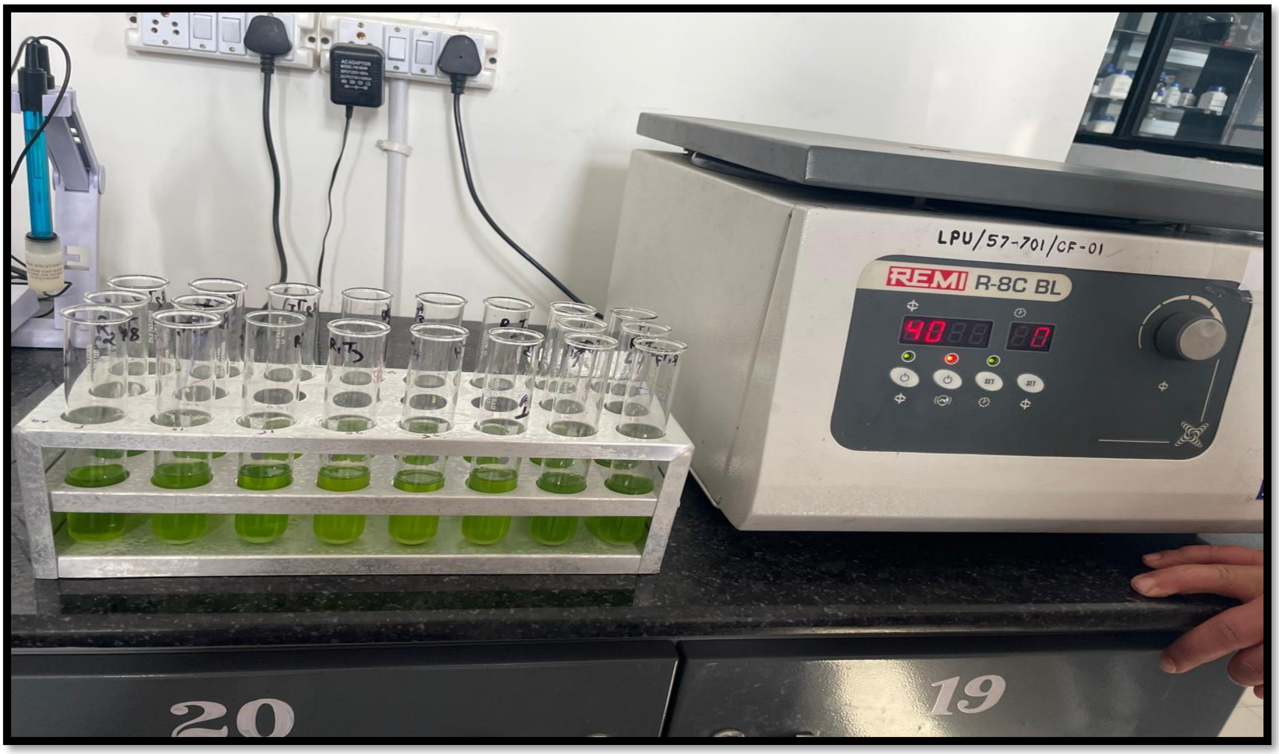
BIOCHEMICAL ANALYSIS IN LAB



BIOCHEMICAL ANALYSIS

CENTRIFUGE

BIOCHEMICAL ANALYSIS



BIOCHEMICAL ANALYSIS

CHAPTER-4

RESULTS AND DISCUSSIONS

The present research work entitled "**Impact of Biopriming, Putrescine and Calcium on growth, yield and quality of green gram (*Vigna radiata* L.)**" was carried out during the *Summer* and *Kharif* season of 2022 and 2023 in the Department of Agronomy as a field experiment in the agriculture farm, School of Agriculture, Lovely Professional University Phagwara. The present study was carried out to determine the impact of seed priming with rhizobium, foliar application with putrescine, and calcium in the variety SML-668 of green gram. There were four main parts of the experiment. In the first part, the investigation was developed to find out the morphological parameters of the green gram crop under all the treatments at 30 and 60 DAS. The second part of the study represents the biochemical responses of green gram plants under control, rhizobium, putrescine and calcium alone and in combination at 30 and 60DAS. The third part was related to the impact of yield and yield attributes of green gram, and lastly, the fourth part is all about seeds. Details of the experimental procedure are given in the previous chapter. In this chapter, an attempt has been made to depict and explain the recorded data. The findings of the two-year research experiments (2022, 2023) *Summer* and *Kharif* season are described under the following headings. The summer season crop shows better results than kharif season crop because the weather conditions are more favourable in summer season as compared to the kharif season. The variety used for the experiment is SML-668 which is summer mung bean. This variety provides better results in summer season.

4.1 **Growth and Developmental Studies**

4.1.1 Plant height

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Plant height was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.1.1.a and 4.1.1.b and illustrated in Figures 4.1.1.a and 4.1.1.b.

In the *Summer* of 2022, treatment T_7 significantly increased plant height, showing improvements of 8.9% and 6.2% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased plant height by 8.6% and 6.1%, while treatment T_6 showed 7.7% and 5.3% reductions. The lowest plant heights were observed in treatment T_1, with 1.0% and 0.5% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in plant height, with 6.0% and 4.3% improvements. Conversely, treatment T_5 decreased by 4.9% and 3.9%, and treatment T_1 recorded the lowest plant heights with 0.4% and 0.1% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest plant height, with 10.2% and 6.4% increases. Treatment T_5 decreased 8.9% and 5.1%, while the lowest plant heights were recorded for treatment T_1, with 2.4% and 3.2% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest plant heights, increasing 6.8% and 5.9%. Treatment T_5 followed with improvements of 5.2% and 5.6%. The minimum plant height in the *Kharif* season was observed in treatment T_1, showing 2.4% and 2.2% reductions compared to the control.

The combined application of putrescine, calcium, and rhizobium positively influences the plant height of green gram by promoting growth through enhanced cell elongation, nutrient availability, and stress management. Calcium and Putrescine can improve cell elongation and stability, resulting in more significant plant height (Kumar *et al.,* 2024). Rhizobium and putrescine can help the plant manage stress and ensure sufficient nitrogen for growth, increasing plant height. Rhizobium and Calcium provide the necessary nitrogen for growth, and calcium ensures the structural integrity and optimal signalling pathways for maximum plant height (Rupali *et al.,* 2018). Putrescine is involved in cell division, differentiation, and elongation, which can lead to increased plant height. Calcium is an essential plant nutrient for maintaining cell wall structure and stability, signalling, and enzyme activity. Rhizobium provides the plant with essential nitrogen and enhances nitrogen availability, increasing biomass and plant height (Chaurasia *et al.,* 2024).

Table 4.1.1.a: Impact of the treatments on plant height (cm) of green gram in *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	42.43^{d}±0.25	41.87^{d}±0.81	62.44^{e}±0.25	61.57^{c}±1.22
Calcium	42.83^{d}±0.25 [1.0]	42.93^{cd}±1.19 [2.4]	62.74^{e}±0.13 [0.5]	63.64^{b}±2.12 [3.2]
Bio-priming with rhizobium	42.50^{d}±0.10 [0.2]	42.55^{d}±0.61 [1.5]	62.47^{e}±0.06 [0.1]	63.10^{bc}±0.44 [2.4]
Putrescine	43.83^{c}±0.55 [3.3]	43.45^{bcd}±0.35 [3.6]	63.71^{d}±0.46 [2.1]	62.92^{bc}±0.47 [2.1]
Calcium + Bio-priming with rhizobium	45.07^{b}±0.42 [5.9]	44.25^{bc}±0.26 [5.3]	64.96^{c}±0.31 [3.9]	63.61^{bc}±0.81 [3.2]
Calcium + Putrescine	46.40^{a}±0.26 [8.6]	46.02^{a}±0.89 [8.9]	66.43^{a}±0.25 [6.1]	64.91^{ab}±0.67 [5.1]
Bio-priming with rhizobium + Putrescine	45.93^{a}±0.47 [7.7]	45.06^{ab}±1.23 [7.0]	65.88^{b}±0.45 [5.3]	64.90^{ab}±1.48 [5.1]
Calcium + Bio priming with rhizobium + Putrescine	46.53^{a}±0.35 [8.9]	46.68^{a}±1.29 [10.2]	66.54^{a}±0.35 [6.2]	65.84^{a}±0.86 [6.4]
C.D at p=0.05	0.61	1.59	0.56	1.83

Note:

1. Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2. Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.1.a: Impact of the treatments on plant height (cm) of green gram in *Summer* season

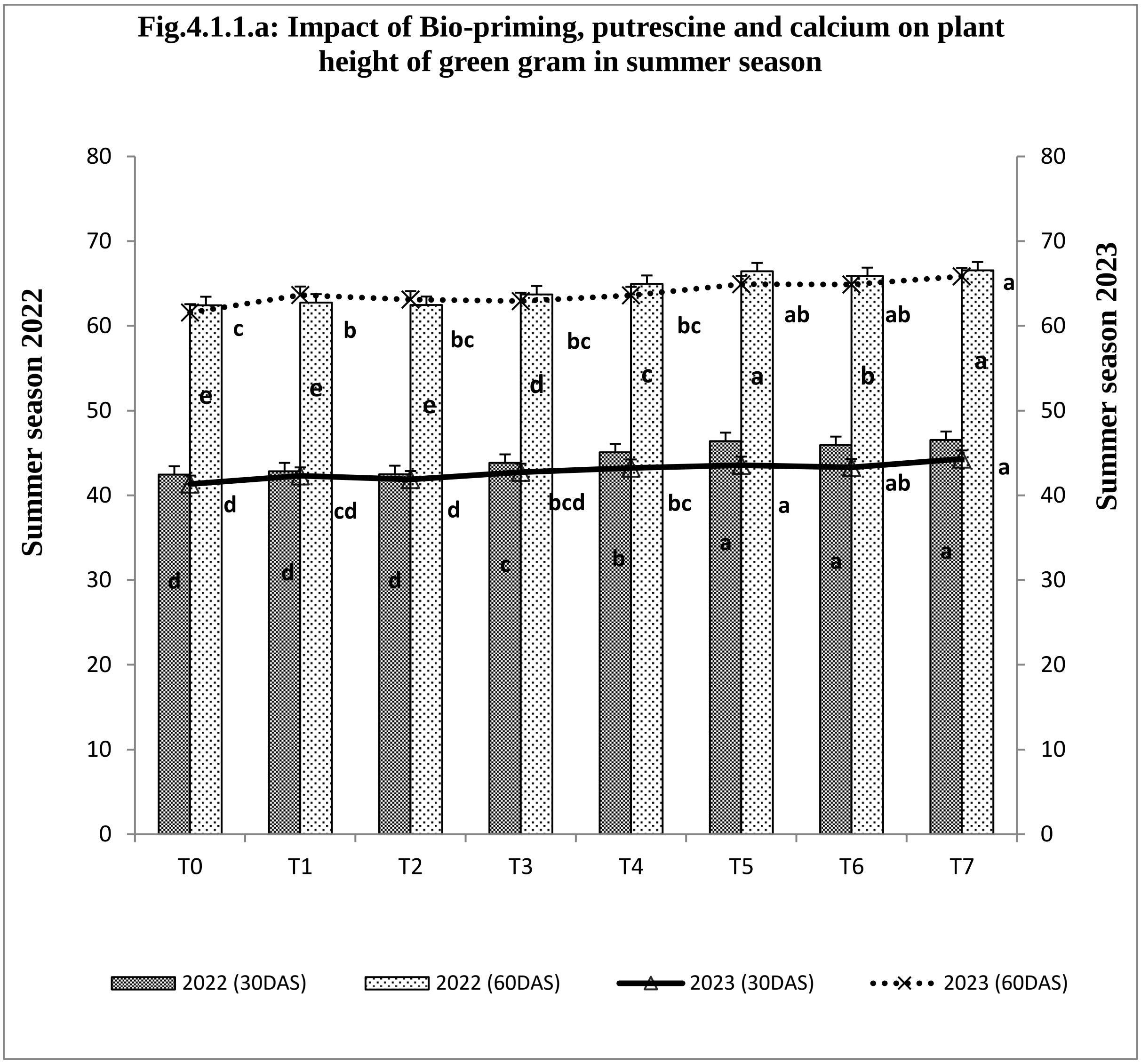


Table- 4.1.1.b: Impact of the treatments on plant height (cm) of green gram in *Kharif* season

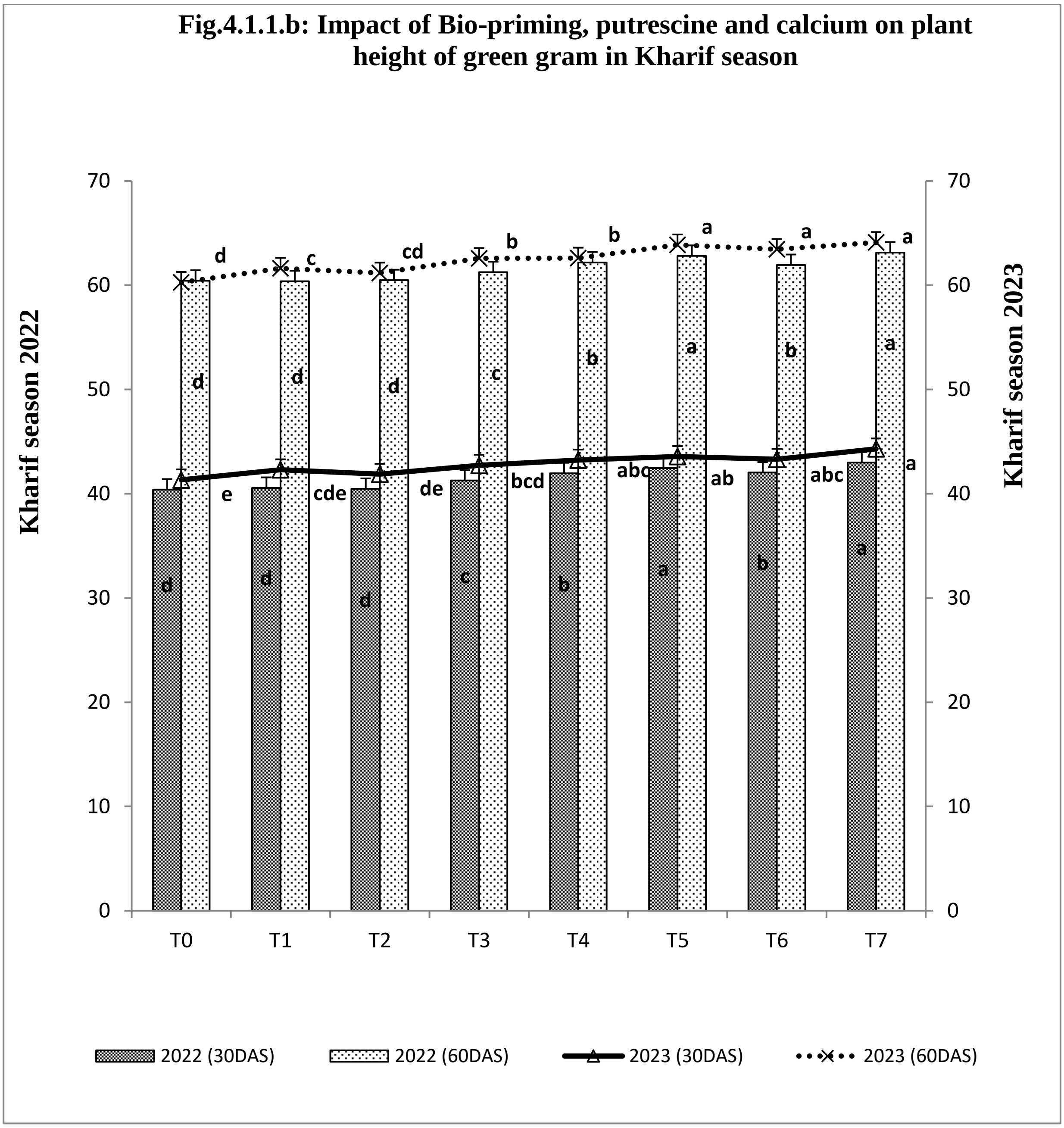
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	$40.40^{d}\pm0.17$	$41.33^{e}\pm0.51$	$60.43^{d}\pm0.15$	$60.27^{d}\pm0.21$
Calcium	$40.57^{d}\pm0.47$ [0.4]	$42.30^{cde}\pm0.40$ [2.4]	$60.39^{d}\pm0.18$ [0.1]	$61.63^{c}\pm0.42$ [2.2]
Bio-priming with rhizobium	$40.47^{d}\pm0.21$ [0.2]	$41.86^{de}\pm0.31$ [1.4]	$60.49^{d}\pm0.20$ [0.1]	$61.17^{cd}\pm0.32$ [1.4]
Putrescine	$41.27^{c}\pm0.65$ [2.1]	$42.73^{bcd}\pm0.40$ [3.4]	$61.26^{c}\pm0.65$ [1.4]	$62.57^{b}\pm0.50$ [3.6]
Calcium + Bio-priming with rhizobium	$41.97^{b}\pm0.59$ [3.7]	$43.23^{abc}\pm0.55$ [4.5]	$62.19^{b}\pm021$ [2.9]	$62.60^{b}\pm0.56$ [3.7]
Calcium + Putrescine	$42.47^{a}\pm1.01$ [4.9]	$43.57^{ab}\pm0.71$ [5.2]	$62.82\pm^{a}0.50$ [3.9]	$63.87^{a}\pm0.58$ [5.6]
Bio-priming with rhizobium + Putrescine	$42.03^{b}\pm0.46$ [3.9]	$43.30^{abc}\pm0.85$ [4.6]	$61.94^{b}\pm0.40$ [2.5]	$63.43^{a}\pm0.35$ [4.9]
Calcium + Bio priming with rhizobium + Putrescine	$43.00^{a}\pm0.53$ [6.0]	$44.30^{a}\pm0.70$ [6.8]	$63.13^{a}\pm0.41$ [4.3]	$64.10^{a}\pm0.53$ [5.9]
C.D at p=0.05	0.92	1.05	0.55	0.83

Note:

1. Calcium, Putrescine and Rhizobium were used in [10 mM, 3mM and 10 ml kg^{-1} of seeds]

2. Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.1.b: Impact of the treatments on plant height (cm) of green gram in *Kharif* season



4.1.2 Total no. of branches

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Total no. of branches was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.1.2.a and 4.1.2.b and illustrated in Figures 4.1.2.a and 4.1.2.b.

In the *Summer* of 2022, treatment T_7 significantly increased in total no. of branches, showing improvements of 33.9% and 32.4% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased total no. of branches by 30.8% and 29.7%, while treatment T_6 showed 22.6% and 27.9% reductions. The lowest total no. of branches was observed in treatment T_1, with 4.0% and 19.9% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in total no. of branches, with 39.4% and 37.7% improvements. Conversely, treatment T_5 decreased by 36.5% and 36.3%, and treatment T_1 recorded the lowest total no. of branches with 34.7% and 34.9% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest total no. of branches, with 46.4% and 34.9% increases. Treatment T_5 decreased by 45.0% and 34.1%, while the lowest total no. of branches was recorded for treatment T_1, with 23.1% and 18.7% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest total no. of branches, increasing 37.3% and 34.9%. Treatment T_5 followed with improvements of 35.3% and 28.3%. The minimum total no. of branches in the *Kharif* season was observed in treatment T_1, showing 31.0% and 27.2% reductions compared to the control.

Calcium increases plant cell structure, strengthening shoots and branches. Since the plant can grow more, it may have more branches (Naeem *et al.*, 2017). Putrescine, a polyamine, regulates cell division, differentiation, and stress response. Nutrient intake and senescence delay are also increases. Putrescine stimulates cell division and elongation, especially early growth, promoting branching. Rhizobium bacteria fix atmospheric nitrogen for legumes. Nitrogen is essential for vegetative development and in amino acids, proteins, and chlorophyll. Rhizobium Bio-priming increases nitrogen availability, promoting vegetative solid growth and branching. These treatments, in combination, can increase green gram plants. Improvements in nutritional availability (calcium and nitrogen), physiological processes (putrescine), and plant health can stimulate branching (Noufal., 2018).

Table-4.1.2.a: Impact of the treatments on total no. of branches of green gram in the *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	2.40^{d}±0.20	2.00^{d}±0.20	3.63^{f}±0.15	3.47^{c}±0.15
Calcium	2.50^{d}±0.10 [4.0]	2.60^{bc}±0.20 [23.1]	4.53^{d}±0.15 [19.9]	4.27^{b}±0.21 [18.7]
Bio-priming with rhizobium	2.53^{d}±0.21 [5.3]	2.30^{cd}±0.20 [13.0]	4.10^{e}±0.20 [11.5]	3.77^{c}±0.25 [7.9]
Putrescine	2.73^{cd}±0.15 [12.2]	2.83^{b}±0.25 [29.4]	4.73^{cd}±0.15 [23.3]	4.33^{b}±0.25 [19.9]
Calcium + Bio-priming with rhizobium	3.00^{bc}±0.20 [20.0]	3.40^{a}±0.20 [41.2]	4.83^{cd}±0.25 [24.9]	4.63^{b}±0.15 [25.1]
Calcium + Putrescine	3.47^{a}±0.15 [30.8]	3.63^{a}±0.15 [45.0]	5.17^{ab}±0.12 [29.7]	5.27^{a}±0.25 [34.1]
Bio-priming with rhizobium + Putrescine	3.10^{b}±0.10 [22.6]	3.50^{a}±0.26 [42.9]	5.03^{bc}±0.15 [27.9]	5.10^{a}±0.20 [32.0]
Calcium + Bio priming with rhizobium + Putrescine	3.63^{a}±0.15 [33.9]	3.73^{a}±0.15 [46.4]	5.37^{a}±0.15 [32.4]	5.33^{a}±0.31 [34.9]
C.D at p=0.05	0.30	0.38	0.30	0.40

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control.

Fig.4.1.2.a: Impact of Bio-priming, putrescine and calcium on total no. of branches of green gram in summer season

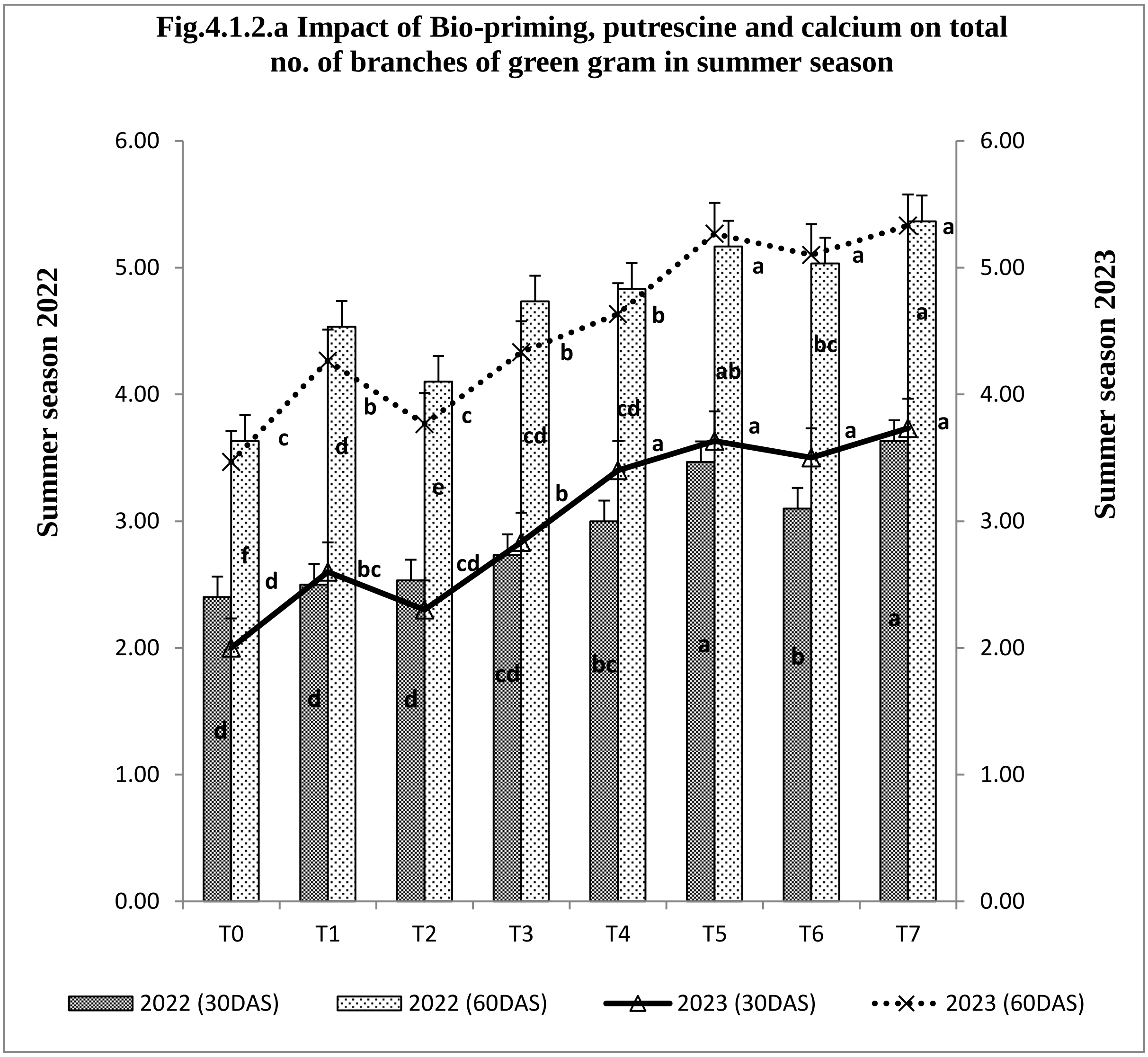


Table-4.1.2.b: Impact of the treatments on total no. of branches of green gram in *Kharif* season

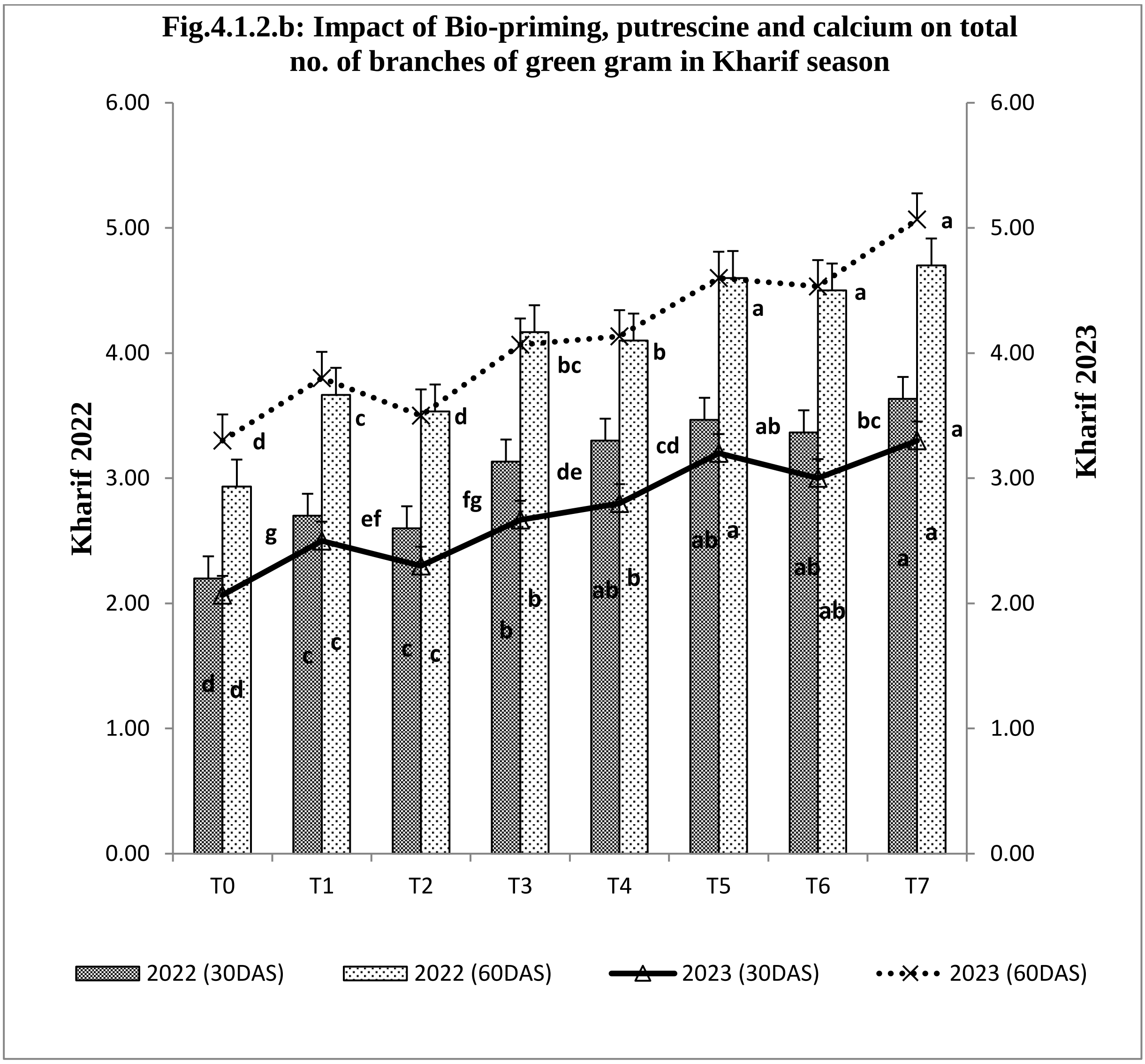
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	$2.20^{d}\pm0.26$	$2.07^{g}\pm0.12$	$2.93^{d}\pm0.32$	$3.30^{d}\pm0.20$
Calcium	$2.70^{c}\pm0.20$ [18.5]	$2.50^{ef}\pm0.10$ [17.2]	$3.67^{c}\pm0.21$ [20.1]	$3.80^{c}\pm0.10$ [13.2]
Bio-priming with rhizobium	$2.60^{c}\pm0.20$ [15.4]	$2.30^{fg}\pm0.10$ [10.0]	$3.53^{c}\pm0.15$ [17.1]	$3.50^{d}\pm0.10$ [5.7]
Putrescine	$3.13^{b}\pm0.15$ [29.8]	$2.67^{de}\pm0.15$ [22.4]	$4.17^{b}\pm0.38$ [29.7]	$4.07^{bc}\pm0.12$ [18.9]
Calcium + Bio-priming with rhizobium	$3.30^{ab}\pm0.10$ [33.3]	$2.80^{cd}\pm0.10$ [26.1]	$4.10^{b}\pm0.10$ [28.5]	$4.13^{b}\pm0.15$ [20.2]
Calcium + Putrescine	$3.47^{ab}\pm0.15$ [36.5]	$3.20^{ab}\pm0.20$ [35.3]	$4.60^{a}\pm0.20$ [36.3]	$4.60^{a}\pm0.20$ [28.3]
Bio-priming with rhizobium + Putrescine	$3.37^{ab}\pm0.15$ [34.7]	$3.00^{bc}\pm0.10$ [31.0]	$4.50^{ab}\pm0.20$ [34.9]	$4.53^{a}\pm0.15$ [27.2]
Calcium + Bio priming with rhizobium + Putrescine	$3.63^{a}\pm0.15$ [39.4]	$3.30^{a}\pm0.20$ [37.3]	$4.70^{a}\pm0.20$ [37.7]	$5.07^{a}\pm0.15$ [34.9]
C.D at p=0.05	0.32	0.25	0.41	0.27

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control.

Fig.4.1.2.b: Impact of Bio-priming, putrescine and calcium on total no. of branches of green gram in *Kharif* season



4.1.3 No. of leaves

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. No. Leaves were measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.1.3.a and 4.1.3.b and illustrated in Figures 4.1.3.a and 4.1.3.b.

In the *Summer* of 2022, treatment T_7 significantly increased the number of leaves, showing improvements of 26.8% and 16.2% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased the number of branches by 25.1% and 14.6%, while treatment T_6 showed 23.8% and 13.5% reductions. The lowest number of leaves was observed in treatment T_1, with 16.6% and 9.1% reductions, respectively.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in no. of leaves, with 28.9% and 17.6% improvements. Conversely, treatment T_5 decreased by 26.3% and 16.8%, and treatment T_1 recorded the lowest no. of leaves with 9.2% and 8.1% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest no. of leaves, with 19.2% and 17.5% increases. Treatment T_5 decreased 18.1% and 17.0%, while the lowest no. of leaves was recorded for treatment T_1, with 8.3% and 8.3% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest no. of leaves, increasing 18.9% and 17.8%. Treatment T_5 followed with improvements of 16.0% and 13.8%. The minimum no. of leaves in the *Kharif* season was observed in treatment T_1, showing 1.9% and 5.8% reductions compared to the control.

The increase in leaf number can be related to the role of calcium in promoting cell division in the leaf meristems, resulting in a larger leaf area and a more significant number of leaves. This study's observed increase in leaf quantity may be attributed to putrescine function in stimulating cell division in the apical and lateral meristems and improving stress tolerance, hence facilitating continuous vegetative development. Rhizobium bacteria, via symbiotic nitrogen fixation, offer vital nitrogen to the plant, which is crucial for vegetative growth and leaf development (Sharma and Dhanda., 2015). The increased nitrogen availability probably enhanced growth and an increased leaf count. Applying calcium and putrescine to the leaves and treating them with Rhizobium by Bio-priming greatly enhances the leaf count in green gram. The increase in the number of leaves is vital for improving the plant's photosynthetic capability, which is directly linked to a more significant potential for yield (Noufal.,2018).

Table-4.1.3.a: Impact of the treatments on no. of leaves of green gram in the *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	16.77^{d}±1.22	17.70^{d}±0.90	26.90^{f}±0.46	26.40^{c}±0.46
Calcium	20.10^{c}±0.60 [16.6]	19.30^{bc}±0.46 [8.3]	29.60^{d}±0.46 [9.1]	28.80^{b}±0.62 [8.3]
Bio-priming with rhizobium	19.80^{c}±0.60 [15.3]	18.70^{cd}±0.46 [5.3]	28.30^{e}±0.60 [0.60]	27.30^{c}±0.75 [8.3]
Putrescine	21.40^{b}±0.46 [21.6]	19.57^{bc}±0.21 [9.5]	30.20^{cd}±0.46 [10.9]	29.00^{b}±0.75 [3.3]
Calcium + Bio-priming with rhizobium	22.17^{ab}±0.49 [24.3]	20.00^{b}±0.46 [11.5]	30.50^{cd}±0.75 [11.8]	29.90^{b}±0.46 [11.7]
Calcium + Putrescine	22.40^{ab}±0.46 [25.1]	21.60^{a}±0.60 [18.1]	31.50^{ab}±0.35 [14.6]	31.80^{a}±0.75 [17.0]
Bio-priming with rhizobium + Putrescine	22.00^{ab}±0.46 [23.8]	20.20^{b}±0.46 [12.4]	31.10^{bc}±0.46 [13.5]	31.30^{a}±0.60 [15.7]
Calcium + Bio priming with rhizobium + Putrescine	22.90^{a}±0.46 [26.8]	21.90^{a}±0.60 [19.2]	32.10^{a}±0.46 [16.2]	32.00^{a}±0.92 [17.5]
C.D at p=0.05	1.12	1.03	0.89	1.19

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.3.a: Impact of the treatments on no. of leaves of green gram in the *Summer* season

Fig. 4.1.3.a: Impact of Bio-priming, putrescine and calcium on no. of leaves of green gram in summer season

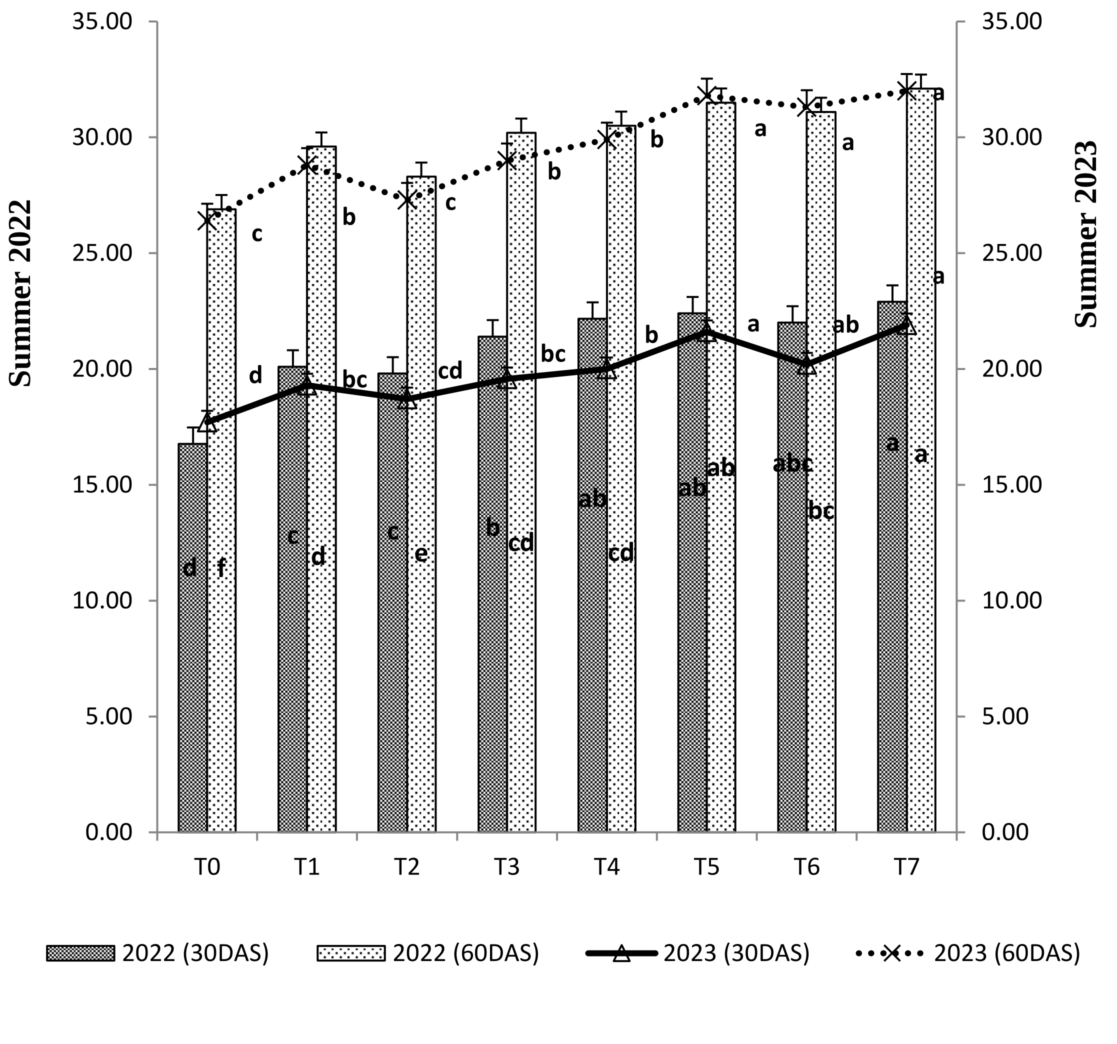


Table 4.1.3.b: Impact of the treatments on no. of leaves of green gram in *Kharif* season

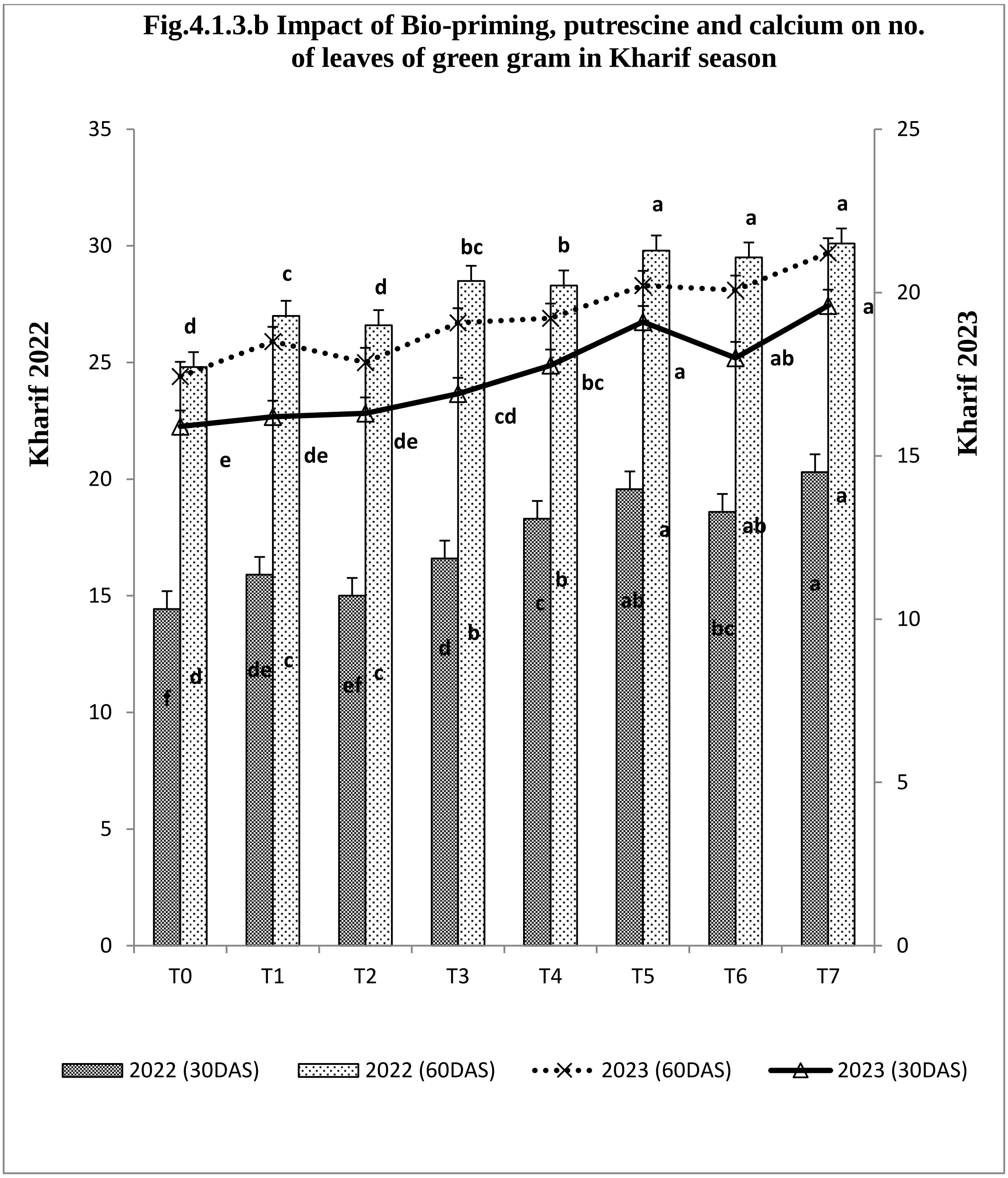
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	14.43^{f}±0.31	15.90^{e}±0.60	24.80^{d}±0.96	24.40^{d}±0.60
Calcium	15.90^{de}±0.60 [9.2]	16.20^{de}±0.30 [1.9]	27.00^{c}±0.62 [8.1]	25.90^{c}±0.30 [5.8]
Bio-priming with rhizobium	15.00^{ef}±0.60 [3.8]	16.30^{de}±0.62 [2.5]	26.60^{c}±0.46 [6.8]	25.00^{d}±0.30 [2.4]
Putrescine	16.60^{d}±0.75 [13.1]	16.90^{cd}±0.46 [5.9]	28.50^{b}±1.14 [13.0]	26.70^{bc}±0.35 [8.6]
Calcium + Bio-priming with rhizobium	18.30^{c}±0.60 [21.1]	17.77^{bc}±0.50 [10.5]	28.30^{b}±0.30 [12.4]	26.90^{b}±0.46 [9.3]
Calcium + Putrescine	19.57^{ab}±0.70 [26.3]	19.10^{a}±0.46 [16.8]	29.80^{a}±0.60 [16.8]	28.30^{a}±0.60 [13.8]
Bio-priming with rhizobium + Putrescine	18.60^{bc}±0.79 [22.4]	18.00^{b}±0.30 [11.7]	29.50^{ab}±0.60 [15.9]	28.10^{a}±0.46 [13.2]
Calcium + Bio priming with rhizobium + Putrescine	20.30^{a}±0.46 [28.9]	19.60^{a}±0.46 [18.9]	30.10^{a}±0.60 [17.6]	29.70^{a}±0.46 [17.8]
C.D at p=0.05	1.13	0.88	1.24	0.81

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.3.b: Impact of the treatments on no. of leaves of green gram in *Kharif* season



4.1.4 Leaf area

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Leaf area was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.1.4.a and 4.1.4.b and illustrated in Figures 4.1.4.a and 4.1.4.b.

In the *Summer* of 2022, treatment T_7 significantly increased leaf area, showing improvements of 16.7% and 19.8% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased leaf area by 13.6% and 17.7%, while treatment T_6 showed 10.2% and 13.0% reductions. The minimum leaf area was observed in treatment T_1, with 1.2% and 3.5% reductions.

During the *Kharif* season 2022, treatment T_7 again demonstrated a notable increase in leaf area, with 11.2% and 19.2% improvements. Conversely, treatment T_5 decreased by 7.7% and 17.7%, and treatment T_1 recorded the lowest leaf area with 2.6% and 1.6% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the maximum leaf area, with 9.6% and 19.2% increases. Treatment T_5 decreased 8.6% and 17.3%, while the minimum leaf area was recorded for treatment T_1, with 3.5% and 2.9% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the maximum leaf area increasing 11.8% and 17.2%. Treatment T_5 followed with improvements of 8.6% and 16.4%. The minimum leaf area in the *Kharif* season was observed in treatment T_1, showing 2.9% and 1.4% reductions compared to the control.

The observed augmentation in leaf area can be linked to the role of calcium in stimulating cellular division and expansion, resulting in a more substantial growth of leaves. Recent studies, like the research conducted by Raddatz *et al.,* (2021), confirmed the beneficial influence of calcium on leaf growth in other plants used in agriculture, corroborating the results of this study. The expanded leaf area improves the plant's photosynthetic capability, enhancing growth and increasing the possibility for higher output. This investigation's observed leaf area increase can be attributed to putrescine's capacity to regulate cell division and elongation, specifically in leaf tissues. Furthermore, the involvement of putrescine in improving stress tolerance may have enabled the plants to sustain healthy leaf growth. The results align with a recent study conducted by Yari *et al.*, (2022), which found that applying external polyamines, such as putrescine, results in notable enhancements in leaf area and overall plant biomass in leguminous crops and using Rhizobium treatment enhanced nitrogen availability, leading to more vigorous leaf growth and increasing leaf area. Recent investigations by Prakash *et al.*, (2023) have shown that Rhizobium treatment significantly improves leaf area and overall vegetative development in leguminous crops.

Table 4.1.4.a: Impact of the treatments on leaf area (cm^2 $plant^{-1}$) of green gram in *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	$283.33^{e}\pm5.63$	$291.93^{e}\pm3.35$	$617.67^{f}\pm2.00$	$620.03^{f}\pm3.17$
Calcium	$286.73^{e}\pm6.91$ [1.2]	$302.57^{d}\pm2.76$ [3.5]	$639.7^{d}\pm2.66$ [3.5]	$638.60^{de}\pm1.75$ [2.9]
Bio-priming with rhizobium	$300.36^{d}\pm2.45$ [5.7]	$301.47^{d}\pm1.23$ [3.2]	$633.73^{e}\pm0.97$ [2.5]	$632.73^{e}\pm2.32$ [2.0]
Putrescine	$305.70^{cd}\pm2.49$ [7.3]	$305.23^{cd}\pm3.54$ [4.4]	$641.57^{cd}\pm1.87$ [3.7]	$641.70^{d}\pm0.92$ [3.4]
Calcium + Bio-priming with rhizobium	$310.02^{bc}\pm2.06$ [8.6]	$310.20^{bc}\pm2.52$ [5.9]	$645.37^{c}\pm2.80$ [4.3]	$687.83^{c}\pm2.76$ [9.9]
Calcium + Putrescine	$327.97^{a}\pm1.99$ [13.6]	$319.27^{a}\pm2.93$ [8.6]	$750.63^{a}\pm2.48$ [17.7]	$749.90^{a}\pm1.23$ [17.3]
Bio-priming with rhizobium + Putrescine	$315.42^{b}\pm2.38$ [10.2]	$312.63^{b}\pm1.15$ [6.6]	$709.67^{b}\pm0.80$ [13.0]	$723.83^{b}\pm7.54$ [14.3]
Calcium + Bio priming with rhizobium + Putrescine	$340.22^{a}\pm6.13$ [16.7]	$322.97^{a}\pm5.05$ [9.6]	$769.87^{a}\pm2.84$ [19.8]	$750.60^{a}\pm5.85$ [17.4]
C.D at p=0.05	7.88	5.24	3.97	6.14

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.4.a: Impact of the treatments on leaf area (cm^2 $plant^{-1}$) of green gram in *Summer* season

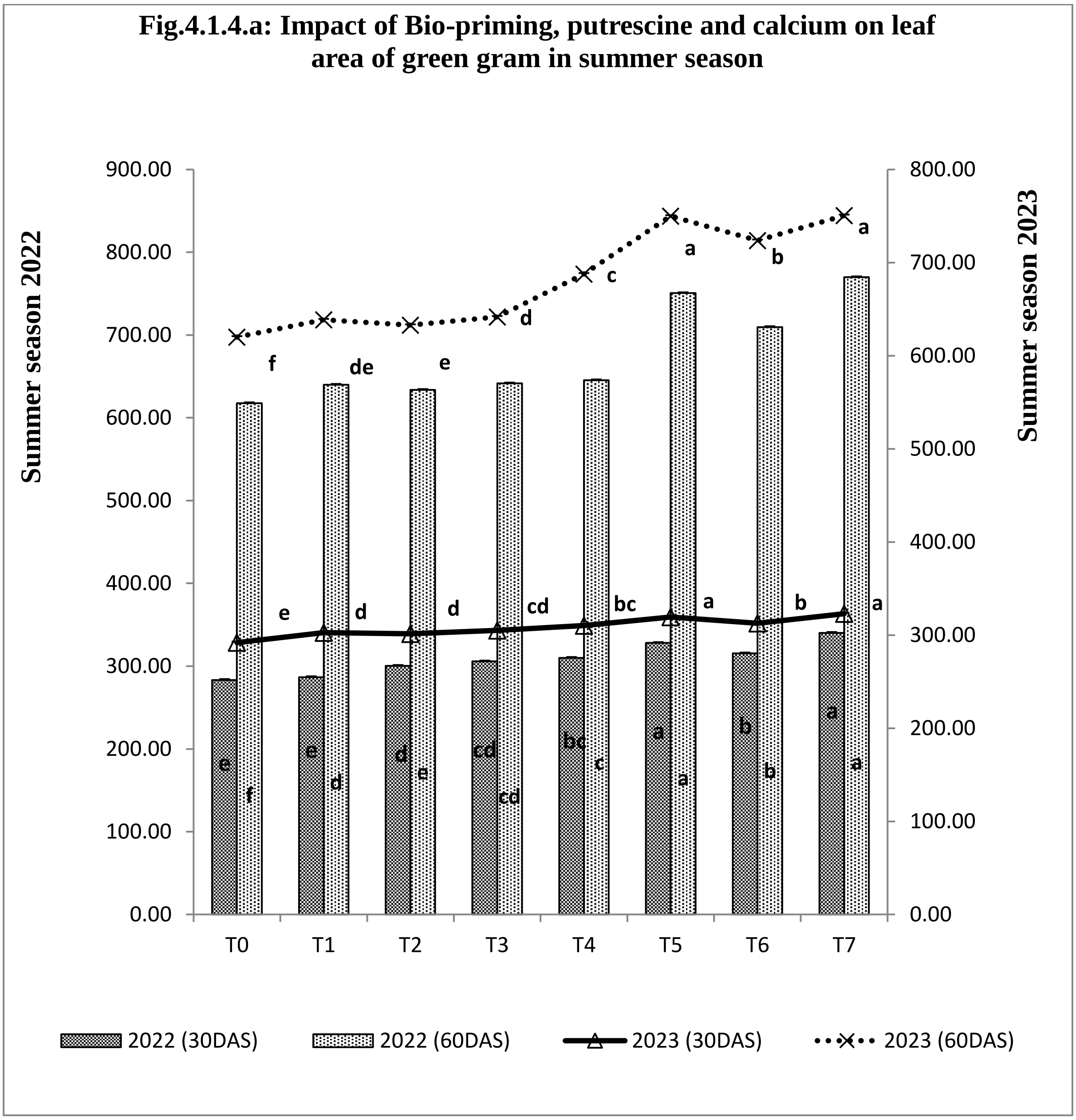


Table 4.1.4.b: Impact of the treatments on leaf area (cm^2 $plant^{-1}$) of green gram in *Kharif* season

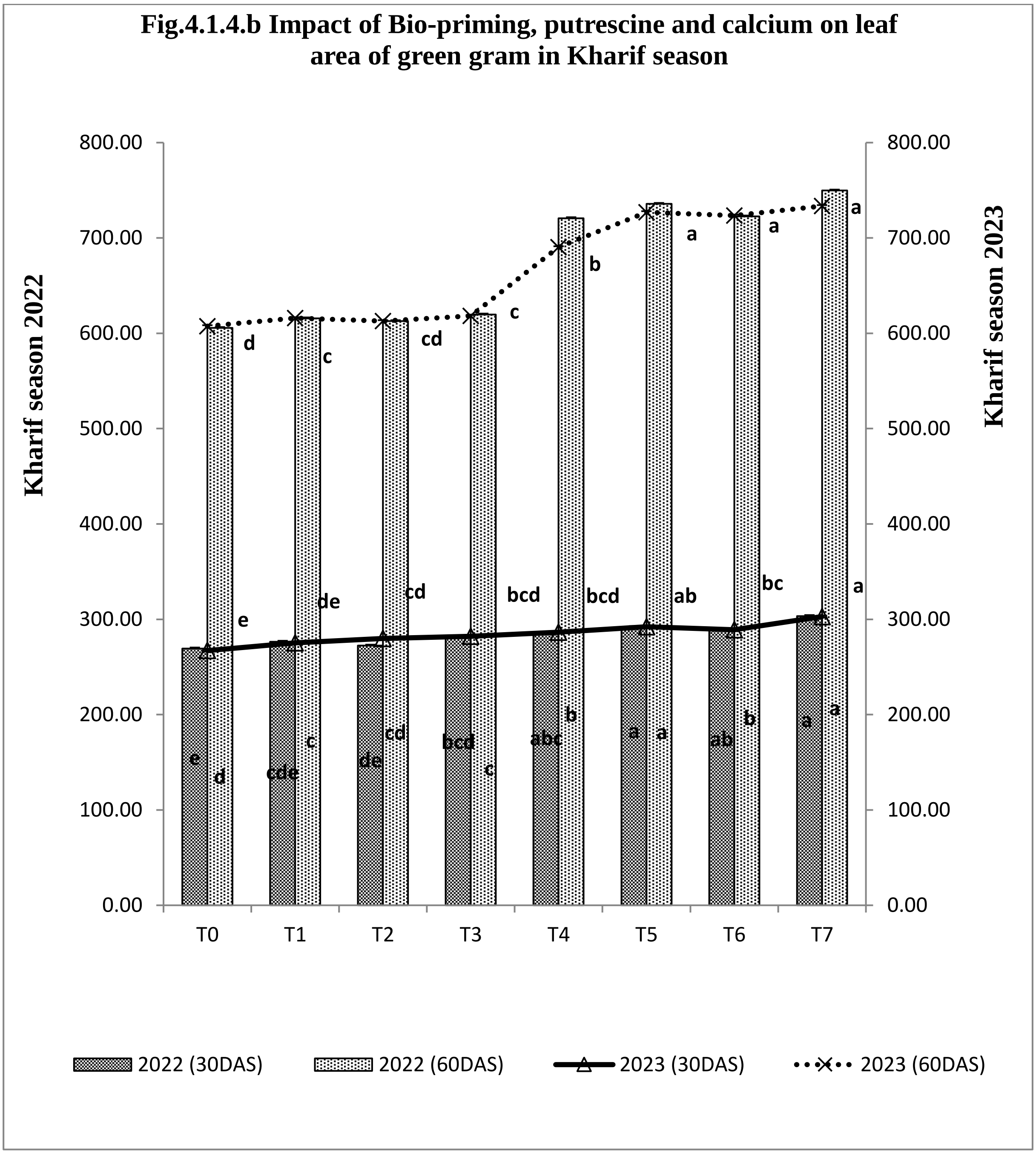
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	269.40^{e}±1.20	267.17^{e}±8.20	605.53^{d}±4.73	607.47^{d}±2.22
Calcium	276.47^{cde}±4.66 [2.6]	275.27^{de}±9.71 [2.9]	615.63^{c}±1.69 [1.6]	615.87^{c}±1.59 [1.4]
Bio-priming with rhizobium	272.40^{de}±6.26 [1.1]	279.90^{cd}±6.01 [4.5]	612.70^{cd}±3.06 [1.2]	612.77^{cd}±2.34 [0.9]
Putrescine	280.43^{bcd}±1.20 [3.9]	282.13^{bcd}±1.59 [5.3]	619.70^{c}±0.95 [2.3]	618.13^{c}±2.87 [1.7]
Calcium + Bio-priming with rhizobium	284.43^{abc}±2.20 [5.3]	286.50^{bcd}±1.18 [6.7]	720.73^{b}±1.88 [16.0]	690.30^{b}±6.90 [12.0]
Calcium + Putrescine	291.87^{a}±1.52 [7.7]	292.23^{ab}±5.24 [8.6]	735.73^{a}±4.82 [17.7]	727.07^{a}±2.14 [16.4]
Bio-priming with rhizobium + Putrescine	288.93^{ab}±5.10 [6.8]	289.00^{bc}±4.76 [7.6]	722.53^{b}±6.82 [16.2]	723.50^{a}±2.93 [16.0]
Calcium + Bio priming with rhizobium + Putrescine	303.43^{a}±10.82 [11.2]	302.87^{a}±5.32 [11.8]	749.73^{a}±9.16 [19.2]	733.47^{a}±5.76 [17.2]
C.D at p=0.05	9.25	10.85	8.71	5.63

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.4.b: Impact of the treatments on leaf area (cm^2 plant^{-1}) of green gram in *Kharif* season



4.1.5 Leaf area index

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. The leaf area index was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.1.5.a and 4.1.5.b and illustrated in Figures 4.1.5.a and 4.1.5.b.

In the *Summer* of 2022, treatment T_7 significantly increased the leaf area index, showing improvements of 16.7% and 19.6% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased leaf area index by 13.6% and 17.6%, while treatment T_6 showed 10.1% and 12.8% reductions. The lowest leaf area index was observed in treatment T_1, with 1.1% and 3.3% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in leaf area index, with 11.0% and 19.3% improvements. Conversely, treatment T_5 decreased by 7.5% and 17.7%, and treatment T_1 recorded the lowest leaf area index with 2.3% and 1.7% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the maximum leaf area index, with 9.4% and 17.3% increases. Treatment T_5 decreased by 8.4% and 16.9%, while the minimum leaf area index was recorded for treatment T_1, with 3.3% and 2.8% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the maximum leaf area index increasing 11.6% and 17.2%. Treatment T_5 followed with improvements of 8.4% and 16.4%. The minimum leaf area index in the *Kharif* season was observed in treatment T_1, showing 2.7% and 1.4% reductions compared to the control.

The leaf area index (LAI) is a critical parameter in plant growth studies, representing the total leaf area relative to the ground area covered by the crop. It influences light interception, photosynthesis, and overall crop productivity. Foliar application of calcium has been shown to improve leaf area index by enhancing leaf cell integrity and promoting vigorous leaf growth. Calcium stabilises cell membranes and supports cell division and elongation, contributing to increase LAI (Morsy *et al.,* 2023). The application of putrescine has been shown to positively impact LAI by enhancing leaf expansion and reducing stress-induced leaf abscission (Hussain *et al.,* 2021).

Putrescine promotes cell division and elongation, contributing to increased leaf area and improved LAI. Bio-priming with Rhizobium enhances nitrogen fixation, which supports better leaf growth and overall plant development. Adequate nitrogen availability promotes increased leaf area and expansion, leading to a higher LAI.

Table 4.1.5.a: Impact of the treatments on leaf area index of green gram in the *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	1.26^{e}±0.03	1.30^{d}±0.01	2.75^{d}±0.01	2.76^{d}±0.01
Calcium	1.27^{e}±0.03 [1.1]	1.34^{c}±0.01 [3.3]	2.84^{cd}±0.01 [3.3]	2.84^{d}±0.01 [2.8]
Bio-priming with rhizobium	1.33^{d}±0.01 [5.6]	1.34^{c}±0.01 [3.0]	2.82^{d}±0.00 [2.4]	2.81^{d}±0.01 [1.9]
Putrescine	1.36^{cd}±0.01 [7.3]	1.36^{c}±0.02 [4.2]	2.85^{cd}±0.01 [3.6]	2.85^{d}±0.01 [3.2]
Calcium + Bio-priming with rhizobium	1.38^{bc}±0.01 [8.6]	1.38^{b}±0.01 [5.7]	3.02^{bc}±0.27 [8.8]	3.02^{c}±0.12 [8.6]
Calcium + Putrescine	1.46^{a}±0.01 [13.6]	1.42^{a}±0.01 [8.4]	3.34^{a}±0.01 [17.6]	3.32^{a}±0.07 [16.9]
Bio-priming with rhizobium + Putrescine	1.40^{b}±0.01 [10.1]	1.39^{b}±0.01 [6.4]	3.15^{b}±0.00 [12.8]	3.22^{b}±0.03 [14.2]
Calcium + Bio priming with rhizobium + Putrescine	1.51^{a}±0.03 [16.7]	1.44^{a}±0.02 [9.4]	3.42^{a}±0.01 [19.6]	3.34^{a}±0.03 [17.3]
C.D at p=0.05	0.03	0.02	0.16	0.09

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.5.a: Impact of the treatments on leaf area index of green gram in the *Summer* season

Fig.4.1.5.a: Impact of Bio-priming, putrescine and calcium on leaf area index of green gram in summer season

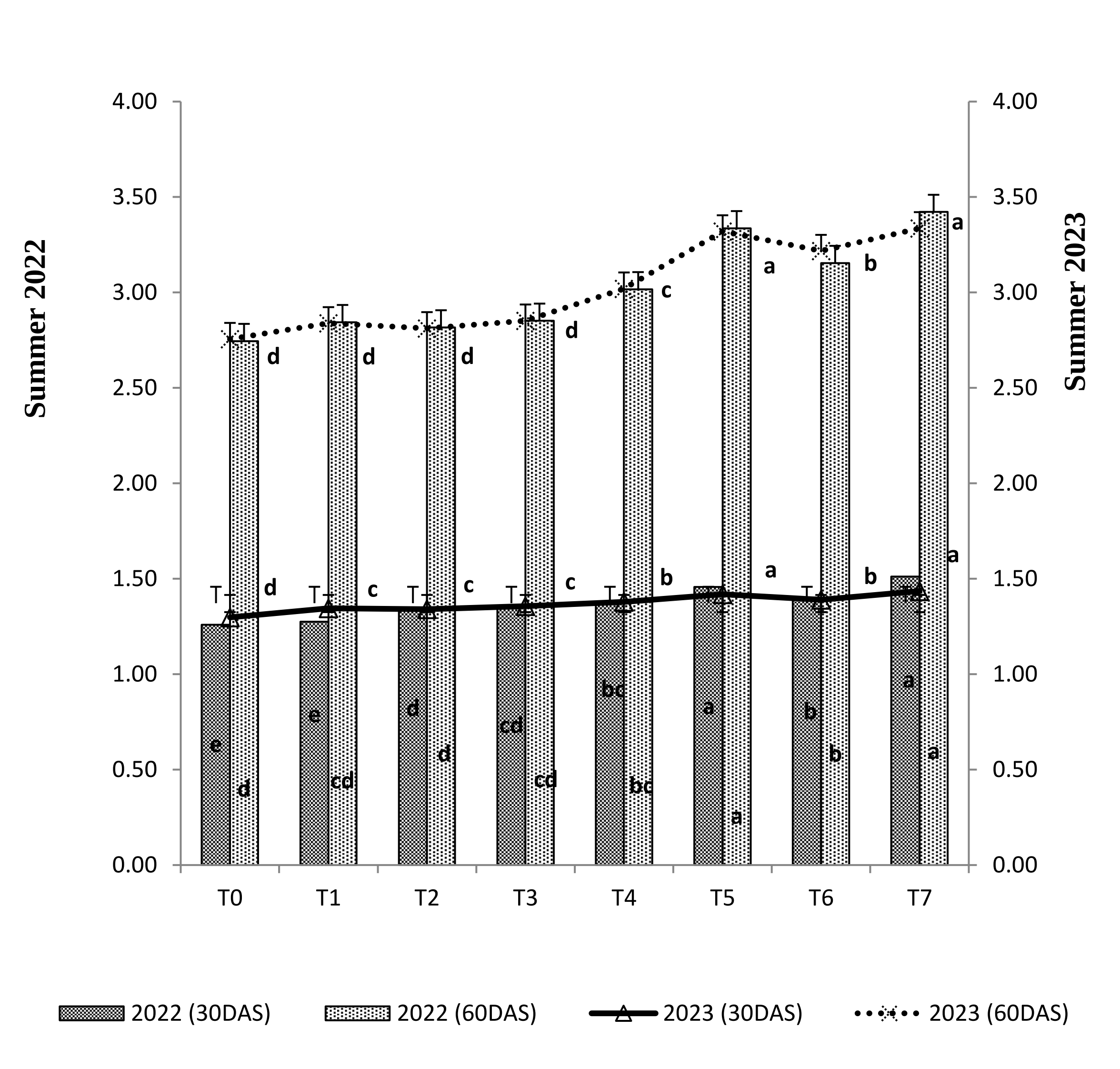


Table 4.1.5.b: Impact of the treatments on leaf area index of green gram in *Kharif* season

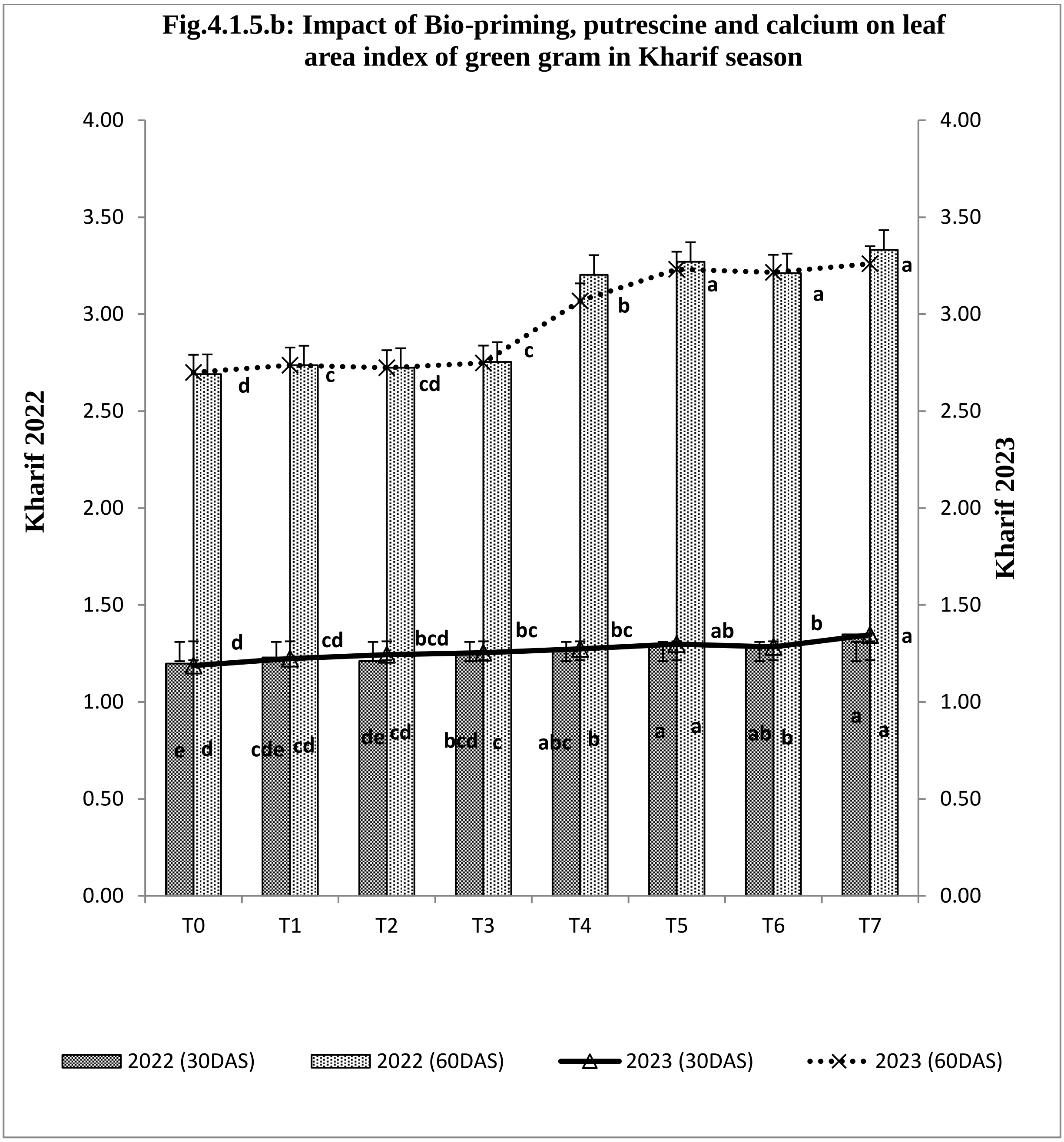
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	1.20^{e}±0.01	1.19^{d}±0.04	2.69^{d}±0.02	2.70^{d}±0.01
Calcium	1.23^{cde}±0.02 [2.3]	1.22^{cd}±0.04 [2.7]	2.74^{cd}±0.01 [1.7]	2.74^{c}±0.01 [1.4]
Bio-priming with rhizobium	1.21^{de}±0.03 [0.9]	1.24^{bcd}±0.03 [4.3]	2.72^{cd}±0.01 [1.2]	2.72^{cd}±0.01 [0.9]
Putrescine	1.25^{bcd}±0.01 [3.7]	1.25^{bc}±0.01 [5.1]	2.75^{c}±0.00 [2.3]	2.75^{c}±0.01 [1.7]
Calcium + Bio-priming with rhizobium	1.26^{abc}±0.01 [5.1]	1.27^{bc}±0.01 [6.5]	3.20^{b}±0.01 [16.0]	3.07^{b}±0.01 [12.0]
Calcium + Putrescine	1.30^{a}±0.01 [7.5]	1.30^{ab}±0.02 [8.4]	3.27^{a}±0.02 [17.7]	3.23^{a}±0.01 [16.4]
Bio-priming with rhizobium + Putrescine	1.28^{ab}±0.02 [6.6]	1.28^{b}±0.02 [7.4]	3.21^{b}±0.03 [16.2]	3.22^{a}±0.01 [16.0]
Calcium + Bio priming with rhizobium + Putrescine	1.35^{a}±0.05 [11.0]	1.35^{a}±0.02 [11.6]	3.33^{a}±0.04 [19.3]	3.26^{a}±0.03 [17.2]
C.D at p=0.05	0.04	0.05	0.04	0.03

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.5.b: Impact of the treatments on leaf area index of green gram in *Kharif* season



4.1.6 Leaf area duration

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Leaf area duration was measured 30 to 60 days after sowing (DAS), with detailed results in Table 4.1.6 and illustrated in Figure 4.1.6.

In the *Summer* of 2022, treatment T_7 significantly increased leaf area duration, showing improvements of 18.8% compared to the control. In contrast, treatment T_5 decreased leaf area duration by 16.5%, while treatment T_6 showed 12.1% reductions. The lowest leaf area duration was observed in treatment T_1, with 2.8% reductions.

During the *Kharif* season 2022, treatment T_7 again demonstrated a notable increase in leaf area duration, with 16.9% improvements. Conversely, treatment T_5 decreased by 14.9%, and treatment T_1 recorded the lowest leaf area duration with 1.9% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest leaf area duration, with a 15.0% increase. Treatment T_5 decreased by 14.5%, while the lowest leaf area duration was recorded for treatment T_1, with 3.1% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest leaf area duration, increasing by 15.6%. Treatment T_5 followed with improvements of 14.2%. The minimum leaf area duration in the *Kharif* season was observed in treatment T_1, showing 1.8% reductions compared to the control.

Leaf Area Duration (LAD) represents the period a plant maintains its leaf area, contributing significantly to photosynthesis and crop yield. Calcium is crucial for maintaining cell membrane integrity and cell wall stability, directly affecting leaf longevity and the overall leaf area duration. Putrescine enhances the strength of cell membranes and regulates stress responses, which contributes to maintaining a higher leaf area for an extended period (Hussain *et al.*, 2021). Rhizobium Bio-priming led to a significant increase in LAD by ensuring better nutrient uptake and leaf health. Similarly, Ahmed *et al.,* (2023) reported that Rhizobium-treated green gram plants exhibited an extended LAD, aligning with the results of this study.

Table 4.1.6: Impact of the treatments on leaf area duration (days) of green gram in *Summer* and *Kharif* season

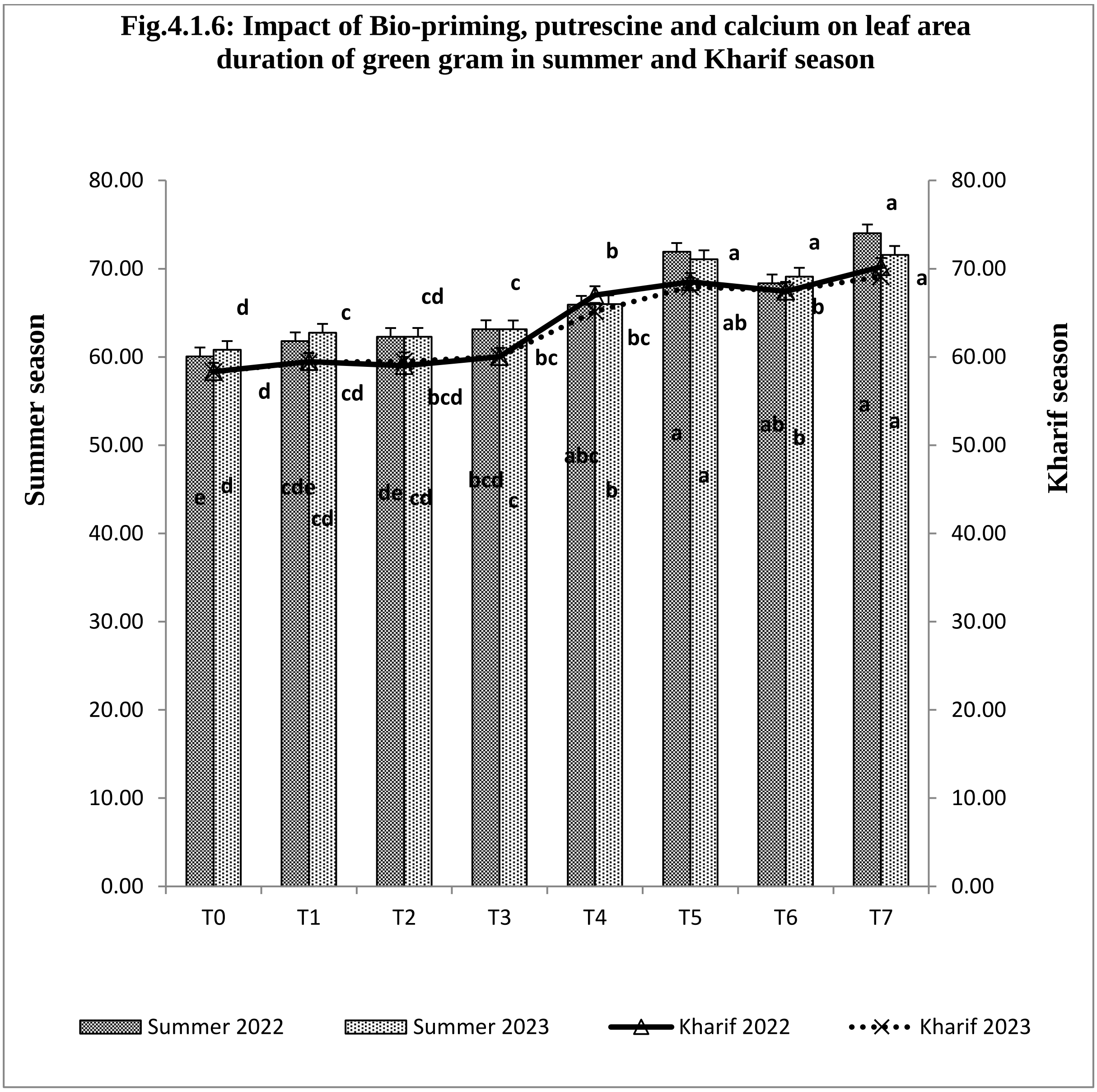
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	60.07^{d}±0.37	60.80^{e}±0.02	58.33^{d}±0.38	58.31^{d}±0.62
Calcium	61.78^{cd}±0.31 [2.8]	62.74^{d}±0.09 [3.1]	59.47^{c}±0.21 [1.9]	59.41^{c}±0.65 [1.8]
Bio-priming with rhizobium	62.27^{cd}±0.20 [3.5]	62.28^{d}±0.16 [2.4]	59.01^{cd}±0.29 [1.1]	59.51^{c}±0.34 [2.0]
Putrescine	63.15^{c}±0.04 [4.9]	63.13^{d}±0.29 [3.7]	60.01^{c}±0.02 [2.8]	60.02^{c}±0.25 [2.8]
Calcium + Bio-priming with rhizobium	65.91^{b}±3.90 [8.9]	65.98^{c}±1.78 [7.9]	67.01^{b}±0.27 [13.0]	65.12^{b}±0.49 [10.5]
Calcium + Putrescine	71.91^{a}±0.26 [16.5]	71.08^{a}±0.87 [14.5]	68.51^{a}±0.22 [14.9]	67.95^{a}±0.21 [14.2]
Bio-priming with rhizobium + Putrescine	68.34^{b}±0.20 [12.1]	69.10^{b}±0.56 [12.0]	67.43^{b}±0.63 [13.5]	67.50^{a}±0.38 [13.6]
Calcium + Bio priming with rhizobium + Putrescine	74.01^{a}±0.32 [18.8]	71.57^{a}±0.42 [15.0]	70.21^{a}±1.33 [16.9]	69.09^{a}±0.73 [15.6]
C.D at p=0.05	2.45	1.30	1.00	0.86

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.6: Impact of the treatments on leaf area duration (days) of green gram in *Summer* and *Kharif* season



4.1.7 Days to 50% flowering

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Days to 50% flowering was measured at the time of flowering, with detailed results in Table 4.1.7 and illustrated in Figure 4.1.7.

In the *Summer* of 2022, treatments significantly increased the number of days to 50% flowering. The treatment T_7 measured the minimum days to 50% flowering by -7.3%. The maximum days to 50% flowering was recorded in the treatment by -0.9% compared to the control.

During the *Kharif* season 2022, treatment T_7 again demonstrated the minimum days to 50% flowering by -4.5 %. Conversely, treatment T_2 recorded the maximum days as 50% flowering, with -0.9 % compared to the control.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the minimum days to 50% flowering, with -6.4%, while the maximum day to 50% flowering was recorded for treatment T_2, with -0.9%.

During the *Kharif* season 2023, treatment T_7 again recorded the lowest days to 50% flowering by -5.5%. The maximum days to 50% flowering in the *Kharif* season were observed in treatment T_2, showing -0.9% compared to the control.

Flowering timing is a crucial phenological phase that substantially affects crop productivity and plant growth. The early flowering in this study may be attributed to calcium's capacity to augment cell division and elongation, expediting the plant's shift from vegetative to reproductive phases. Rhizobium treatment enhances both the growth of the plant and the timing of flowering in leguminous crops by optimising the absorption and utilisation of nutrients. Applying calcium, rhizobium and putrescine in combination effectively decreases the time it takes for green gram plants to reach 50% flowering. Increasing the initial stages of flowering is crucial in optimising the potential crop yield.

Table 4.1.7: Impact of the treatments on days to 50% flowering of green gram in *Summer* and *Kharif* season

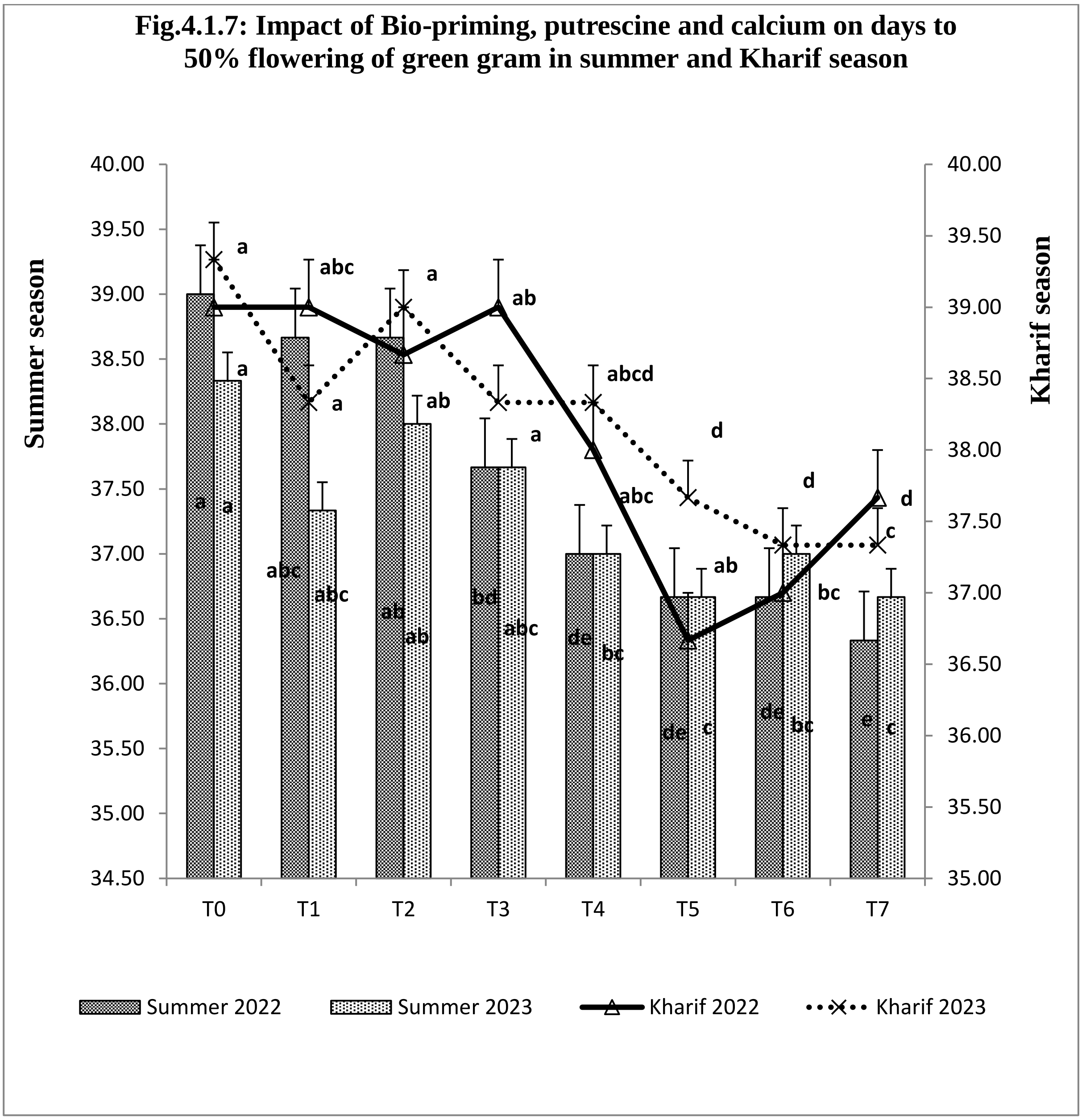
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	$39.00^{a}\pm0.00$	$38.33^{a}\pm0.58$	$39.00^{a}\pm1.00$	$39.2^{a}\pm1.2$
Calcium	$38.67^{abc}\pm0.58$ [-0.9]	$37.33^{abc}\pm0.58$ [-2.7]	$39.00^{a}\pm1.00$ [0.0]	$38.7^{abc}\pm0.6$ [-1.4]
Bio-priming with rhizobium	$38.67^{ab}\pm0.58$ [-0.9]	$38.00^{ab}\pm1.00$ [-0.9]	$38.67^{ab}\pm0.58$ [-0.9]	$38.8^{a}\pm0.3$ [-0.9]
Putrescine	$37.67^{bd}\pm0.58$ [-3.5]	$37.67^{abc}\pm0.58$ [-1.8]	$39.00^{a}\pm1.00$ [0.0]	$38.7^{ab}\pm0.6$ [-1.4]
Calcium + Bio-priming with rhizobium	$37.00^{de}\pm0.00$ [-5.4]	$37.00^{bc}\pm0.00$ [-3.6]	$38.00^{abc}\pm1.00$ [-2.6]	$38.2^{abcd}\pm0.3$ [-2.7]
Calcium + Putrescine	$36.67^{de}\pm0.58$ [-6.4]	$36.67^{c}\pm0.58$ [-4.5]	$37.67^{abc}\pm0.58$ [-3.5]	$37.5^{d}\pm0.5$ [-4.5]
Bio-priming with rhizobium + Putrescine	$36.67^{de}\pm0.58$ [-6.4]	$37.00^{bc}\pm0.00$ [-3.6]	$37.00^{bc}\pm1.00$ [-5.4]	$37.2^{d}\pm0.3$ [-5.5]
Calcium + Bio-priming with rhizobium + Putrescine	$36.33^{e}\pm1.15$ [-7.3]	$36.67^{c}\pm0.58$ [-4.5]	$36.67^{c}\pm0.58$ [-6.4]	$37.2^{d}\pm0.6$ [-5.5]
C.D at p=0.05	0.98	1.07	1.55	1.10

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.7: Impact of the treatments on days to 50% flowering of green gram in *Summer* and *Kharif* season



4.1.8 Days to physiological maturity

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Days to physiological maturity were measured at maturity, with detailed results in Table 4.1.8 and illustrated in Figure 4.1.8.

In the *Summer* of 2022, treatments significantly increased days to physiological maturity. The treatments T_6 and T_5 measured minimum days to physiological maturity by -3.3%. The maximum days to physiological maturity were recorded in the treatment T_1 by -0.5% compared to the control.

During the *Kharif* season in 2022, treatment T_7 recorded the minimum days to physiological maturity by -4.5%. Conversely, treatment T_2 recorded the maximum days to physiological maturity at -1.6% compared to the control.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the minimum days to physiological maturity, with -2.7%, while treatment T_2 recorded the maximum day to physiological maturity, with -0.5%.

During the *Kharif* season 2023, treatment T_7 again recorded the lowest days to physiological maturity by -3.1%. The maximum days to physiological maturity in the *Kharif* season were observed in treatment T_2, showing -0.9% compared to the control.

Physiological maturity is a critical stage in crop development, marking the point at which the plant's growth processes have ceased, and the seeds have achieved their maximum dry weight. The improved structural integrity provided by calcium ensures the plant can maintain its physiological functions until the seeds reach full maturity. Polyamines like putrescine help delay senescence and extend the active seed-filling period, contributing to more synchronised and timely physiological maturity (Hussain *et al.,* 2021). Recent research indicates that Rhizobium Bio-priming helps synchronise the maturation process by ensuring adequate nutrient supply throughout the growing season, facilitating a more uniform physiological maturity (Prakash *et al.,* 2023).

Table 4.1.8: Impact of the treatments on days to physiological maturity of green gram in *Summer* and *kharif* season

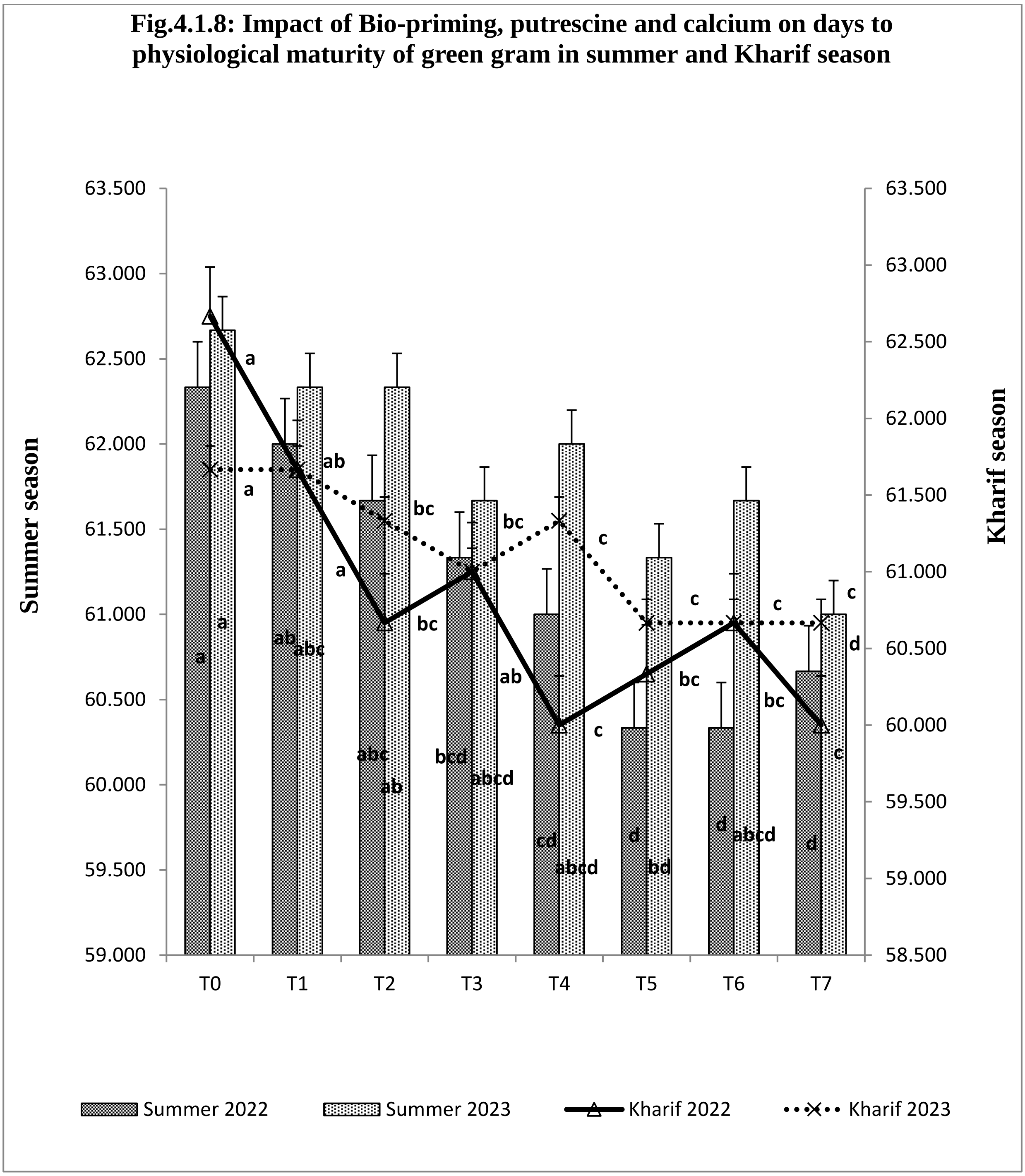
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	62.33^{a}±0.58	62.67^{a}±0.58	62.67^{a}±0.58	62.2^{a}±0.3
Calcium	62.00^{ab}±0.00 [-0.5]	62.33^{abc}±0.58 [-0.5]	61.67^{a}±0.58 [-1.6]	61.7^{ab}±0.6 [-0.9]
Bio-priming with rhizobium	61.67^{abc}±0.58 [-1.1]	62.33^{ab}±0.58 [-0.5]	60.67^{bc}±0.58 [-3.3]	61.0^{bc}±0.5 [-2.0]
Putrescine	61.33^{bcd}±0.58 [-1.6]	61.67^{abcd}±0.58 [-1.6]	61.00^{ab}±0.00 [-2.7]	61.0^{bc}±0.0 [-2.0]
Calcium + Bio-priming with rhizobium	61.00^{cd}±0.00 [-2.2]	62.00^{abcd}±0.00 [-1.1]	60.00^{c}±0.00 [-4.5]	60.7^{c}±0.3 [-2.5]
Calcium + Putrescine	60.33^{d}±0.58 [-3.3]	61.33^{bd}±0.58 [-2.2]	60.33^{bc}±0.58 [-3.9]	60.5^{c}±0.5 [-2.8]
Bio-priming with rhizobium + Putrescine	60.33^{d}±0.58 [-3.3]	61.67^{abcd}±0.58 [-1.6]	60.67^{bc}±0.58 [-3.3]	60.7^{c}±0.6 [-2.5]
Calcium + Bio priming with rhizobium + Putrescine	60.67^{d}±0.58 [-2.7]	61.00^{d}±0.00 [-2.7]	60.00^{c}±0.00 [-4.5]	60.3^{c}±0.3 [-3.1]
C.D at p=0.05	0.96	0.91	0.82	0.79

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.8: Impact of the treatments on days to physiological maturity of green gram in *Summer* and *Kharif* season



4.1.9 Root length

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Root length was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.1.9.a and 4.1.9.b and illustrated in Figures 4.1.9.a and 4.1.9.b.

In the *Summer* of 2022, treatment T_7 significantly increased root length, showing 55.8% and 42.0% improvements at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_4 decreased root length by 51.5% at 30 DAS and T_6 by 38.6% at 60 DAS, while treatment T_6 showed 50.0% at 30 DAS and T_4 by 33.5% reductions at 60 DAS. The lowest root length was observed in treatment T_1, with 13.6% and 15.1% reductions.

During the *Kharif* season 2022, treatment T_7 again demonstrated a notable increase in leaf area, with 59.8% and 41.6% improvements. Conversely, treatment T_4 decreased by 51.4% and 34.0%, and treatment T_1 recorded the lowest root length with 14.5% and 7.4% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the maximum root length, with 59.7% and 35.0% increases. Treatment T_4 decreased by 56.3% at 30 DAS and T_6 by 29.0% at 60 DAS, while the lowest root length was recorded for treatment T_1, with 20.5% and 9.2% reductions. During the *Kharif* season 2023, treatment T_7 recorded the highest root length, increasing by 61.1% and 41.4%. Treatment T_4 followed with improvements of 56.6% and 33.6%. The minimum root length in the *Kharif* season was observed in treatment T_1, showing 19.2% and 10.6% reductions compared to the control.

The results showed that Rhizobium inoculation significantly enhanced root length compared to the control. This can be attributed to the improved nitrogen availability in the rhizosphere due to biological nitrogen fixation. Enhanced nitrogen levels promote better root growth, facilitating more excellent nutrient and water uptake, thus contributing to overall plant vigour. The symbiotic relationship between rhizobium and mung bean roots likely stimulated root proliferation, leading to longer roots. Putrescine, involved in plant growth regulation, also positively affects root length. Its role in cell division, elongation, and differentiation could explain the increased root length observed in the mung beans treated with putrescine. Calcium's role in maintaining the integrity of cell walls and its involvement in signalling pathways that regulate root growth.

Table 4.1.9.a: Impact of the treatments on root length (cm) of green gram in the *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	$6.77^{d}\pm0.75$	$6.07^{e}\pm0.81$	$12.17^{e}\pm0.76$	$13.83^{e}\pm0.87$
Calcium	$7.83^{cd}\pm0.85$ [13.6]	$7.63^{d}\pm0.23$ [20.5]	$14.33^{d}\pm0.65$ [15.1]	$15.23^{d}\pm0.81$ [9.2]
Bio-priming with rhizobium	$12.60^{a}\pm0.46$ [46.3]	$12.80^{b}\pm0.69$ [52.6]	$17.80^{b}\pm0.44$ [31.6]	$17.03^{b}\pm0.45$ [18.8]
Putrescine	$8.33^{bc}\pm0.55$ [18.8]	$8.27^{bc}\pm0.51$ [26.6]	$16.30^{c}\pm0.96$ [25.3]	$16.73^{bc}\pm0.40$ [17.4]
Calcium + Bio-priming with rhizobium	$13.97^{a}\pm0.50$ [51.5]	$13.90^{a}\pm0.79$ [56.3]	$18.30^{b}\pm0.96$ [33.5]	$18.80^{a}\pm0.62$ [26.4]
Calcium + Putrescine	$9.23^{b}\pm0.70$ [26.7]	$9.87^{cd}\pm0.91$ [38.5]	$15.73^{c}\pm0.40$ [22.6]	$15.77^{cd}\pm0.51$ [12.3]
Bio-priming with rhizobium + Putrescine	$13.53^{a}\pm0.81$ [50.0]	$13.73^{a}\pm1.70$ [55.8]	$19.83^{a}\pm0.25$ [38.6]	$19.47^{a}\pm0.31$ [29.0]
Calcium + Bio priming with rhizobium + Putrescine	$15.33^{a}\pm1.06$ [55.8]	$15.17^{a}\pm0.70$ [59.7]	$20.97^{a}\pm0.81$ [42.0]	$21.27^{a}\pm0.42$ [35.0]
C.D at p=0.05	1.31	1.54	1.03	1.08

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.9.a: Impact of the treatments on root length (cm) of green gram in the *Summer* season

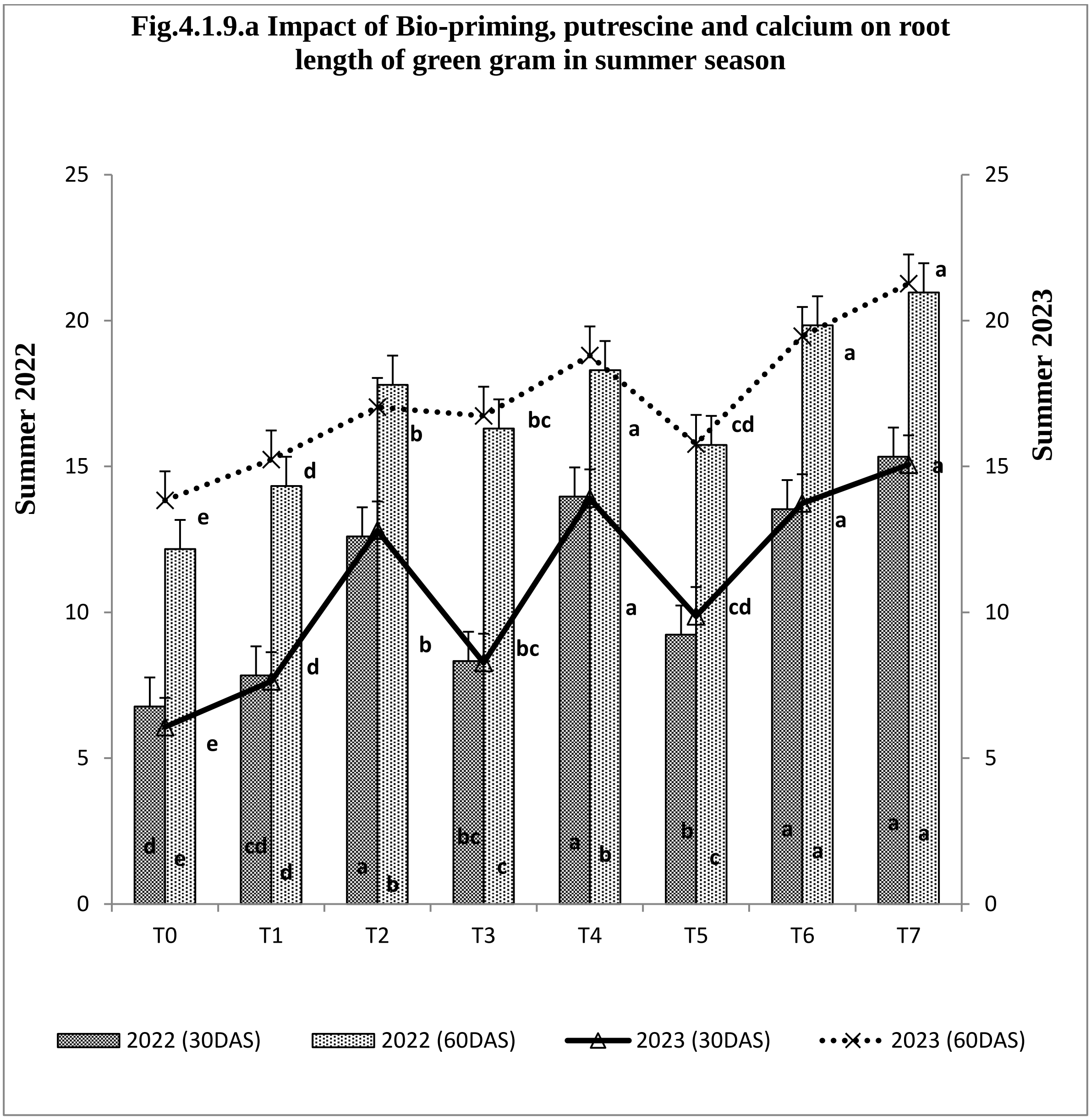


Table 4.1.9.b: Impact of the treatments on root length (cm) of green gram in *Kharif* season

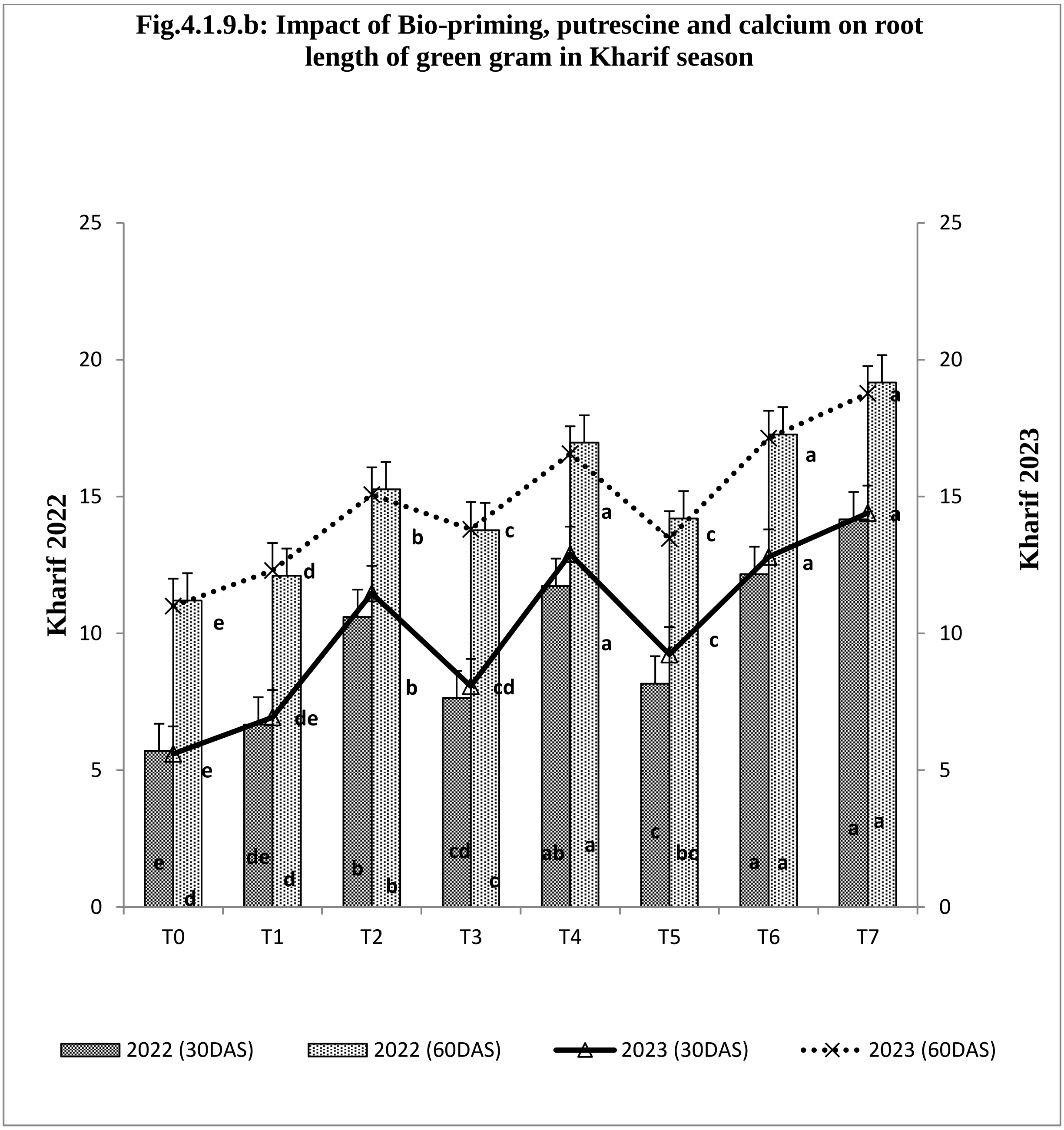
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	5.70^{e}±0.17	5.60^{e}±0.78	11.20^{d}±0.26	11.00^{e}±0.20
Calcium	6.67^{de}±0.51 [14.5]	6.93^{de}±0.70 [19.2]	12.10^{d}±0.36 [7.4]	12.30^{d}±0.72 [10.6]
Bio-priming with rhizobium	10.60^{b}±0.40 [46.2]	11.47^{b}±0.65 [51.2]	15.27^{b}±0.70 [26.6]	15.07b±0.21 [27.0]
Putrescine	7.63^{cd}±0.75 [25.3]	8.07^{cd}±1.27 [30.6]	13.77^{c}±0.68 [18.6]	13.80^{c}±0.75 [20.3]
Calcium + Bio-priming with rhizobium	11.73^{ab}±1.20 [51.4]	12.90^{a}±0.79 [56.6]	16.97^{a}±0.32 [34.0]	16.57^{a}±0.45 [33.6]
Calcium + Putrescine	8.17^{c}±0.60 [30.2]	9.23^{c}±1.07 [39.4]	14.20^{bc}±0.96 [21.1]	13.47^{c}±0.45 [18.3]
Bio-priming with rhizobium + Putrescine	12.17^{a}±0.65 [53.2]	12.80^{a}±0.92 [56.3]	17.27^{a}±0.50 [35.1]	17.13^{a}±0.45 [35.8]
Calcium + Bio priming with rhizobium + Putrescine	14.17^{a}±0.75 [59.8]	14.40^{a}±0.26 [61.1]	19.17^{a}±1.02 [41.6]	18.77^{a}±0.51 [41.4]
C.D at p=0.05	1.22	1.27	1.21	0.90

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.9.b: Impact of the treatments on root length (cm) of green gram in *Kharif* season



4.1.10 No. of nodules

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. No. of nodules was measured at (vegetative stage and during reproductive stage) 30 and 60 days after sowing (DAS), with detailed results in Table 4.1.10.a and 4.1.10.b and illustrated in Figures 4.1.10.a and 4.1.10.b.

In the *Summer* of 2022, treatment T_7 significantly increased the number of nodules, showing improvements of 57.7% and 48.3% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_6 decreased the number of nodules by 53.8% and 44.7%, while treatment T_4 showed 52.1% and 42.6% reductions. The lowest number of nodules was observed in treatment T_1, with 14.2% and 10.6% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in no. of nodules, with 69.5% and 60.8% improvements. Conversely, treatment T_6 decreased by 68.9% and 59.0%, and treatment T_1 recorded the lowest no. of nodules with 4.0% and 4.3% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest no. of nodules, with 65.0% and 51.5% increases. Treatment T_6 decreased 63.6% and 47.8%, while the lowest no. of nodules was recorded for treatment T_1, with 18.6% and 18.5% reductions. During the *Kharif* season 2023, treatment T_6 again recorded the highest number of nodules, increasing by 64.7% and 62.4%. Treatment T_4 followed with improvements of 62.4% and 60.3%. The minimum no. of nodules in the *Kharif* season was observed in treatment T_1, showing 9.5% and 3.9% reductions compared to the control.

The increase in nodule number is likely due to the successful colonisation of the rhizosphere by the Rhizobium strain used in the study, which enhanced the symbiotic interaction. Putrescine is known to regulate nodule formation and function, possibly through its roles in cell division and differentiation and its protective effects against stress. Calcium plays a crucial role in cell wall stability and signalling, which is essential in establishing and developing nodules. Additionally, calcium regulates cytoskeletal dynamics and membrane stability, which are critical during infection and nodule development.

Table-4.1.10.a: Impact of the treatments on no. of nodules of green gram in the *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	$30.33^{d}\pm2.52$	$27.67^{e}\pm2.52$	$45.00^{e}\pm4.36$	$44.00^{e}\pm2.65$
Calcium	$35.33^{cd}\pm4.51$ [14.2]	$34.00^{d}\pm2.65$ [18.6]	$50.33^{de}\pm4.04$ [10.6]	$54.00^{d}\pm2.65$ [18.5]
Bio-priming with rhizobium	$62.67^{d}\pm8.33$ [51.6]	$69.33^{b}\pm1.53$ [60.1]	$77.33^{b}\pm7.09$ [41.8]	$76.67^{b}\pm1.53$ [42.6]
Putrescine	$39.33^{bc}\pm5.03$ [22.9]	$40.00^{c}\pm2.00$ [30.8]	$55.00^{d}\pm3.61$ [18.2]	$55.33^{bc}\pm3.79$ [20.5]
Calcium + Bio-priming with rhizobium	$63.33^{a}\pm8.50$ [52.1]	$74.33^{b}\pm5.13$ [62.8]	$78.33^{ab}\pm8.74$ [42.6]	$77.33^{a}\pm2.08$ [43.1]
Calcium + Putrescine	$42.33^{b}\pm5.13$ [28.4]	$48.67^{c}\pm3.51$ [43.1]	$59.67^{c}\pm6.81$ [24.6]	$59.33^{cd}\pm3.06$ [25.8]
Bio-priming with rhizobium + Putrescine	$65.67^{a}\pm7.23$ [53.8]	$76.00^{a}\pm4.58$ [63.6]	$81.33^{ab}\pm6.66$ [44.7]	$84.33^{a}\pm4.51$ [47.8]
Calcium + Bio priming with rhizobium + Putrescine	$71.67^{b}\pm3.79$ [57.7]	$79.00^{a}\pm3.61$ [65.0]	$87.00^{a}\pm3.46$ [48.3]	$90.67^{a}\pm2.52$ [51.5]
C.D at p=0.05	1.31	1.54	1.03	1.08

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.10.a: Impact of the treatments on no. of nodules of green gram in the *Summer* season

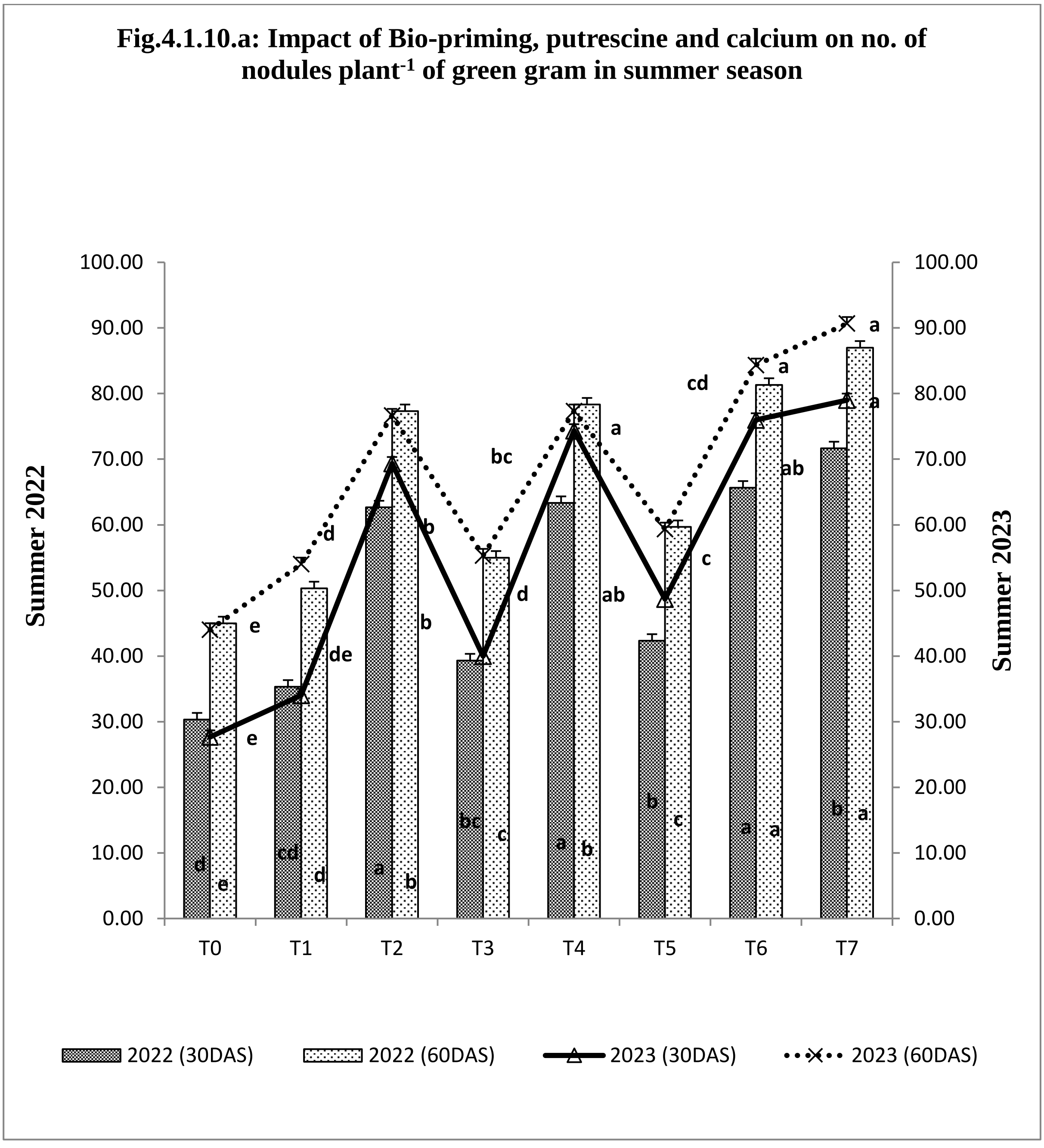


Table-4.1.10.b: Impact of the treatments on no. of nodules of green gram in *Kharif* season

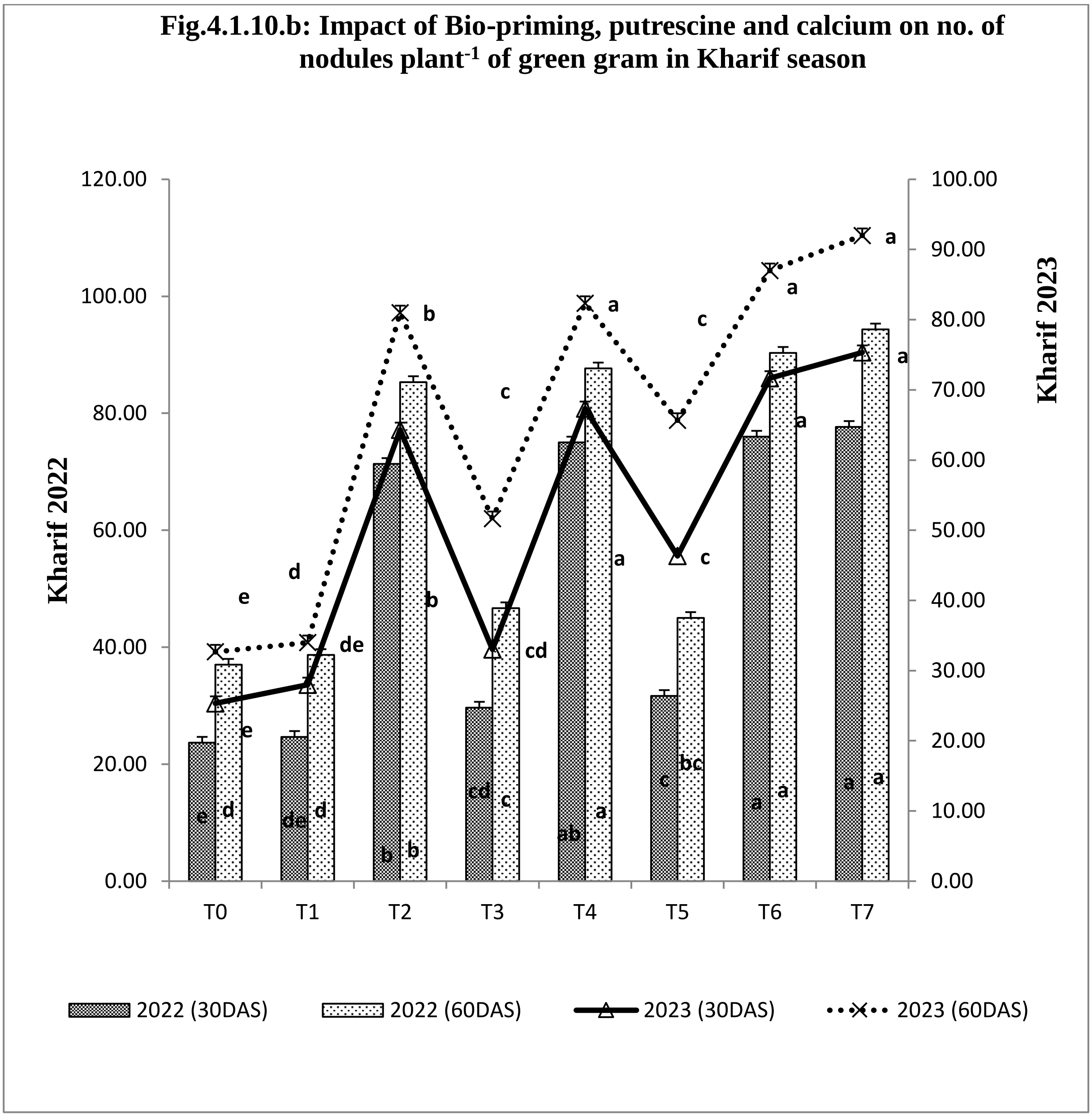
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	23.67^{e}±2.52	25.33^{d}±3.06	37.00^{e}±1.00	32.67^{e}±3.06
Calcium	24.67^{de}±3.51 [4.0]	28.00^{d}±3.61 [9.5]	38.67^{de}±2.08 [4.3]	34.00^{d}±3.00 [3.9]
Bio-priming with rhizobium	71.33^{b}±3.21 [66.8]	64.33^{b}±2.52 [60.6]	85.33^{b}±4.51 [56.6]	81.00^{b}±2.65 [59.7]
Putrescine	29.67^{cd}±3.06 [20.2]	33.00^{c}±4.36 [23.2]	46.67^{cd}±1.53 [20.7]	51.67^{c}±2.89 [36.8]
Calcium + Bio-priming with rhizobium	75.00^{ab}±4.00 [68.4]	67.33^{a}±2.08 [62.4]	87.67^{a}±3.06 [57.8]	82.33^{a}±4.73 [60.3]
Calcium + Putrescine	31.67^{c}±3.06 [25.3]	46.33^{bc}±1.53 [45.3]	45.00^{c}±2.00 [17.8]	65.67^{c}±2.08 [50.2]
Bio-priming with rhizobium + Putrescine	76.00^{a}±5.00 [68.9]	71.67^{a}±4.04 [64.7]	90.33^{a}±6.81 [59.0]	87.00^{a}±4.58 [62.4]
Calcium + Bio priming with rhizobium + Putrescine	77.67^{a}±2.52 [69.5]	75.33^{a}±3.51 [66.4]	94.33^{a}±3.06 [60.8]	92.00^{a}±3.61 [64.5]
C.D at p=0.05	1.22	1.27	1.21	0.90

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.1.10.b: Impact of the treatments on no. of nodules of green gram in *Kharif* season



4.2 Biochemical parameters

4.2.1 SPAD index

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. SPAD index was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.2.1.a and 4.2.1.b and illustrated in Figures 4.2.1.a and 4.2.1.b. In the *Summer* of 2022, treatment T_7 significantly increased the SPAD index, showing improvements of 24.4% and 11.7% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased the SPAD index by 22.3% and 9.4%, while treatment T_6 showed 20.5% and 7.1% reductions. The lowest SPAD index was observed in treatment T_1, with 12.0% and 4.6% reductions. During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in the SPAD index, with 26.1% and 17.6% improvements. Conversely, treatment T_5 decreased by 22.4% and 15.9%, and treatment T_1 recorded the lowest SPAD index with 18.3% and 14.2% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest SPAD index, with 21.6% and 14.9% increases. Treatment T_5 decreased by 20.4% and 13.4%, while the lowest SPAD index was recorded for treatment T_1, with 8.7% and 5.4% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest SPAD index, increasing 20.9% and 19.7%. Treatment T_5 followed with improvements of 19.7% and 18.2%. The minimum SPAD index in the *Kharif* season was observed in treatment T_1, showing 17.7% and 16.2% reductions compared to the control.

The SPAD meter measures the relative chlorophyll content in plant leaves, providing a quick and non-destructive way to assess plant health and photosynthetic capacity. Rhizobium inoculation improved chlorophyll content in mung bean leaves, likely due to enhanced nitrogen availability. Nitrogen is a critical component of chlorophyll molecules, and Rhizobium's role in biological nitrogen fixation likely contributed to higher nitrogen levels in the plant, subsequently increasing chlorophyll synthesis. Recent studies support these findings, demonstrating that Rhizobium inoculation can significantly enhance chlorophyll content and SPAD values in leguminous crops by improving nitrogen nutrition (Kumar *et al.,* 2022; Singh *et al.,* 2023). Recent research has shown that polyamines, including putrescine, can enhance chlorophyll content under stress conditions, which aligns with the findings of this study (Ali *et al.,* 2021; Zafar *et al.,* 2023). The increase in SPAD values with putrescine application suggests that it supports the plant's photosynthetic machinery, potentially leading to improved growth and yield. Studies have reported that calcium can enhance chlorophyll content by stabilising chloroplast structures and protecting them from damage under stress conditions (Gupta and Kumar, 2022; Huang *et al.,* 2023). The results of this study corroborate these findings, suggesting that calcium is vital for sustaining chlorophyll levels in mung bean leaves.

Table 4.2.1.a: Impact of the treatments on SPAD index of green gram in *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	30.83^{g}±1.55	30.77^{f}±0.25	52.57^{d}±1.06	56.00^{f}±0.56
Calcium	35.03^{e}±0.87 [12.0]	33.70^{d}±0.56 [8.7]	55.10^{c}±0.10 [4.6]	59.20^{d}±0.61 [5.4]
Bio-priming with rhizobium	33.73^{f}±0.32 [8.6]	32.40^{e}±0.92 [5.0]	54.43^{cd}±0.21 [3.4]	57.70^{e}±0.66 [2.9]
Putrescine	36.60^{d}±0.40 [15.8]	35.33^{b}±0.61 [12.9]	55.77^{bc}±0.25 [5.7]	62.33^{b}±1.0 [10.2]
Calcium + Bio-priming with rhizobium	37.60^{cd}±0.50 [18.0]	36.63^{c}±0.68 [16.0]	56.10^{bc}±0.10 [6.3]	60.77^{c}±0.38 [7.8]
Calcium + Putrescine	39.67^{ab}±0.50 [22.3]	38.63^{a}±0.49 [20.4]	58.03^{ab}±1.68 [9.4]	64.70^{a}±0.46 [13.4]
Bio-priming with rhizobium + Putrescine	38.77^{bc}±0.35 [20.5]	37.23^{b}±0.96 [17.4]	56.57^{bc}±0.47 [7.1]	63.37^{b}±0.81 [11.6]
Calcium + Bio priming with rhizobium + Putrescine	40.77^{a}±0.15 [24.4]	39.23^{a}±0.65 [21.6]	59.57^{a}±2.70 [11.7]	65.80^{a}±0.87 [14.9]
C.D at p=0.05	1.20	1.19	2.21	1.32

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the percent increase /decrease over the control

Fig. 4.2.1.a: Impact of the treatments on SPAD index of green gram in *Summer* season

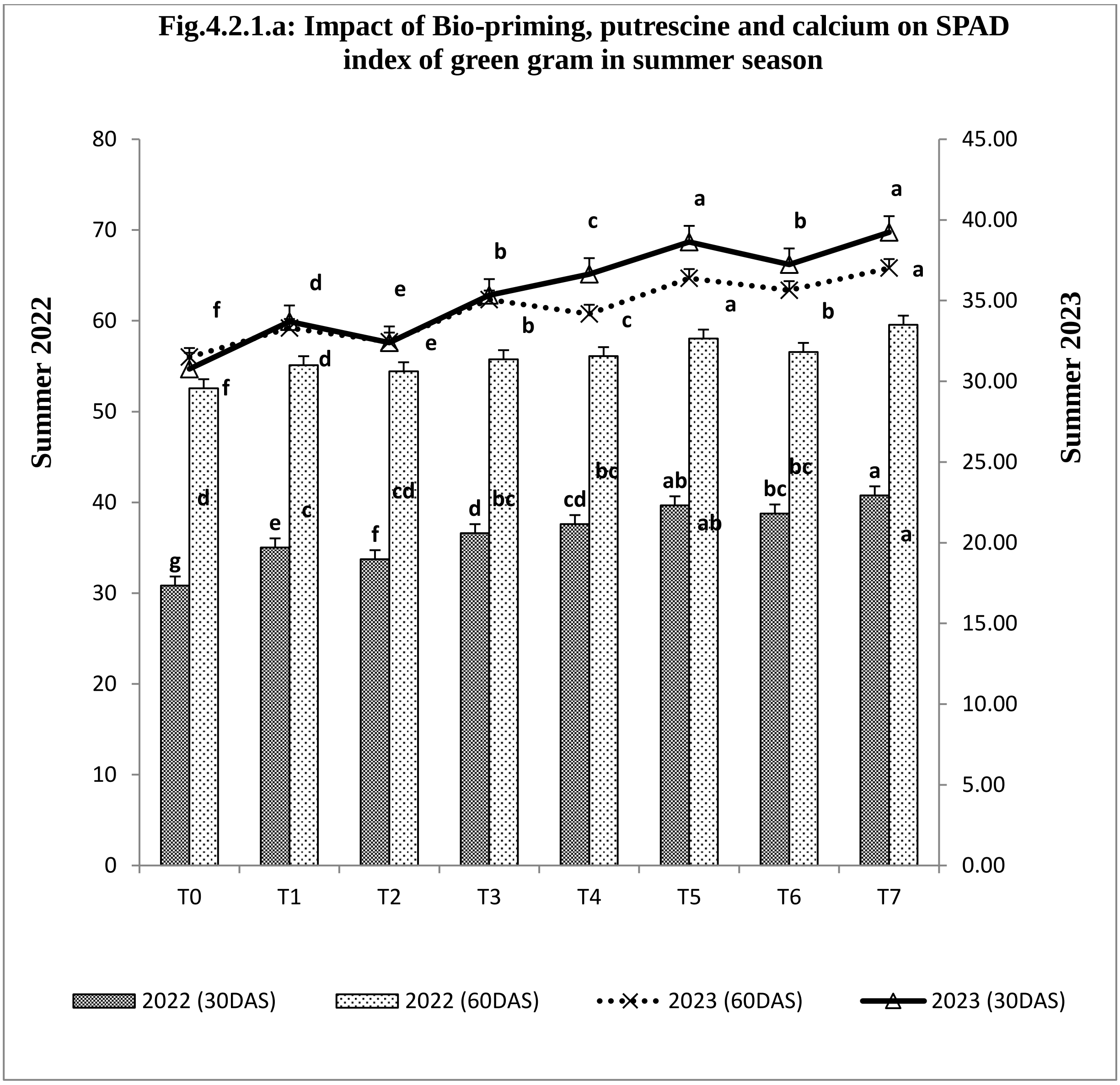


Table-4.2.1.b: Impact of the treatments on SPAD index of green gram in *Kharif* season

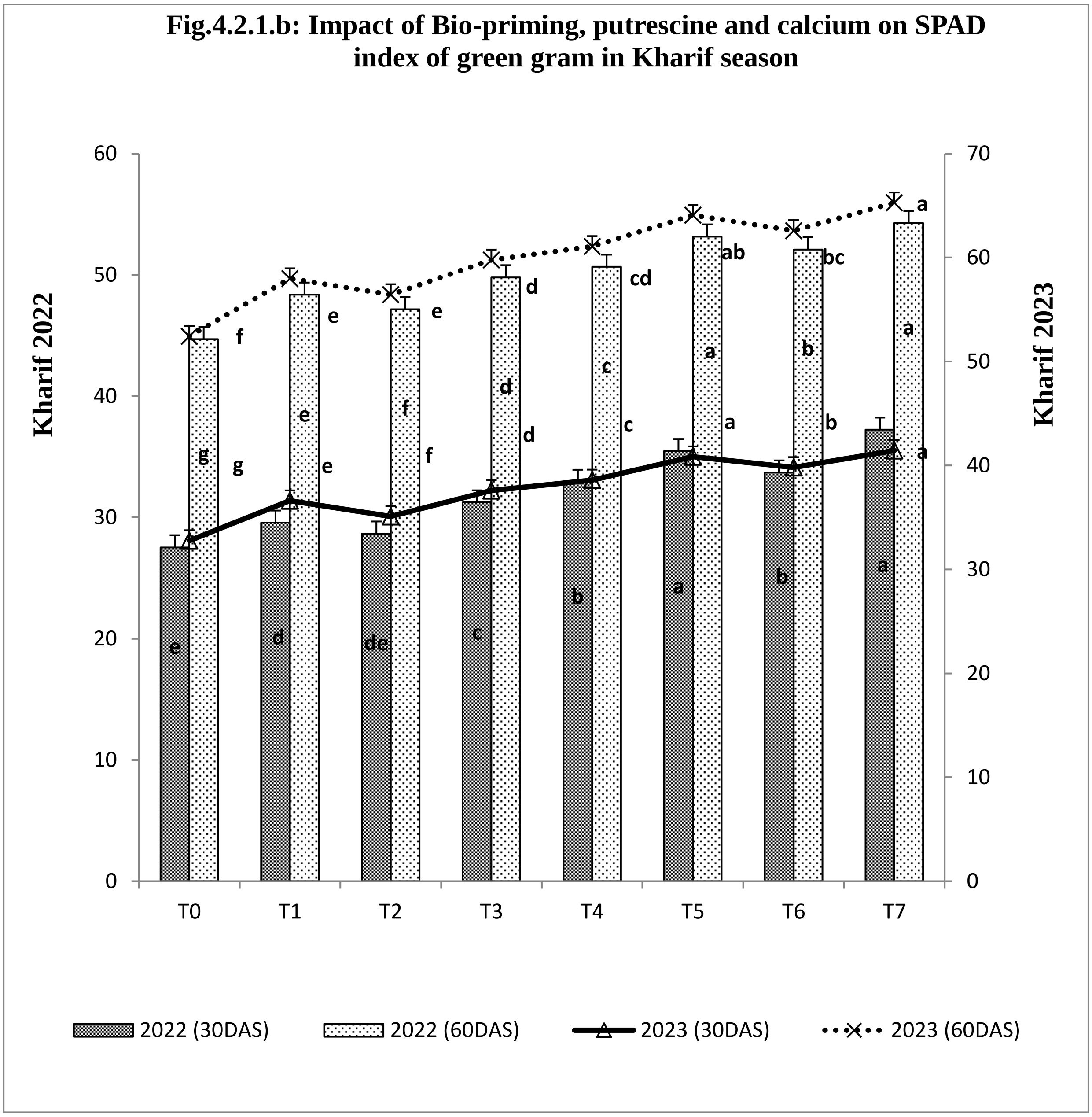
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	27.53^{e}±0.45	32.77^{g}±0.67	44.70^{g}±0.62	52.43^{f}±1.85
Calcium	29.57^{d}±0.64 [6.9]	36.60^{e}±0.40 [10.5]	48.37^{e}±0.25 [7.6]	57.97^{e}±0.78 [9.6]
Bio-priming with rhizobium	28.67^{de}±0.42 [4.0]	35.10^{f}±0.79 [6.6]	47.17^{f}±0.45 [5.2]	56.43^{e}±1.20 [7.1]
Putrescine	31.23^{c}±0.38 [11.9]	37.60^{d}±0.40 [12.8]	49.80^{d}±0.30 [10.2]	59.77^{d}±0.40 [12.3]
Calcium + Bio-priming with rhizobium	32.98^{b}±0.31 [16.4]	38.60^{c}±0.50 [15.1]	50.67^{c}±0.40 [11.8]	61.07^{cd}±0.35 [14.1]
Calcium + Putrescine	35.47^{a}±1.42 [22.4]	40.83^{a}±0.45 [19.7]	53.17^{a}±0.35 [15.9]	64.07^{ab}±0.51 [18.2]
Bio-priming with rhizobium + Putrescine	33.70^{b}±0.46 [18.3]	39.80^{b}±0.50 [17.7]	52.10^{b}±0.40 [14.2]	62.60^{bc}±0.50 [16.2]
Calcium + Bio priming with rhizobium + Putrescine	37.23^{a}±0.32 [26.1]	41.43^{a}±0.42 [20.9]	54.27^{a}±0.45 [17.6]	65.27^{a}±0.42 [19.7]
C.D at p=0.05	1.17	0.99	0.78	1.57

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the percent increase /decrease over the control

Fig. 4.2.1.a: Impact of the treatments on SPAD index of green gram in *kharif* season



4.2.2 Chlorophyll content

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Chlorophyll content was measured at 30 and 60 days after sowing (DAS), with detailed results in Table 4.2.2.a and 4.2.2.b and illustrated in Figures 4.2.2.a and 4.2.2.b.

In the *Summer* of 2022, treatment T_7 significantly increased chlorophyll content, showing improvements of 38.2% and 40.1% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased chlorophyll content by 33.3% and 38.0%, while treatment T_6 showed 27.6% and 36.5% reductions. The lowest chlorophyll content was observed in treatment T_1, with 12.9% and 23.8% reductions. During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in chlorophyll content, with 41.5% and 33.0% improvements. Conversely, treatment T_5 decreased by 34.2% and 30.9%, and treatment T_1 recorded the lowest chlorophyll content with 17.5% and 16.1% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest chlorophyll content, with 38.8% and 46.2% increases. Treatment T_5 decreased 33.1% and 43.6%, while the lowest chlorophyll content was recorded for treatment T_1, with 14.8% and 31.4% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest chlorophyll content, increasing 35.6% and 28.1%. Treatment T_5 followed with improvements of 32.0% and 25.9%. The minimum chlorophyll content in the *Kharif* season was observed in treatment T_1, showing 11.8% and 12.7% reductions compared to the control.

Rhizobium improved nitrogen availability resulting from the biological nitrogen fixation process facilitated by Rhizobium. Nitrogen is a vital component of chlorophyll molecules, and its increased availability likely led to higher chlorophyll synthesis in the inoculated plants. The enhanced chlorophyll content suggests that Rhizobium inoculation not only improves nitrogen supply but also enhances the photosynthetic capacity of the plants, contributing to better growth and productivity.

The application of putrescine resulted in an increase in chlorophyll levels, which can be linked to its role in promoting stress tolerance, cell division, and overall plant growth. Putrescine has been shown to protect plants from oxidative stress and stabilize chlorophyll molecules, which could explain the observed increase in chlorophyll content. Calcium plays a critical role in maintaining cellular structure and function, including the stability of chloroplast membranes where chlorophyll is housed. Additionally, calcium is involved in various signalling pathways that regulate chlorophyll synthesis and degradation. The observed increase in chlorophyll content with calcium treatment suggests that adequate calcium levels are essential for maintaining healthy chloroplasts and ensuring efficient photosynthesis.

Table-4.2.2.a: Impact of the treatments on total chlorophyll content (mg g^{-1}FW) of green gram in *Summer* season

Treatments	30DAS		60DAS	
	*Summer*2022	*Summer*2023	*Summer*2022	*Summer*2023
Control	$0.63^{c}\pm0.05$	$0.62^{f}\pm0.03$	$1.39^{c}\pm0.32$	1.39 ± 0.24
Calcium	$0.72^{bc}\pm0.18$ [12.9]	$0.73^{def}\pm0.01$ [14.8]	$1.82^{abc}\pm0.24$ [23.8]	$2.02^{b}\pm0.16$ [31.4]
Bio-priming with rhizobium	$0.70^{c}\pm0.10$ [10.0]	$0.68^{ef}\pm0.07$ [8.4]	$1.67^{bc}\pm0.43$ [16.9]	$1.81^{b}\pm0.47$ [23.1]
Putrescine	$0.76^{bc}\pm0.16$ [16.9]	$0.77^{cde}\pm0.05$ [19.6]	$2.04^{ab}\pm0.29$ [31.9]	$2.28^{b}\pm0.13$ [39.1]
Calcium + Bio-priming with rhizobium	$0.84^{abc}\pm0.00$ [25.2]	$0.82^{bcd}\pm0.07$ [24.3]	$2.10^{ab}\pm0.23$ [33.9]	$2.35^{ab}\pm0.10$ [40.9]
Calcium + Putrescine	$0.95^{ab}\pm0.14$ [33.3]	$0.93^{ab}\pm0.08$ [33.1]	$2.24^{a}\pm0.28$ [38.0]	$2.46^{ab}\pm0.27$ [43.6]
Bio-priming with rhizobium + Putrescine	$0.87^{abc}\pm0.05$ [27.6]	$0.85^{bc}\pm0.08$ [27.4]	$2.19^{ab}\pm0.33$ [36.5]	$2.38^{ab}\pm0.13$ [41.5]
Calcium + Bio priming with rhizobium + Putrescine	$1.02^{a}\pm0.22$ [38.2]	$1.01^{a}\pm0.08$ [38.8]	$2.32^{a}\pm0.32$ [40.1]	$2.58^{a}\pm0.13$ [46.2]

C.D at p=0.05	0.21	0.11	0.50	0.38

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the percent increase /decrease over the control

Fig. 4.2.2.a: Impact of the treatments on total chlorophyll content (mg g^{-1} FW) of green gram in *Summer* season

Fig.4.2.2.a Impact of Bio-priming, putrescine and calcium on chlorophyll content of green gram in summer season

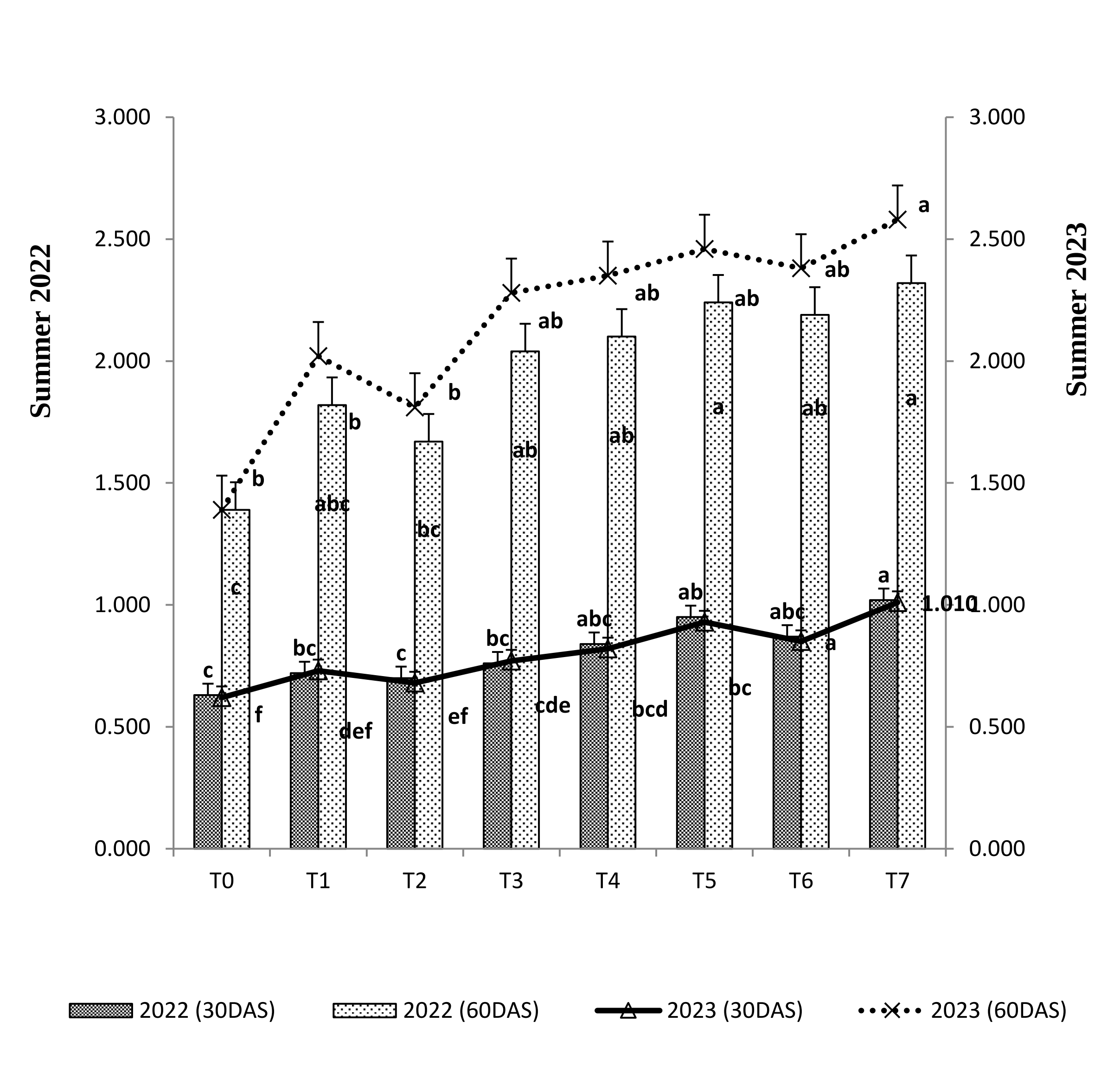


Table-4.2.2.b: Impact of the treatments on total chlorophyll content (mg g^{-1} FW) of green gram in *Kharif* season

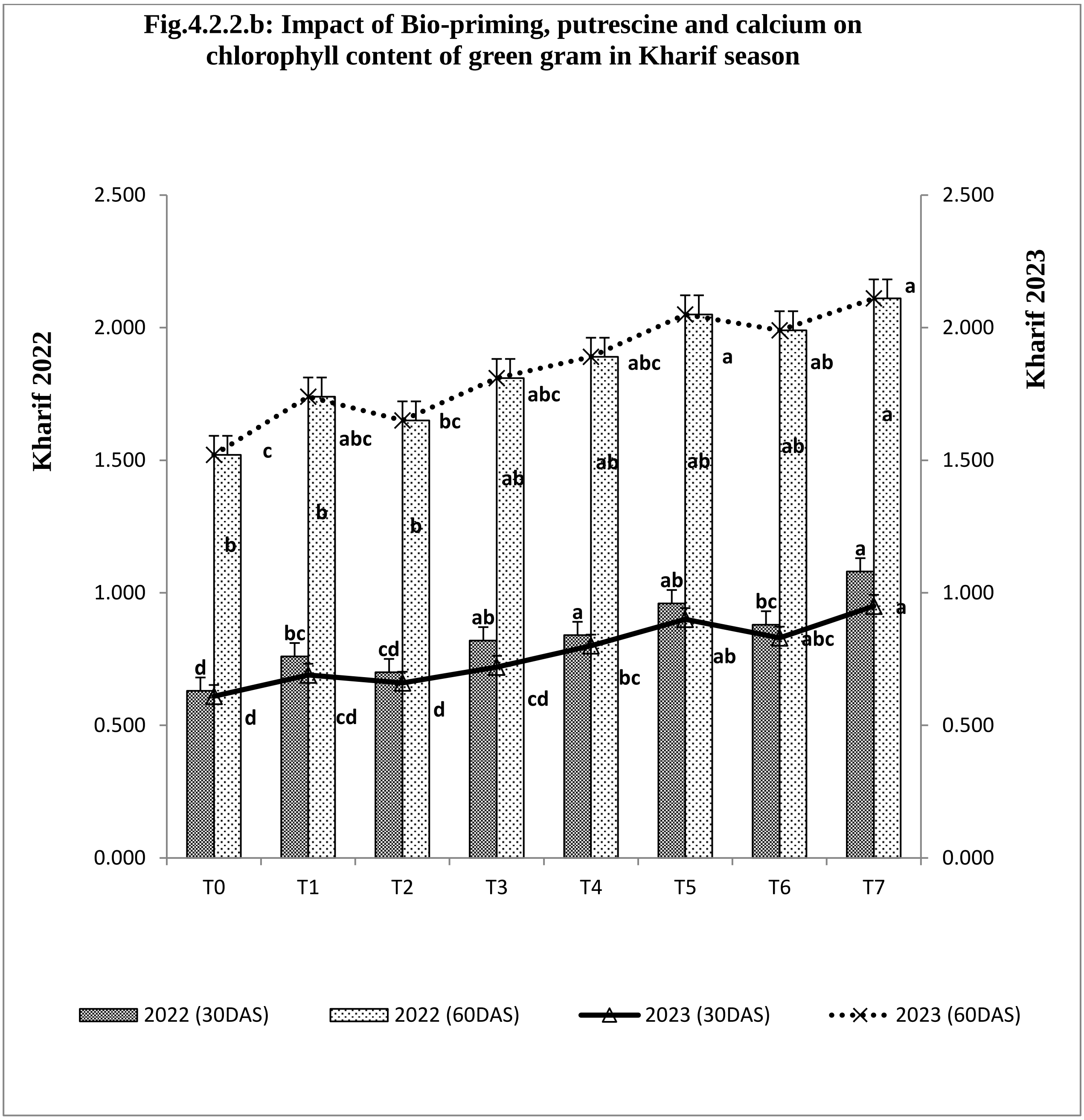
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	0.63^{d}±0.06	0.61^{d}±0.00	1.52^{b}±0.14	1.52^{c}±0.14
Calcium	0.76^{bc}±0.04 [17.5]	0.69^{cd}±0.03 [11.8]	1.74^{b}±0.24 [16.1]	1.74^{abc}±0.24 [12.7]
Bio-priming with rhizobium	0.70^{cd}±0.10 [9.5]	0.66^{d}±0.05 [7.3]	1.65^{b}±0.29 [14.2]	1.65^{bc}±0.29 [7.6]
Putrescine	0.82^{ab}±0.05 [23.1]	0.72^{cd}±0.05 [15.6]	1.81^{ab}±0.42 [23.6]	1.81^{abc}±0.42 [16.1]
Calcium + Bio-priming with rhizobium	0.84^{a}±0.12 [25.0]	0.80^{bc}±0.04 [23.6]	1.89^{ab}±0.08 [25.1]	1.89^{abc}±0.08 [19.4]
Calcium + Putrescine	0.96^{ab}±0.05 [34.2]	0.90^{ab}±0.17 [32.0]	2.05^{ab}±0.02 [30.9]	2.05^{a}±0.02 [25.9]
Bio-priming with rhizobium + Putrescine	0.88^{bc}±0.08 [28.5]	0.83^{abc}±0.11 [26.3]	1.99^{ab}±0.17 [27.7]	1.99^{ab}±0.17 [23.5]
Calcium + Bio priming with rhizobium + Putrescine	1.08^{a}±0.06 [41.5]	0.95^{a}±0.10 [35.6]	2.11^{a}±0.24 [33.0]	2.11^{a}±0.24 [28.1]
C.D at p=0.05	0.14	0.13	0.35	0.35

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.2.b: Impact of the treatments on total chlorophyll content (mg g^{-1} FW) of green gram in *Kharif* season



4.2.3 Total protein content in leaves

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Total protein content was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.2.3.a and 4.2.3.b and illustrated in Figures 4.2.3.a and 4.2.3.b. In the *Summer* of 2022, treatment T_7 significantly increased protein content, showing improvements of 8.3% and 15.4% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased protein content by 7.0% and 12.5%, while treatment T_6 showed 6.6% and 12.4% reductions. The lowest protein content was observed in treatment T_1, with 1.6% and 9.5% reductions. During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in protein content, with 9.2% and 17.2% improvements. Conversely, treatment T_5 decreased by 8.4% and 16.5%, and treatment T_1 recorded the lowest protein content with 2.7% and 13.1% reductions. In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest protein content, with 9.5% and 15.9% increases. Treatment T_5 decreased 7.9% and 12.9%, while the lowest protein content was recorded for treatment T_1, with 1.7% and 12.8% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest protein content, increasing 12.0% and 17.9%. Treatment T_5 followed with improvements of 11.1% and 17.2%. The minimum protein content in the *Kharif* season was observed in treatment T_1, showing 5.3% and 13.6% reductions compared to the control.

Nitrogen is a critical element in amino acids, which are the building blocks of proteins. The increased nitrogen fixation likely led to higher nitrogen assimilation into amino acids and, consequently, into proteins. Recent studies have shown that Rhizobium inoculation can enhance protein content in leguminous crops by improving nitrogen uptake and utilization (Chaudhary *et al.*, 2021; Singh *et al.*, 2022). The application of putrescine increased protein levels, which can be linked to its role in enhancing nitrogen metabolism and stress tolerance. Polyamines like putrescine are known to modulate the activities of enzymes involved in protein synthesis and nitrogen assimilation, leading to improved protein accumulation in plant tissues (Gill and Tuteja, 2022; Gupta *et al.*, 2023). Calcium is essential for various cellular processes, including protein synthesis and enzyme activation. It plays a crucial role in maintaining cellular integrity and function, vital for efficient protein biosynthesis. Recent research has indicated that adequate calcium levels can enhance the protein content in crops by stabilising cellular structures and supporting the activities of enzymes involved in protein metabolism (Huang *et al.*, 2023; Patel *et al.*, 2023). This finding underscores the importance of using a multifaceted strategy to enhance protein content in mung beans. The results suggest that while Rhizobium plays a critical role in providing the nitrogen necessary for protein synthesis, adding putrescine and calcium further supports this process by enhancing nitrogen metabolism and maintaining cellular functions.

Table 4.2.3.a: Impact of the treatments on total protein content (mg g^{-1} FW) in leaves of green gram in the *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	21.12^{e}±0.04	20.13^{e}±0.04	18.05^{c}±1.15	17.27^{c}±1.15
Calcium	21.47^{d}±0.08 [1.6]	20.49^{d}±0.08 [1.7]	19.95^{b}±0.04 [9.5]	19.16^{b}±0.04 [9.9]
Bio-priming with rhizobium	21.19^{e}±0.11 [0.3]	20.21^{e}±0.11 [0.4]	19.63^{b}±0.28 [8.0]	18.84^{b}±0.28 [8.3]
Putrescine	21.99^{c}±0.08 [3.9]	21.00^{c}±0.08 [4.1]	19.99^{b}±0.05 [9.7]	19.20^{b}±0.05 [10.1]
Calcium + Bio-priming with rhizobium	22.43^{b}±0.05 [5.8]	21.44^{b}±0.05 [6.1]	20.53^{ab}±0.05 [12.1]	19.74^{ab}±0.05 [12.5]
Calcium + Putrescine	22.72^{a}±0.06 [7.0]	21.85^{a}±0.08 [7.9]	20.63^{ab}±0.03 [12.5]	19.84^{ab}±0.03 [12.9]
Bio-priming with rhizobium + Putrescine	22.60^{a}±0.09 [6.6]	21.62^{ab}±0.09 [6.8]	20.59^{ab}±0.05 [12.4]	19.81^{ab}±0.05 [12.8]
Calcium + Bio priming with rhizobium + Putrescine	23.02^{a}±0.02 [8.3]	22.25^{a}±0.35 [9.5]	21.33^{a}±1.13 [15.4]	20.54^{a}±1.13 [15.9]
C.D at p=0.05	0.14	0.26	1.01	1.01

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.3.a: Impact of the treatments on total protein content (mg g^{-1} FW) in leaves of green gram in the *Summer* season

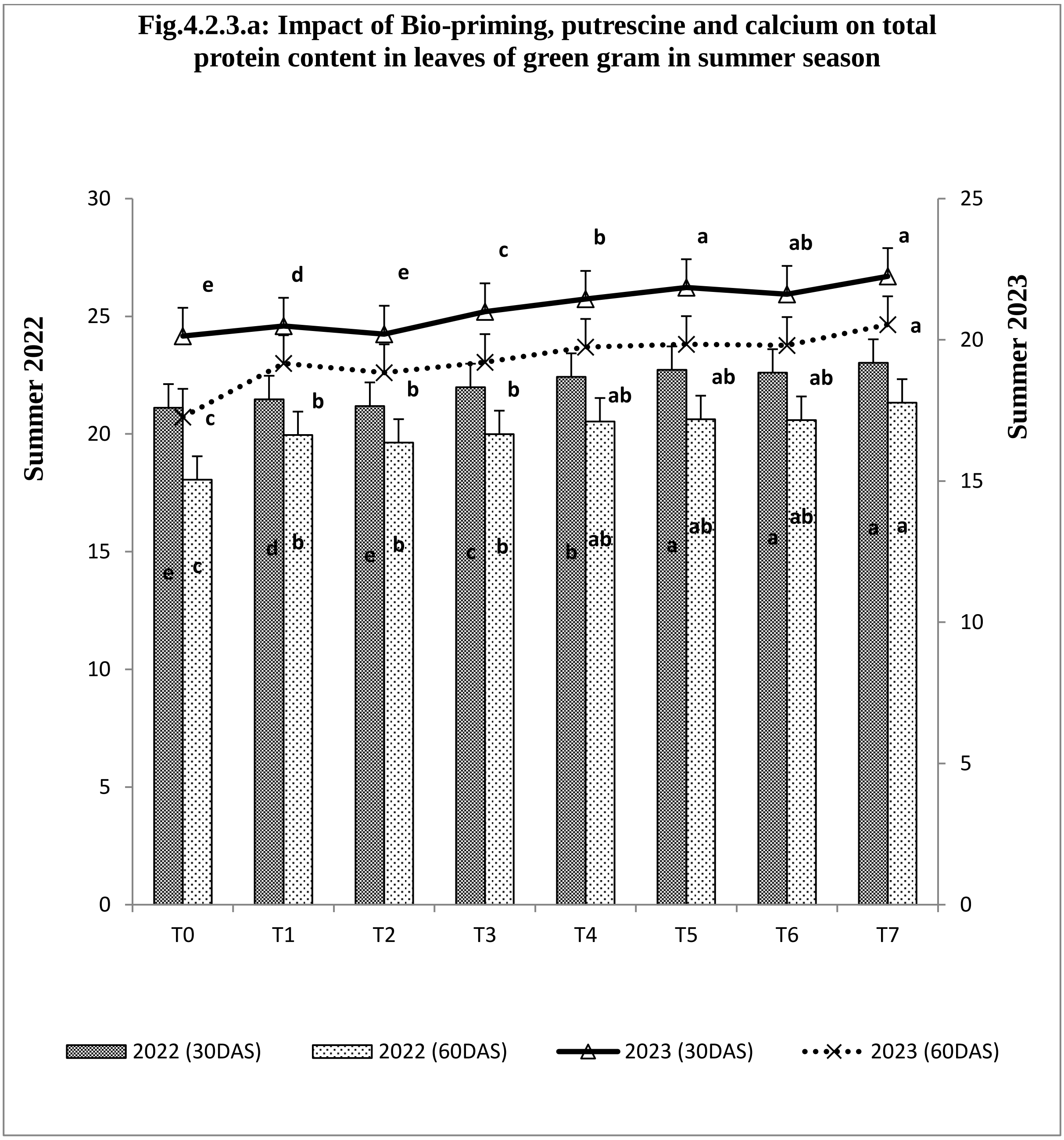


Table 4.2.3.b: Impact of the treatments on total protein content (mg g^{-1} FW) in leaves of green gram in the *Kharif* season

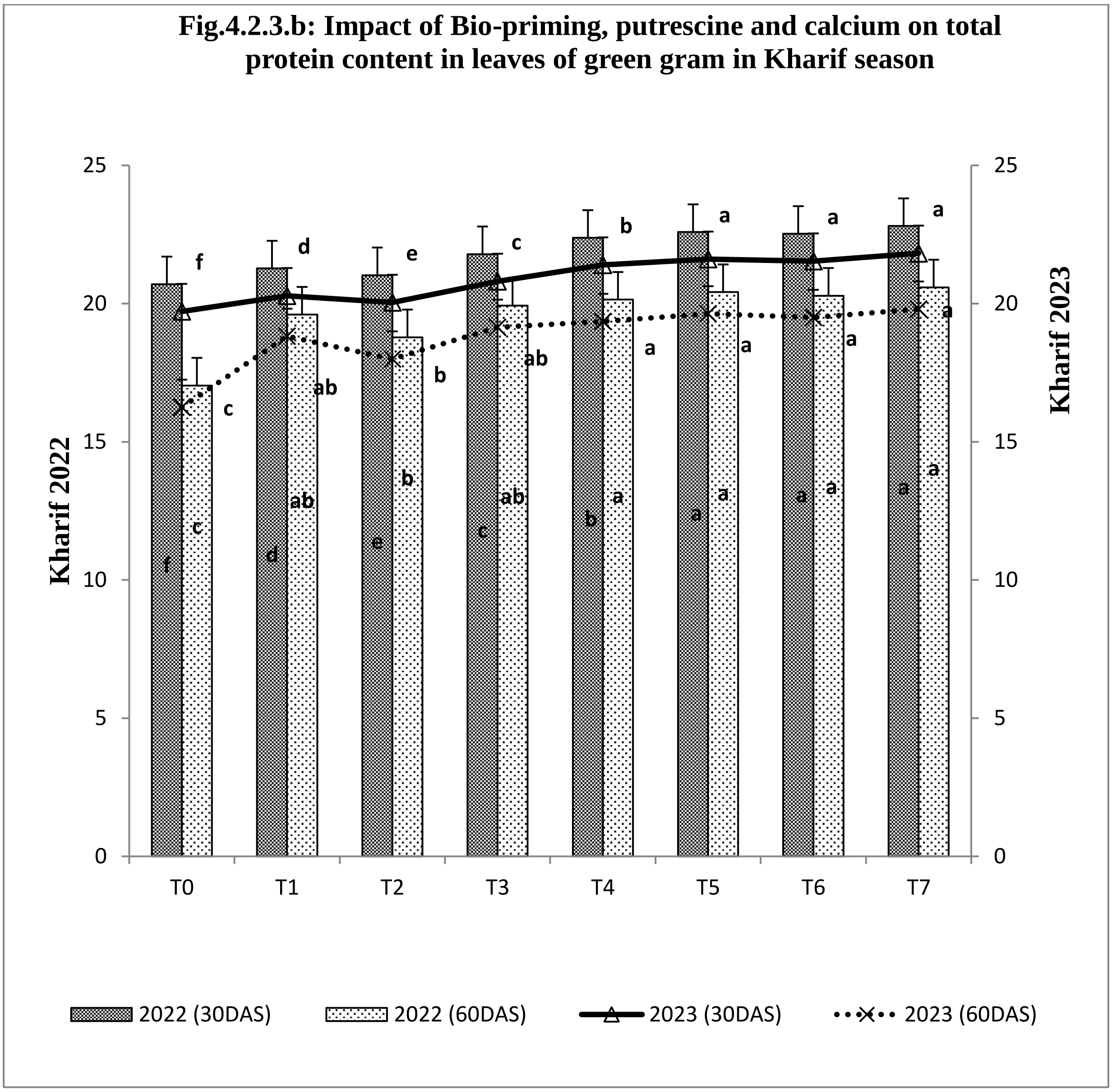
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	20.70^{f}±0.04	19.71^{f}±0.04	17.04^{c}±1.40	16.25^{c}±1.40
Calcium	21.27^{d}±0.04 [2.7]	20.29^{d}±0.04 [5.3]	19.60^{ab}±0.25 [13.1]	18.82^{ab}±0.25 [13.6]
Bio-priming with rhizobium	21.03^{e}±0.13 [1.6]	20.04^{e}±0.13 [4.1]	18.78^{b}±0.98 [9.3]	17.99^{b}±0.98 [9.7]
Putrescine	21.79^{c}±0.16 [5.0]	20.80^{c}±0.16 [7.7]	19.93^{ab}±0.08 [14.5]	19.14^{ab}±0.08 [15.1]
Calcium + Bio-priming with rhizobium	22.38^{b}±0.09 [7.5]	21.40^{b}±0.09 [10.2]	20.14^{a}±0.03 [15.4]	19.35^{a}±0.03 [16.0]
Calcium + Putrescine	22.59^{a}±0.04 [8.4]	21.61^{a}±0.04 [11.1]	20.42^{a}±0.05 [16.5]	19.63^{a}±0.05 [17.2]
Bio-priming with rhizobium + Putrescine	22.52^{a}±0.05 [8.1]	21.54^{a}±0.05 [10.8]	20.29^{a}±0.09 [16.0]	19.50^{a}±0.09 [16.7]
Calcium + Bio priming with rhizobium + Putrescine	22.81^{a}±0.04 [9.2]	21.82^{a}±0.04 [12.0]	20.59^{a}±0.06 [17.2]	19.80^{a}±0.06 [17.9]
C.D at p=0.05	0.14	0.14	1.14	1.14

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.3.b: Impact of the treatments on total protein content (mg g^{-1} FW) in leaves of green gram in the *Kharif* season



4.2.4 Total carbohydrate content in leaves

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Total carbohydrate content was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.2.4.a and 4.2.4.b and illustrated in Figures 4.2.4.a and 4.2.4.b.

In the *Summer* of 2022, treatment T_7 significantly increased carbohydrate content, showing improvements of 37.6% and 40.0% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased carbohydrate content by 34.9% and 37.2%, while treatment T_6 showed 31.5% and 33.1% reductions. The lowest carbohydrate content was observed in treatment T_1, with 13.3% and 11.0% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in carbohydrate content, with 44.6% and 41.8% improvements. Conversely, treatment T_5 decreased by 41.5% and 40.7%, and treatment T_1 recorded the lowest carbohydrate content with 18.2% and 17.3% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest carbohydrate content, with 38.0% and 34.8% increases. Treatment T_5 decreased 35.4% and 31.7%, while the lowest carbohydrate content was recorded for treatment T_1, with 17.2% and 9.4% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest carbohydrate content, increasing 38.3% and 42.8%. Treatment T_5 followed with improvements of 34.2% and 41.7%. The minimum carbohydrate content in the *Kharif* season was observed in treatment T_1, showing 18.5% and 17.8% reductions compared to the control.

Carbohydrates are essential plant storage compounds, contributing significantly to seed quality and energy supply. Enhanced photosynthesis leads to the production of more carbohydrates, as the photosynthetic process converts carbon dioxide into sugars and starches that are stored in the seeds. Recent studies have demonstrated that Rhizobium inoculation can significantly increase carbohydrate content in leguminous crops by promoting nitrogen assimilation and photosynthetic efficiency (Sharma *et al.*, 2022; Patel *et al.*, 2023). Polyamines like putrescine have been shown to improve photosynthetic efficiency and carbon metabolism, leading to higher carbohydrate accumulation (Kumar *et al.*, 2022; Gupta *et al.*, 2023). The results suggest that putrescine enhances carbohydrate synthesis by stabilising photosynthesis. Calcium is crucial for various cellular processes, including carbohydrate metabolism. It plays a role in regulating enzymes that synthesise and break down carbohydrates, and it helps maintain cell wall integrity, which is essential for storing carbohydrates in seeds (Huang *et al.*, 2023; Singh and Verma, 2023).

Table 4.2.4.a: Impact of the treatments on total carbohydrates (mg g^{-1}FW) of green gram in the *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	5.06^{f}±0.10	5.06^{f}±0.10	4.50^{d}±0.33	4.78^{d}±0.19
Calcium	5.83^{e}±0.50 [13.3]	6.11^{e}±0.25 [17.2]	5.06^{d}±0.25 [11.0]	5.28^{d}±0.35 [9.4]
Bio-priming with rhizobium	5.78^{e}±0.35 [12.4]	5.89^{e}±0.25 [14.1]	4.78^{d}±0.25 [5.8]	5.11^{d}±0.25 [6.5]
Putrescine	6.44^{d}±0.25 [21.5]	6.67^{d}±0.17 [24.1]	5.78^{c}±0.25 [22.1]	6.22^{c}±0.19 [23.2]
Calcium + Bio-priming with rhizobium	7.00^{cd}±0.33 [27.7]	7.11^{c}±0.19 [28.8]	6.17^{c}±0.33 [27.0]	6.33^{c}±0.17 [24.5]
Calcium + Putrescine	7.78^{ab}±0.25 [34.9]	7.83^{ab}±0.29 [35.4]	7.17^{ab}±0.33 [37.2]	7.00^{ab}±0.33 [31.7]
Bio-priming with rhizobium + Putrescine	7.39^{bc}±0.25 [31.5]	7.56^{b}±0.35 [33.0]	6.72^{b}±0.25 [33.1]	6.67^{bc}±0.33 [28.3]
Calcium + Bio priming with rhizobium + Putrescine	8.11^{a}±0.25 [37.6]	8.17^{a}±0.17 [38.0]	7.50^{a}±0.17 [40.0]	7.33^{a}±0.17 [34.8]
C.D at p=0.05	0.56	0.43	0.49	0.44

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.4.a: Impact of the treatments on total carbohydrates (mg g^{-1}FW) in leaves of green gram in the *Summer* season

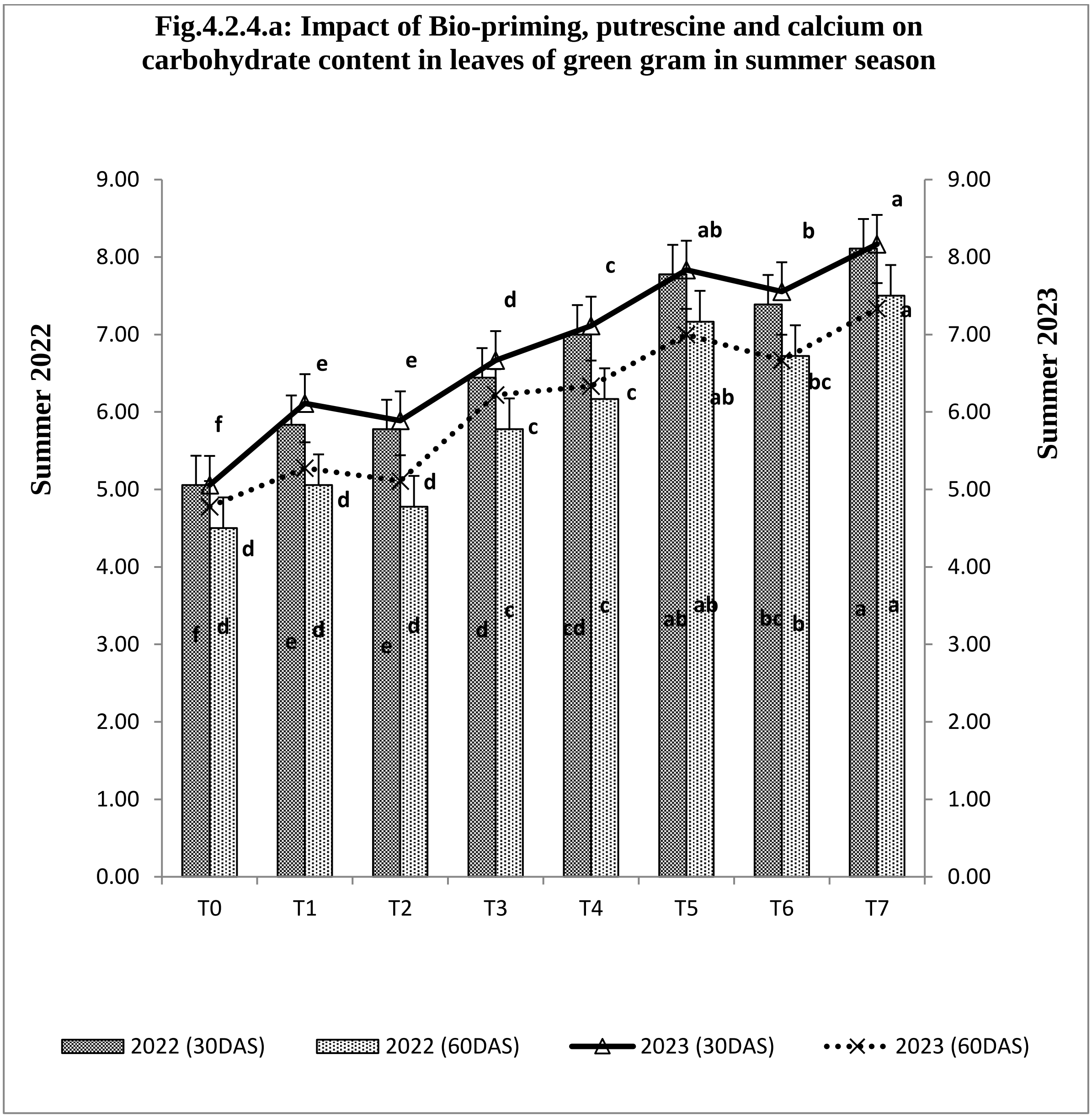


Table 4.2.4.b: Impact of the treatments on total carbohydrates (mg g^{-1}FW) of green gram in *Kharif* season

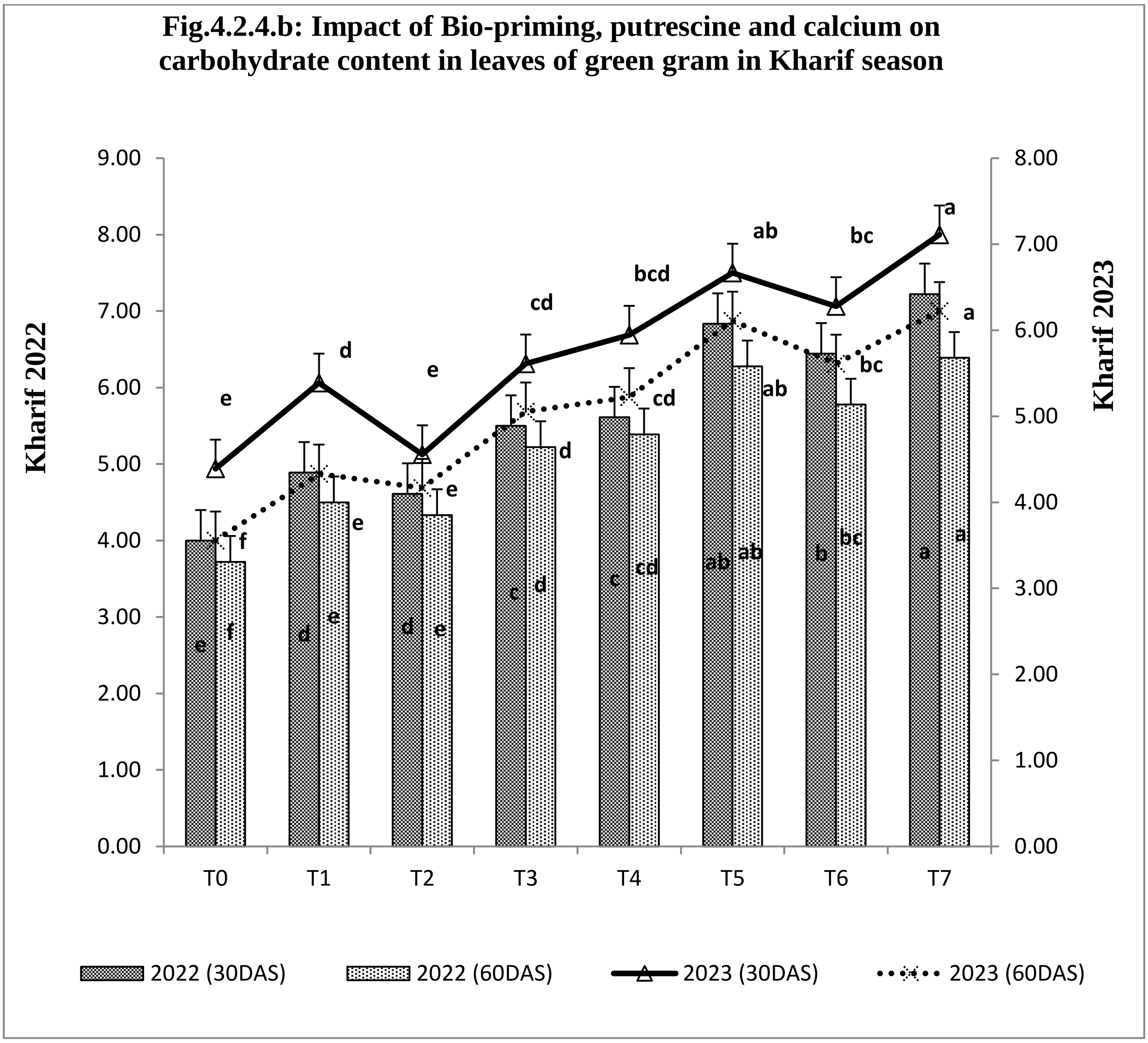
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	$4.00^{e}\pm0.33$	$4.39^{e}\pm0.38$	$3.72^{f}\pm0.25$	$3.56^{f}\pm0.25$
Calcium	$4.89^{d}\pm0.25$ [18.2]	$5.39^{d}\pm0.42$ [18.5]	$4.50^{e}\pm0.33$ [17.3]	$4.33^{e}\pm0.33$ [17.8]
Bio-priming with rhizobium	$4.61^{d}\pm0.25$ [13.3]	$4.56^{e}\pm0.63$ [3.6]	$4.33^{e}\pm0.33$ [14.2]	$4.17^{e}\pm0.33$ [14.6]
Putrescine	$5.50^{c}\pm0.17$ [27.3]	$5.61^{cd}\pm0.19$ [21.8]	$5.22^{d}\pm0.25$ [28.8]	$5.06^{d}\pm0.25$ [29.6]
Calcium + Bio-priming with rhizobium	$5.61^{c}\pm0.25$ [28.7]	$5.94^{bcd}\pm0.25$ [26.1]	$5.39^{cd}\pm0.25$ [31.0]	$5.22^{cd}\pm0.25$ [31.8]
Calcium + Putrescine	$6.83^{ab}\pm0.17$ [41.5]	$6.67^{ab}\pm0.17$ [34.2]	$6.28^{ab}\pm0.25$ [40.7]	$6.11^{ab}\pm0.25$ [41.7]
Bio-priming with rhizobium + Putrescine	$6.44^{b}\pm0.25$ [37.9]	$6.28^{bc}\pm0.25$ [30.1]	$5.78^{bc}\pm0.25$ [35.6]	$5.61^{bc}\pm0.25$ [36.6]
Calcium + Bio priming with rhizobium + Putrescine	$7.22^{a}\pm0.25$ [44.6]	$7.11^{a}\pm0.51$ [38.3]	$6.39^{a}\pm0.25$ [41.8]	$6.22^{a}\pm0.25$ [42.8]
C.D at p=0.05	0.42	0.69	0.51	0.51

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.4.b: Impact of the treatments on total carbohydrates (mg g^{-1}FW) in leaves of green gram in *Kharif* season



4.2.5 Total phenylalanine ammonia-lyase (PAL) activity in leaves

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. PAL activity was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.2.5.a and 4.2.5.b and illustrated in Figures 4.2.5.a and 4.2.5.b.

In the *Summer* of 2022, treatment T_7 significantly increased PAL activity, showing improvements of 5.1% and 3.9% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased PAL activity by 4.5% and 3.5%, while treatment T_6 showed 4.1% and 3.3% reductions. The lowest PAL activity was observed in treatment T_1, with 1.9% and 1.5% reductions. During the *Kharif* season 2022, treatment T_7 again demonstrated a notable increase in PAL activity, with 3.8% and 5.7% improvements. Conversely, treatment T_5 decreased by 3.1% and 5.7%, and treatment T_1 recorded the lowest PAL activity with 1.4% and 1.5% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest PAL activity, with 3.0% and 4.9% increases. Treatment T_5 decreased by 2.3% and 4.2%, while the lowest PAL activity was recorded for treatment T_1, with 0.6% and 1.7% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest PAL activity, increasing 4.4% and 4.6%. Treatment T_5 followed with improvements of 3.4% and 3.7%. The minimum PAL activity in the *Kharif* season was observed in treatment T_1, showing 1.4% and 0.4% reductions compared to the control.

PAL is a crucial enzyme in the phenylpropanoid pathway, which synthesises various secondary metabolites, including flavonoids, lignin, and phytoalexins. These compounds play essential roles in plant defence, growth, and development. Rhizobium inoculation can enhance PAL activity by promoting nitrogen assimilation and triggering systemic acquired resistance, which involves the phenylpropanoid pathway (Kumar *et al.,* 2022; Sharma *et al.,* 2023). Putrescine, a polyamine involved in plant growth and stress responses, is known to modulate various enzymatic activities, including those in the phenylpropanoid pathway. The observed increase in PAL activity suggests that putrescine may enhance phenylpropanoid synthesis, contributing to improved plant defence mechanisms and secondary metabolite production. Polyamines like putrescine have been shown to interact with critical enzymes, such as PAL, to enhance their activity, particularly under stress conditions (Gill and Tuteja, 2022; Zafar *et al.,* 2023). The observed increase in PAL activity with calcium treatment suggests that calcium acts as a secondary messenger, triggering the activation of PAL and subsequent phenylpropanoid synthesis. Calcium's role in stabilising cellular structures and protecting cells from oxidative stress may also contribute to maintaining high PAL activity (Gupta and Kumar, 2022; Huang *et al.,* 2023)

Table 4.2.5.a: Impact of the treatments on total phenylalanine ammonia-lyase (PAL) activity (μ^{-1} mg^{-1} $min.^{-1}$) in leaves of green gram in *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	0.070^{g}±0.005	0.071^{g}±0.005	0.075^{g}±0.005	0.074^{g}±0.003
Calcium	0.071^{e}±0.005 [1.9]	0.071^{e}±0.002 [0.6]	0.076^{e}±0.005 [1.5]	0.075^{e}±0.006 [1.7]
Bio-priming with rhizobium	0.070^{f}±0.005 [0.5]	0.071^{f}±0.005 [0.0]	0.075^{f}±0.005 [0.5]	0.074^{f}±0.006 [0.5]
Putrescine	0.072^{d}±0.005 [2.3]	0.072^{d}±0.005 [1.0]	0.076^{d}±0.005 [1.8]	0.076^{d}±.005 [2.3]
Calcium + Bio-priming with rhizobium	0.073^{c}±0.005 [4.0]	0.072^{c}±0.005 [1.6]	0.077^{c}±0.005 [3.0]	0.076^{c}±0.005 [3.2]
Calcium + Putrescine	0.073^{a}±0.005 [4.5]	0.072^{a}±0.003 [2.3]	0.078^{a}±0.005 [3.5]	0.077^{a}±0.004 [4.2]
Bio-priming with rhizobium + Putrescine	0.073^{b}±0.005 [4.1]	0.0730^{b}±0.005 [2.2]	0.078^{b}±0.005 [3.3]	0.077^{b}±0.003 [3.7]
Calcium + Bio priming with rhizobium + Putrescine	0.074^{a}±0.003 [5.1]	0.07^{a}±0.005 [3.0]	0.078^{a}±0.005 [3.9]	0.078^{a}±0.003 [4.9]
C.D at p=0.05	0.001	0.001	0.001	0.001

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.5.a: Impact of the treatments on total phenylalanine ammonia-lyase (PAL) activity (μ^{-1} mg^{-1} $min.^{-1}$) in leaves of green gram in *Summer* season

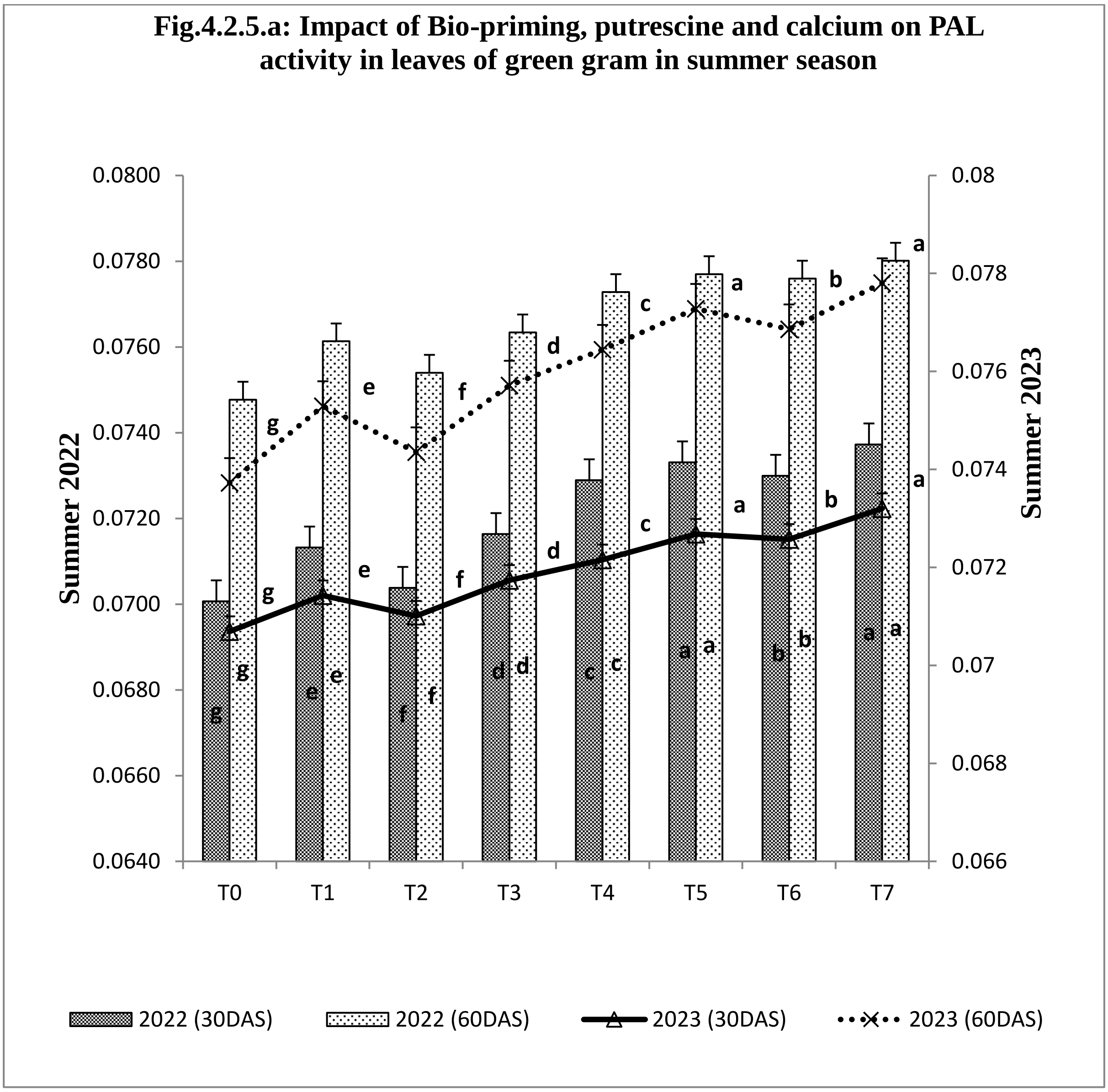


Table 4.2.5.b: Impact of the treatments on total phenylalanine ammonia-lyase (PAL) activity (μ^{-1} mg^{-1} $min.^{-1}$) in leaves of green gram in *Kharif* season

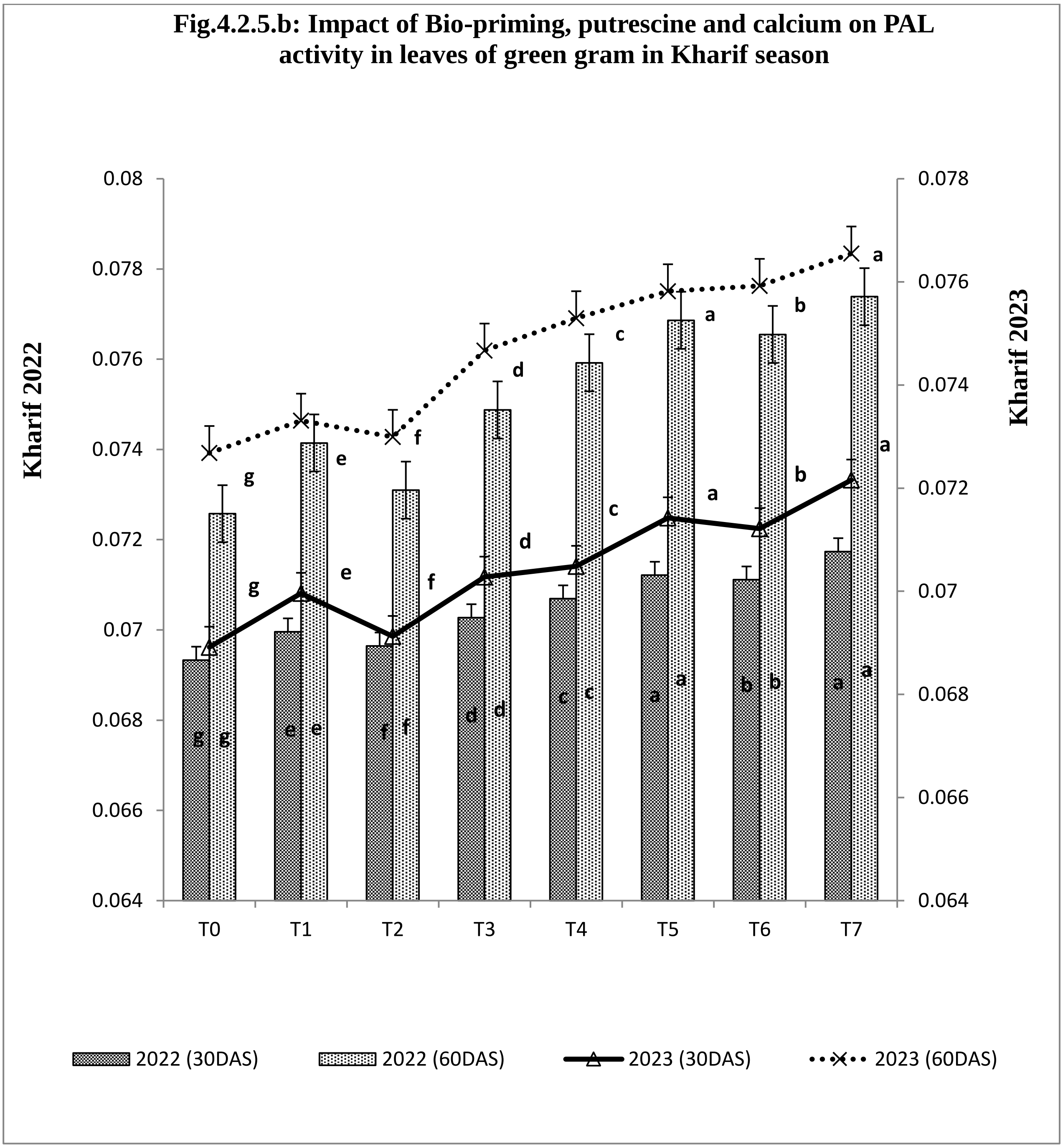
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	0.069^{g}±0.003	0.069^{g}±0.002	0.073^{g}±0.005	0.073^{g}±0.005
Calcium	0.070^{e}±0.003 [1.4]	0.070^{e}±0.003 [1.4]	0.074^{e}±0.005 [1.5]	0.073^{e}±0.005 [0.4]
Bio-priming with rhizobium	0.070^{f}±0.005 [0.9]	0.069^{f}±0.002 [0.2]	0.073^{f}±0.003 [0.1]	0.073^{f}±0.005 [0.0]
Putrescine	0.070^{d}±0.005 [1.8]	0.070d±0.002 [1.8]	0.075^{d}±0.005 [2.5]	0.075^{d}±0.005 [2.2]
Calcium + Bio-priming with rhizobium	0.071^{c}±0.005 [2.4]	0.070^{c}±0.003 [2.1]	0.076^{c}±0.003 [3.8]	0.075^{c}±0.006 [3.0]
Calcium + Putrescine	0.071^{a}±0.005 [3.1]	0.071^{a}±0.005 [3.4]	0.077^{a}±0.003 [5.0]	0.076^{a}±0.005 [3.7]
Bio-priming with rhizobium + Putrescine	0.071^{b}±0.005 [3.0]	0.071^{b}±0.003 [3.1]	0.077^{b}±0.003 [4.6]	0.076^{b}±0.008 [3.8]
Calcium + Bio priming with rhizobium + Putrescine	0.072^{a}±0.005 [3.8]	0.072^{a}±0.003 [4.4]	0.077^{a}±0.002 [5.7]	0.077^{a}±0.003 [4.6]
C.D at p=0.05	0.001	0.001	0.001	0.001

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.5.b: Impact of the treatments on total phenylalanine ammonia-lyase (PAL) activity (μ^{-1} mg^{-1} $min.^{-1}$) in leaves of green gram in *Kharif* season



4.2.6 Total flavanol content in leaves

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Total flavanol content was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.2.6.a and 4.2.6.b and illustrated in Figures 4.2.6.a and 4.2.6.b.

In the *Summer* of 2022, treatment T_7 significantly increased flavanol content, showing 6.8% and 4.8% improvements at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased flavanol content by 6.5% and 4.5%, while treatment T_6 showed 5.8% and 4.2% reductions. The lowest flavanol content was observed in treatment T_1, with 4.2% and 1.4% reductions.

During the *Kharif* season 2022, treatment T_7 again demonstrated a notable increase in flavanol content, with 20.0% and 5.6% improvements. Conversely, treatment T_5 decreased by 18.0% and 4.0%, and treatment T_1 recorded the lowest flavanol content with 16.0% and 0.7% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest flavanol content, with 16.7% and 7.6% increases. Treatment T_5 decreased by 15.8% and 7.5%, while the lowest flavanol content was recorded for treatment T_1, with 6.4% and 3.6% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest flavanol content, increasing 4.6% and 18.8%. Treatment T_5 followed with improvements of 4.1% and 16.6%. The minimum flavanol content in the *Kharif* season was observed in treatment T_1, showing 1.3% and 6.4% reductions compared to the control.

Flavanols are a group of polyphenol compounds that play a crucial role in plant defence mechanisms, antioxidant activity, and overall plant health. The enhancement in flavanol content can be attributed to the improved nitrogen fixation by Rhizobium, which supports the overall metabolic activities of the plant, including the phenylpropanoid pathway responsible for flavanol biosynthesis. Nitrogen is a critical component in amino acids and other organic molecules that serve as precursors in secondary metabolite synthesis, including flavanol (Kumar *et al.*, 2022; Singh *et al.*, 2023). The increase in flavanol content observed with putrescine treatment suggests that it may enhance the biosynthesis of flavanol by promoting the activity of critical enzymes such as phenylalanine ammonia-lyase (PAL), which catalyses the first step in the phenylpropanoid pathway. Additionally, putrescence's role in improving stress tolerance may lead to an upregulation of flavanol synthesis as a protective mechanism against abiotic and biotic stresses (Gill and Tuteja, 2022; Zafar *et al.*, 2023). The increased flavanol content with calcium treatment suggests calcium may act as a secondary messenger in signal transduction pathways that regulate flavanol synthesis. Calcium activates PAL and other enzymes in the phenylpropanoid pathway, enhancing flavanol production (Huang *et al.*, 2023; Gupta and Kumar, 2023).

Table 4.2.6.a: Impact of the treatments on total flavanol content (mg g^{-1} FW) in leaves of green gram in *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	$0.465^{b}\pm0.006$	$0.433^{g}\pm0.008$	$0.535^{g}\pm0.004$	$0.480^{g}\pm0.005$
Calcium	$0.486^{ab}\pm0.009$ [4.2]	$0.463^{e}\pm0.010$ [6.4]	$0.542^{e}\pm0.004$ [1.4]	$0.498^{e}\pm0.004$ [3.6]
Bio-priming with rhizobium	$0.482^{ab}\pm0.013$ [3.5]	$0.444^{f}\pm0.006$ [2.6]	$0.542^{f}\pm0.003$ [1.3]	$0.488^{f}\pm0.004$ [1.6]
Putrescine	$0.488^{a}\pm0.007$ [4.6]	$0.480^{d}\pm0.004$ [9.9]	$0.545^{d}\pm0.006$ [1.9]	$0.503^{d}\pm0.003$ [4.6]
Calcium + Bio-priming with rhizobium	$0.492^{a}\pm0.007$ [5.5]	$0.491^{c}\pm0.006$ [11.8]	$0.553^{c}\pm0.004$ [3.2]	$0.510^{c}\pm0.004$ [5.8]
Calcium + Putrescine	$0.497^{a}\pm0.007$ [6.5]	$0.514^{a}\pm0.002$ [15.8]	$0.560^{a}\pm0.004$ [4.5]	$0.519^{a}\pm0.005$ [7.5]
Bio-priming with rhizobium + Putrescine	$0.493^{a}\pm0.004$ [5.8]	$0.503^{b}\pm0.005$ [14.0]	$0.558^{b}\pm0.004$ [4.2]	$0.514^{b}\pm0.003$ [6.6]
Calcium + Bio priming with rhizobium + Putrescine	$0.499^{a}\pm0.006$ [6.8]	$0.520^{a}\pm0.006$ [16.7]	$0.562^{a}\pm0.004$ [4.8]	$0.520^{a}\pm0.006$ [7.6]
C.D at p=0.05	0.014	0.012	0.008	0.008

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.6.a: Impact of the treatments on total flavanol content (mg g^{-1}FW) in leaves of green gram in *Summer* season

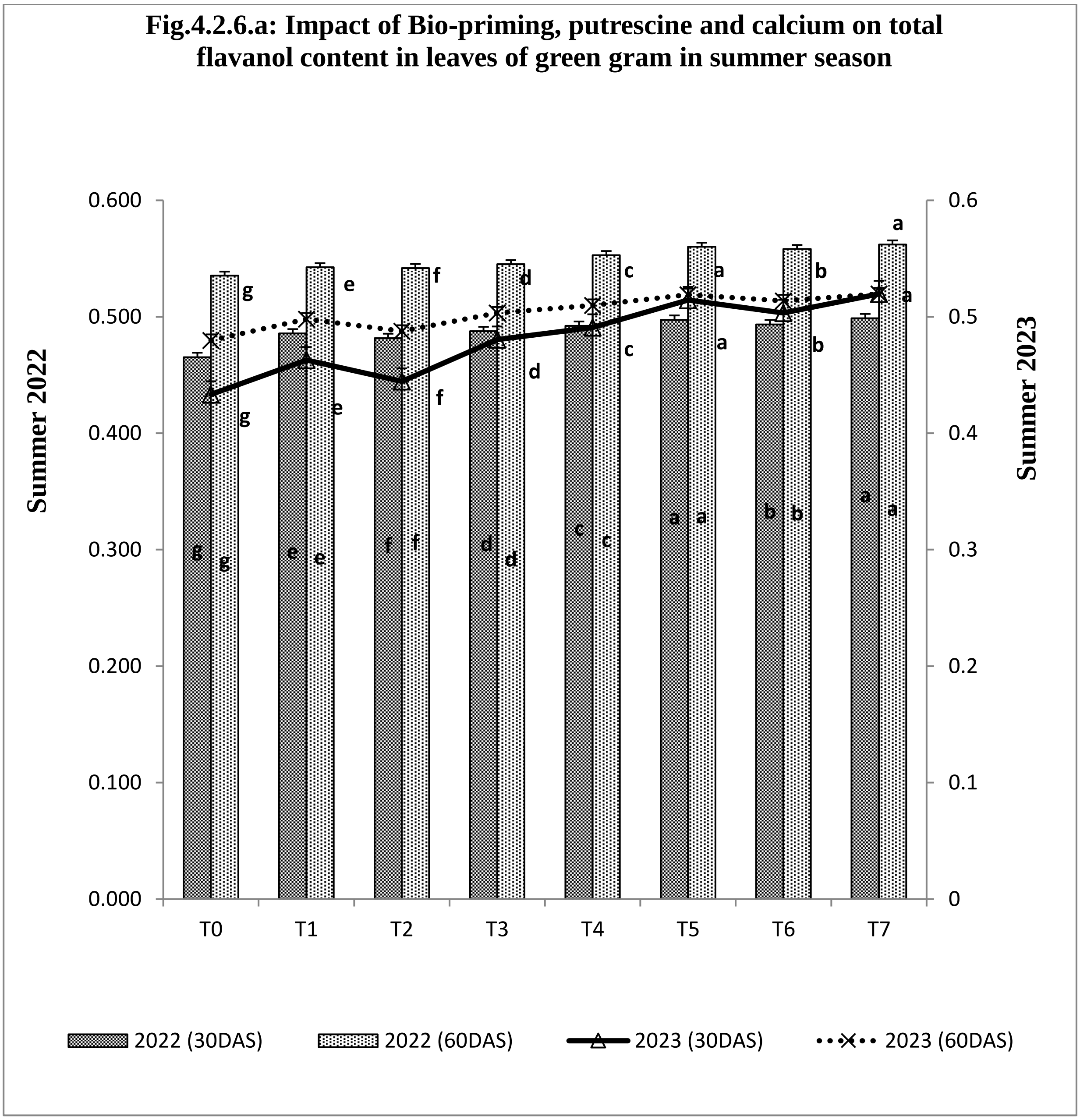


Table 4.2.6.b: Impact of the treatments on total flavanol content (mg g^{-1}FW) in leaves of green gram in *Kharif* season

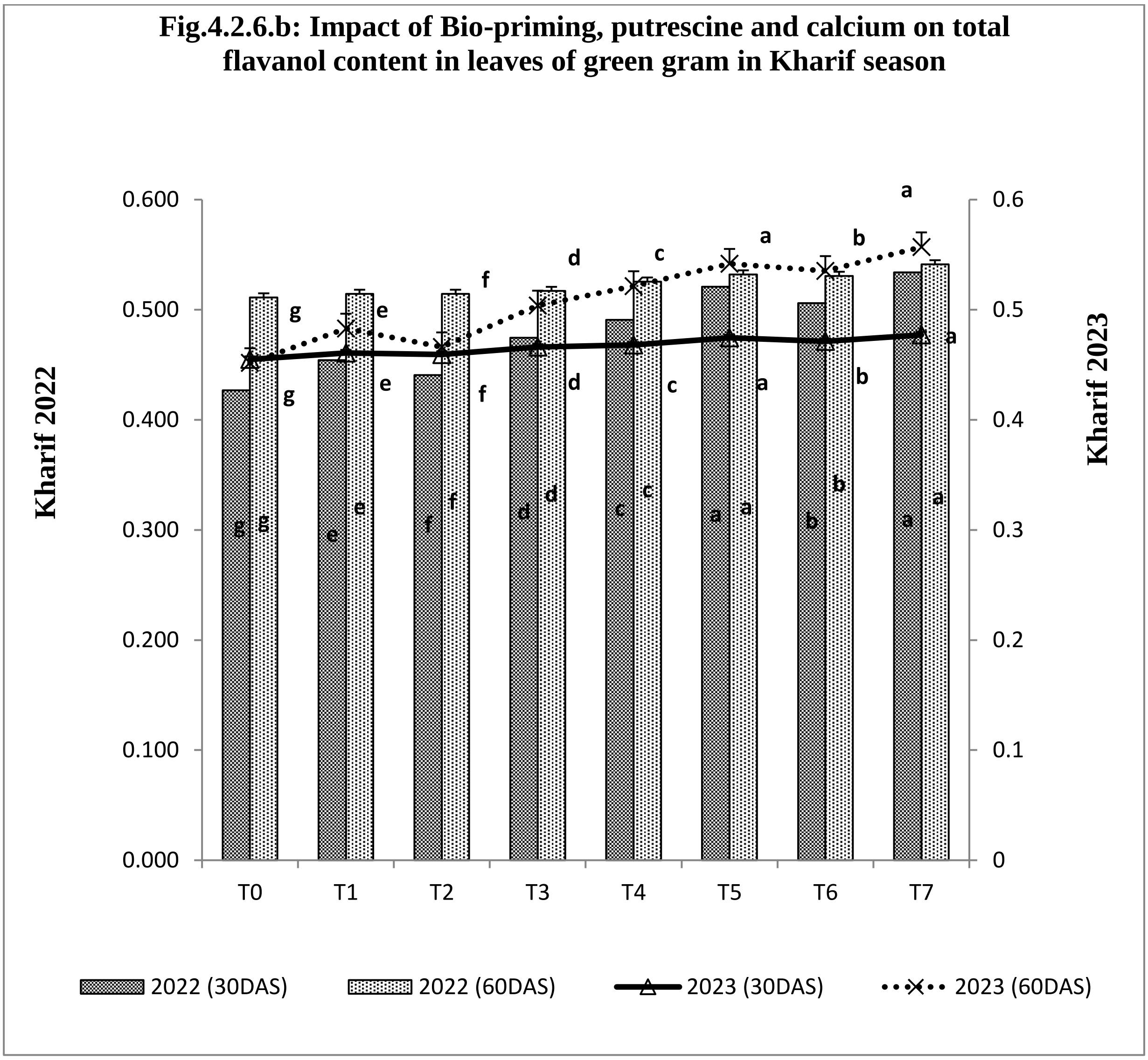
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	0.427^{g}±0.005	0.455^{g}±0.003	0.511^{g}±0.003	0.452^{f}±0.004
Calcium	0.454^{e}±0.006 [6.0]	0.461^{e}±0.004 [1.3]	0.514^{f}±0.002 [0.7]	0.483^{e}±0.009 [6.4]
Bio-priming with rhizobium	0.441^{f}±0.007 [3.1]	0.459^{f}±0.003 [1.0]	0.514^{e}±0.003 [0.7]	0.466^{ef}±0.006 [3.0]
Putrescine	0.475^{d}±0.005 [10.0]	0.466^{d}±0.003 [2.4]	0.517^{d}±0.003 [1.2]	0.504^{d}±0.016 [10.3]
Calcium + Bio-priming with rhizobium	0.491^{c}±0.004 [13.0]	0.468^{c}±0.003 [2.8]	0.525^{c}±0.005 [2.8]	0.522^{c}±0.005 [13.3]
Calcium + Putrescine	0.521^{a}±0.005 [18.0]	0.475^{a}±0.005 [4.1]	0.532^{a}±0.003 [4.0]	0.542^{ab}±0.005 [16.6]
Bio-priming with rhizobium + Putrescine	0.506^{b}±0.006 [15.6]	0.471^{b}±0.005 [3.4]	0.531^{b}±0.006 [3.7]	0.535^{bc}±0.004 [15.6]
Calcium + Bio priming with rhizobium + Putrescine	0.534^{a}±0.003 [20.0]	0.477^{a}±0.005 [4.6]	0.541^{a}±0.003 [5.6]	0.557^{a}±0.005 [18.8]
C.D at p=0.05	0.010	0.007	0.007	0.014

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.6.b: Impact of the treatments on total flavanol content (mg g^{-1}FW) in leaves of green gram in *Kharif* season



4.2.7 Total flavonoid content in leaves

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Total flavonoid content was measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.2.7.a and 4.2.7.b and illustrated in Figures 4.2.7.a and 4.2.7.b.

In the *Summer* of 2022, treatment T_7 significantly increased flavonoid content, showing improvements of 4.5% and 9.4% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased flavonoid content by 3.8% and 9.0%, while treatment T_6 showed 2.5% and 8.1% reductions. The lowest flavonoid content was observed in treatment T1, with 1.1% and 3.8% reductions, respectively.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in flavonoid content, with 6.7% and 8.7% improvements. Conversely, treatment T_5 decreased by 5.8% and 7.8%, and treatment T_1 recorded the lowest flavonoid content with 1.8% and 3.5% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest flavonoid content, with 14.5% and 5.0% increases. Treatment T_5 decreased 13.6% and 4.7%, while the lowest flavonoid content was recorded for treatment T_1, with 6.5% and 0.5% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest flavonoid content, increasing by 11.4% and 14.0%. Treatment T_5 followed with improvements of 9.0% and 12.7%. The minimum flavonoid content in the *Kharif* season was observed in treatment T_1, showing 2.9% and 5.8% reductions compared to the control.

Flavonoids are a group of polyphenol compounds that play critical roles in plant defence, pigmentation, and overall health while contributing to crops' nutritional and medicinal value. Rhizobium inoculation can enhance the synthesis of flavonoids by improving nitrogen availability and activating defence-related metabolic pathways (Kumar *et al.,* 2023; Singh *et al.,* 2023). Putrescine may help mitigate oxidative stress and stabilise cellular structures, leading to an up regulation of flavonoid synthesis as a protective mechanism. Putrescine can significantly boost flavonoid production, particularly under stress conditions, by enhancing enzymatic activities and secondary metabolite pathways (Gill and Tuteja, 2022; Zafar *et al.,* 2023). Calcium is vital in maintaining cell wall integrity and mitigating oxidative stress, which may further support flavonoid accumulation in plant tissues (Huang *et al.,* 2023; Gupta and Kumar, 2023). These findings are consistent with recent research indicating that calcium supplementation can enhance the production of flavonoids and other polyphenol compounds, contributing to improved plant health and resilience.

Table 4.2.7.a: Impact of the treatments on total flavonoid content (mg g^{-1}FW) in leaves of green gram in *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	0.628^{g}±0.002	0.706^{f}±0.009	0.666^{e}±0.007	0.851^{g}±0.005
Calcium	0.635^{d}±0.005 [1.1]	0.755^{d}±0.008 [6.5]	0.692^{cd}±0.008 [3.8]	0.855^{e}±0.008 [0.5]
Bio-priming with rhizobium	0.633^{f}±0.009 [0.7]	0.729^{e}±0.008 [3.1]	0.680^{de}±0.007 [2.0]	0.854^{f}±0.005 [0.4]
Putrescine	0.635^{e}±0.004 [1.1]	0.778^{c}±0.009 [9.3]	0.700^{c}±0.006 [4.8]	0.867^{d}±0.005 [1.8]
Calcium + Bio-priming with rhizobium	0.640^{c}±0.006 [1.9]	0.793^{bc}±0.007 [10.9]	$0.708b^{c}$±0.005 [5.9]	0.878^{c}±0.006 [3.1]
Calcium + Putrescine	0.653^{a}±0.006 [3.8]	0.817^{a}±0.007 [13.6]	0.732^{a}±0.007 [9.0]	0.893^{a}±0.005 [4.7]
Bio-priming with rhizobium + Putrescine	0.644^{b}±0.007 [2.5]	0.797^{b}±0.009 [11.4]	0.725^{ab}±0.006 [8.1]	0.890^{b}±0.005 [4.4]
Calcium + Bio priming with rhizobium + Putrescine	0.658^{a}±0.010 [4.5]	0.826^{a}±0.007 [14.5]	0.735^{a}±0.011 [9.4]	0.896^{a}±0.007 [5.0]
C.D at p=0.05	0.011	0.015	0.013	0.010

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.7.a: Impact of the treatments on total flavonoid content (mg g^{-1}FW) in leaves of green gram in *Summer* season

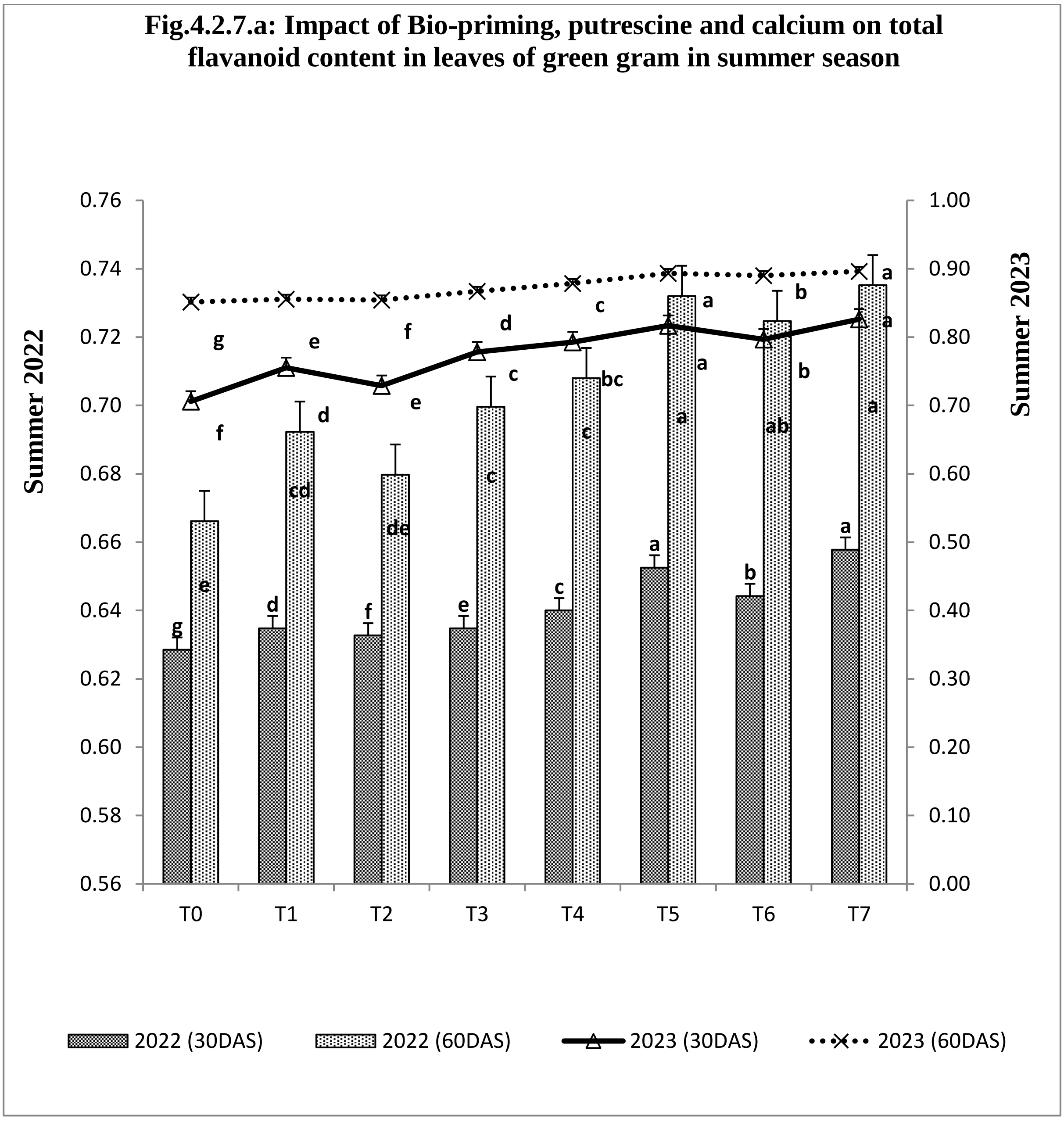


Table 4.2.7.b: Impact of the treatments on total flavonoid content (mg g^{-1} FW) in leaves of green gram in *Kharif* season

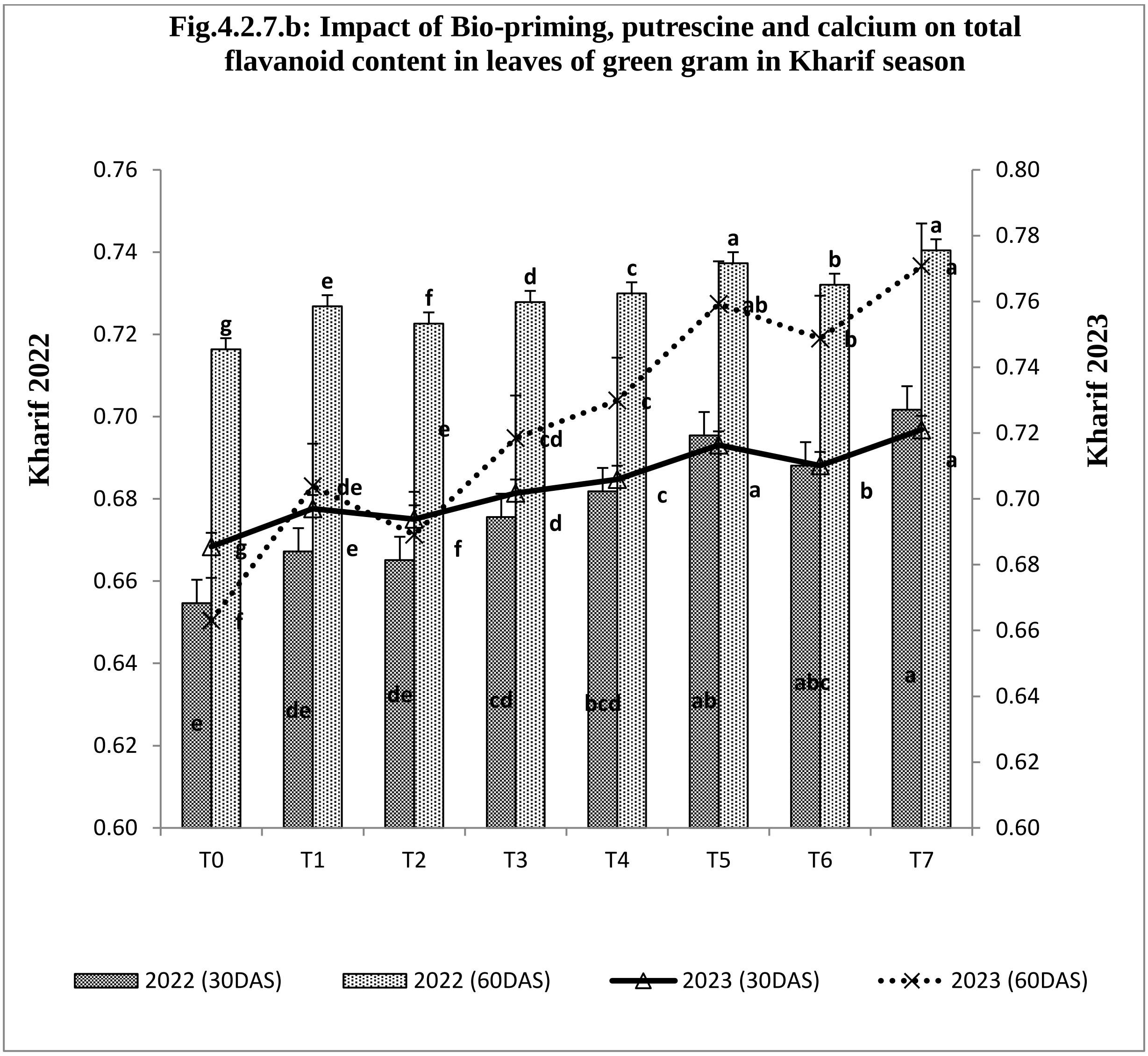
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	$0.655^{e}\pm0.010$	$0.663^{g}\pm0.005$	$0.690^{g}\pm0.002$	$0.655^{f}\pm0.010$
Calcium	$0.667^{de}\pm0.007$ [1.8]	$0.675^{e}\pm0.006$ [2.9]	$0.715^{e}\pm0.007$ [3.5]	$0.704^{de}\pm0.005$ [5.8]
Bio-priming with rhizobium	$0.665^{de}\pm0.006$ [1.5]	$0.669^{f}\pm0.008$ [2.1]	$0.706^{f}\pm0.008$ [2.3]	$0.689^{e}\pm0.005$ [3.8]
Putrescine	$0.676^{cd}\pm0.010$ [3.0]	$0.686^{d}\pm0.008$ [4.5]	$0.723^{d}\pm0.004$ [4.6]	$0.718^{cd}\pm0.006$ [7.7]
Calcium + Bio-priming with rhizobium	$0.682^{bcd}\pm0.007$ [3.9]	$0.703^{c}\pm0.006$ [6.8]	$0.730^{c}\pm0.002$ [5.5]	$0.730^{c}\pm0.007$ [9.2]
Calcium + Putrescine	$0.688^{ab}\pm0.007$ [5.8]	$0.719^{a}\pm0.008$ [9.0]	$0.748^{a}\pm0.007$ [7.8]	$0.759^{ab}\pm0.008$ [12.7]
Bio-priming with rhizobium + Putrescine	$0.702^{abc}\pm0.008$ [4.8]	$0.708^{b}\pm0.005$ [7.5]	$0.740^{b}\pm0.000$ [6.8]	$0.749^{b}\pm0.005$ [11.5]
Calcium + Bio priming with rhizobium + Putrescine	$0.702^{a}\pm0.008$ [6.7]	$0.739^{a}\pm0.007$ [11.4]	$0.756^{a}\pm0.008$ [8.7]	$0.771^{a}\pm0.011$ [14.0]
C.D at p=0.05	0.013	0.012	0.011	0.014

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.7.b: Impact of the treatments on total flavonoid content (mg g^{-1}FW) in leaves of green gram in *Kharif* season



4.2.8 Total phenols content in leaves

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Phenols were measured 30 and 60 days after sowing (DAS), with detailed results in Table 4.2.8.a and 4.2.8.b and illustrated in Figures 4.2.8.a and 4.2.8.b.

In the *Summer* of 2022, treatment T_7 significantly increased phenols, showing improvements of 11.1% and 8.2% at 30 and 60 DAS, respectively, compared to the control. In contrast, treatment T_5 decreased phenols by 9.8% and 7.5%, while treatment T_6 showed 9.3% and 7.6% reductions. The lowest phenols were observed in treatment T_1, with 6.8% and 4.4% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in phenols, with 10.7% and 10.7% improvements. Conversely, treatment T_5 decreased by 9.5% and 8.7%, and treatment T_1 recorded the lowest phenols with 3.8% and 4.3% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest phenols, with 10.5% and 8.2% increases. Treatment T_5 decreased 9.7% and 7.5%, while the lowest phenols were recorded for treatment T_1, with 6.1% and 3.3% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest phenols, increasing 11.7% and 9.7%. Treatment T_5 followed with improvements of 9.2% and 8.4%. The minimum phenols in the *Kharif* season were observed in treatment T_1, showing 3.5% and 3.8% reductions compared to the control.

Phenols are phenolic compounds linked to cell wall components or other macromolecules, playing a crucial role in plant structural integrity, defence mechanisms, and antioxidant activity. Rhizobium can induce systemic acquired resistance (SAR) in plants, leading to increased synthesis of secondary metabolites, including phenols. This induction helps plants better respond to environmental stresses and pathogens, producing higher phenol accumulation. The combined effects of putrescine stress mitigation, calcium's cell wall stabilisation, and Rhizobium's nitrogen-fixing ability create a more favourable environment for phenolic compound synthesis and accumulation.

Table 4.2.8.a: Impact of the treatments on phenols (mg g^{-1}FW) in leaves of green gram in the *Summer* season

Treatments	30DAS		60DAS	
	Summer 2022	*Summer* 2023	*Summer* 2022	*Summer* 2023
Control	0.199^{g}±0.003	0.201^{g}±0.003	0.210^{g}±0.007	0.212^{g}±0.001
Calcium	0.214^{e}±0.003 [6.8]	0.214^{e}±0.002 [6.1]	0.220^{e}±0.005 [4.4]	0.219^{e}±0.001 [3.3]
Bio-priming with rhizobium	0.207^{f}±0.003 [3.8]	0.207^{f}±0.002 [3.0]	0.214^{f}±0.001 [1.7]	0.214^{f}±0.002 [1.0]
Putrescine	0.215^{d}±0.002 [7.3]	0.215^{d}±0.003 [6.6]	0.222^{d}±0.002 [5.4]	0.223^{d}±0.002 [4.9]
Calcium + Bio-priming with rhizobium	0.217^{c}±0.003 [8.4]	0.218^{c}±0.004 [7.9]	0.224^{c}±0.005 [6.4]	0.226^{b}±0.002 [6.0]
Calcium + Putrescine	0.221^{a}±0.002 [9.8]	0.222^{a}±0.002 [9.7]	0.227^{b}±0.004 [7.5]	0.229^{a}±0.001 [7.5]
Bio-priming with rhizobium + Putrescine	0.219^{b}±0.002 [9.3]	0.221^{b}±0.001 [8.9]	0.227^{a}±0.003 [7.6]	0.225^{c}±0.005 [5.9]
Calcium + Bio priming with rhizobium + Putrescine	0.224^{a}±0.000 [11.1]	0.225^{a}±0.001 [10.5]	0.229^{a}±0.003 [8.2]	0.231^{a}±0.002 [8.2]
C.D at p=0.05	0.004	0.004	0.007	0.004

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.8.a: Impact of the treatments on phenols (mg g^{-1}FW) in leaves of green gram in the *Summer* season

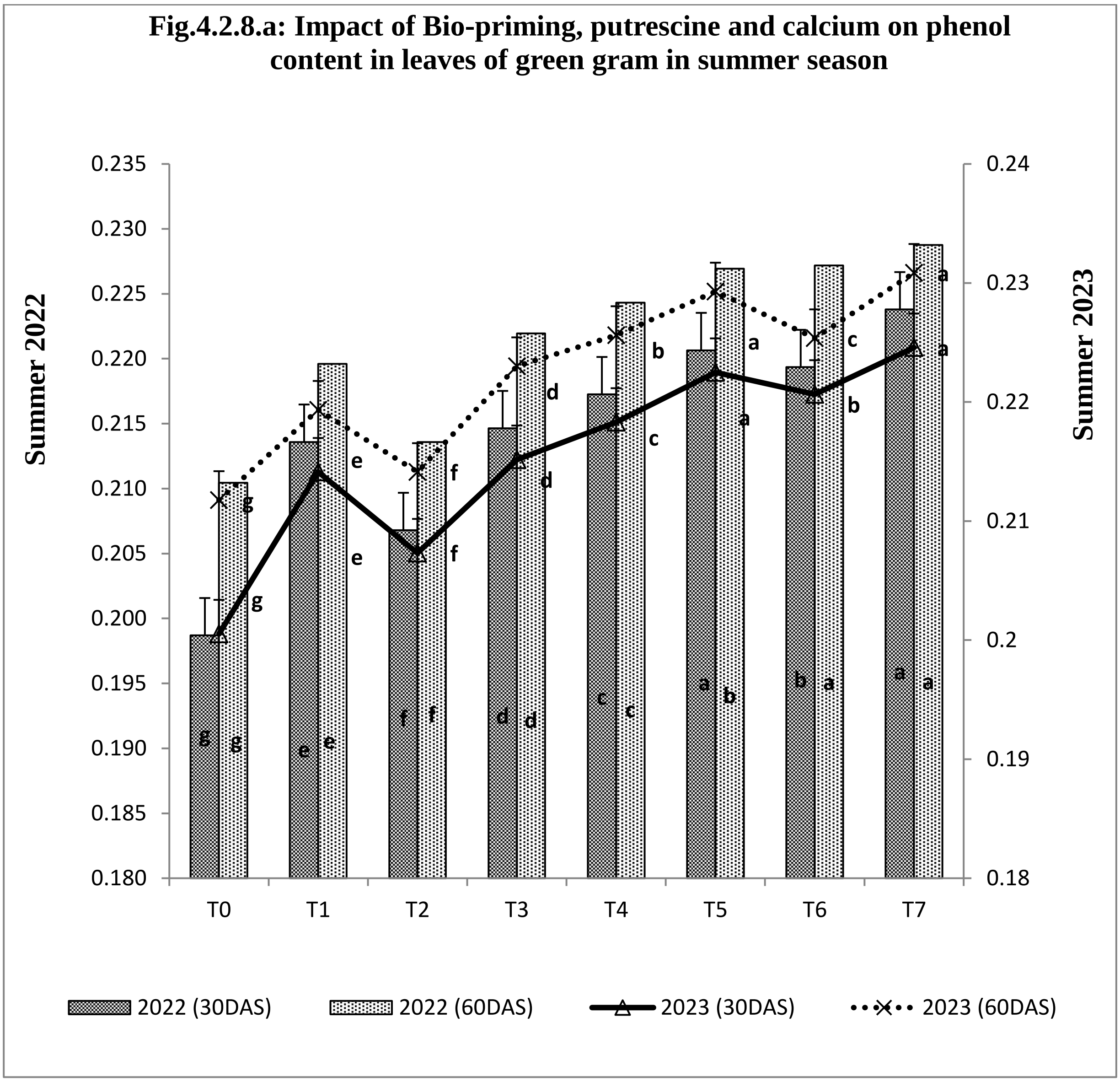


Table 4.2.8.b: Impact of the treatments on phenols (mg g^{-1}FW) in leaves of green gram in the *Kharif* season

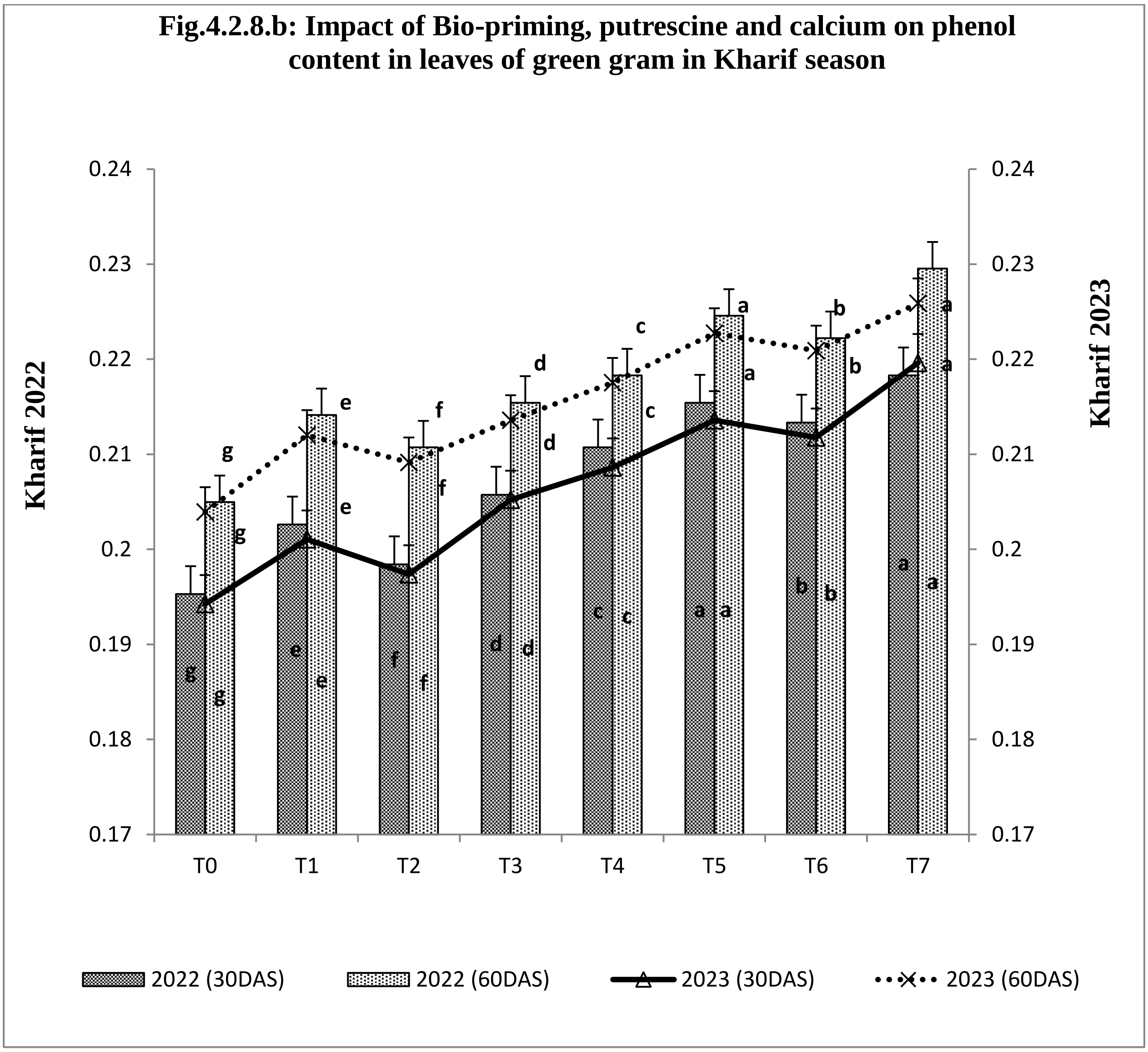
Treatments	30DAS		60DAS	
	Kharif 2022	*Kharif* 2023	*Kharif* 2022	*Kharif* 2023
Control	$0.195^{g}\pm0.002$	$0.194^{g}\pm0.002$	$0.205^{g}\pm0.001$	$0.204^{g}\pm0.001$
Calcium	$0.203^{e}\pm0.002$ [3.8]	$0.201^{e}\pm0.003$ [3.5]	$0.214^{e}\pm0.002$ [4.3]	$0.212^{e}\pm0.001$ [3.8]
Bio-priming with rhizobium	$0.198^{f}\pm0.003$ [1.7]	$0.197^{f}\pm0.003$ [1.7]	$0.211^{f}\pm0.001$ [2.7]	$0.209^{f}\pm0.002$ [2.5]
Putrescine	$0.206^{d}\pm0.002$ [5.2]	$0.205^{d}\pm0.001$ [5.5]	$0.215^{d}\pm0.001$ [4.8]	$0.214^{d}\pm0.001$ [4.5]
Calcium + Bio-priming with rhizobium	$0.211^{c}\pm0.001$ [7.5]	$0.209^{c}\pm0.002$ [7.0]	$0.218^{c}\pm0.002$ [6.1]	$0.218^{c}\pm0.002$ [6.2]
Calcium + Putrescine	$0.215^{a}\pm0.002$ [9.5]	$0.214^{a}\pm0.002$ [9.2]	$0.225^{a}\pm0.002$ [8.7]	$0.223^{a}\pm0.001$ [8.4]
Bio-priming with rhizobium + Putrescine	$0.213^{b}\pm0.002$ [8.6]	$0.212^{b}\pm0.001$ [8.4]	$0.222^{b}\pm0.001$ [7.8]	$0.221^{b}\pm0.002$ [7.7]
Calcium + Bio priming with rhizobium + Putrescine	$0.218^{a}\pm0.002$ [10.7]	$0.220^{a}\pm0.002$ [11.7]	$0.230^{a}\pm0.002$ [10.7]	$0.226^{a}\pm0.002$ [9.7]
C.D at p=0.05	0.004	0.004	0.003	0.003

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.2.8.b: Impact of the treatments on phenols (mg g^{-1}FW) in leaves of green gram in the *Kharif* season



4.3 Quality parameters

4.3.1 NPK content

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons. NPK content was measured with detailed results in Table 4.3.1. a, 4.3.1.b, 4.3.1.c and illustrated in Figure 4.3.1.a, 4.3.1.b, 4.3.1.c.

In the *Summer* season of 2022, treatment T_7 significantly increased NPK content, with nitrogen content showing improvements of 4.46%, phosphorus content by 0.096%, and potassium content by 0.702%, followed by treatment T_5 by 4.45%, 0.0935%, and 0.702% NPK content. In the summer of 2023, treatment T_7 significantly increased the NPK content by 4.49%, 0.094%, and 0.70% compared to the control.

During the *Kharif* season 2022, treatment T_7 again demonstrated a notable increase in NPK content with 4.4% N, 0.091% P and 0.701% K improvements followed by treatment T_5 decreased by 24.38% N, 10.13% P and 1.70% K. Conversely, treatment T_6 decreased by 4.43% N, 0.09% P and 0.701% K and treatment T_1 recorded the lowest nitrogen content with 3.42% N, 0.081% P and 0.65% K reductions. Meanwhile, in *Kharif* 2023, the treatment T_7 significantly increased the NPK content by 4.2%, 0.09% and 0.7% compared to the control.

Nutrients like nitrogen (N), phosphorus (P), and potassium (K) are crucial for plant growth, development, and yield. Calcium application can boost nitrogen content by improving the efficiency of nitrogen uptake and assimilation in various crops. Calcium also affects phosphorus uptake by promoting root growth and function, essential for phosphorus absorption. Calcium helps form root structures that are more efficient in absorbing phosphorus (Raddatz *et al.,* 2022). Studies by Ali *et al.,* (2023) support this, indicating that calcium application enhances phosphorus uptake and utilisation in legumes. The role of calcium in potassium uptake is linked to its effect on cell membrane stability and nutrient transport (Malik *et al.,* 2022). Putrescine influences nitrogen metabolism by enhancing enzymatic activities related to nitrogen assimilation and uptake (Hussain *et al.,* 2021). The positive impact of putrescine on phosphorus content observed in this study aligns with findings by Singh *et al.*, (2022), who reported improved phosphorus absorption in plants treated with polyamines. Putrescine's role in potassium uptake is related to its effects on stress mitigation and improved root development, which enhances potassium absorption (Yari *et al.*, 2022). Rhizobium injection is known for its ability to fix atmospheric nitrogen, making it available to the plant. This directly increases the nitrogen content in legumes (Udvardi and Poole, 2020). Recent studies, such as those by Ahmed *et al.*, (2023), confirm that Rhizobium inoculation enhances nitrogen content by improving nitrogen fixation efficiency. Rhizobium can indirectly influence phosphorus uptake by improving root development and function, which helps absorb phosphorus (Prakash *et al.*, 2023). Rhizobium’s impact on potassium content is less direct but can be attributed to overall improvements in plant health and root function, which enhance potassium uptake (Khan *et al.*, 2022).

Table 4.3.1.a: Impact of the treatments on nitrogen content (%) of green gram in *Summer* and *Kharif* season

Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	3.39	3.52	3.35	3.4
Calcium	3.43	3.85	3.42	3.47
Bio-priming with rhizobium	3.42	3.82	3.4	3.42
Putrescine	3.45	3.97	3.43	3.49
Calcium + Bio-priming with rhizobium	3.47	4.38	3.45	4.28
Calcium + Putrescine	4.45	4.47	4.43	4.38
Bio-priming with rhizobium + Putrescine	4.39	4.45	4.38	4.31
Calcium + Bio priming with rhizobium + Putrescine	4.46	4.49	4.4	4.2

Note: Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

Fig. 4.3.1.a: Impact of the treatments on nitrogen content (%) of green gram in *Summer* and *Kharif* season

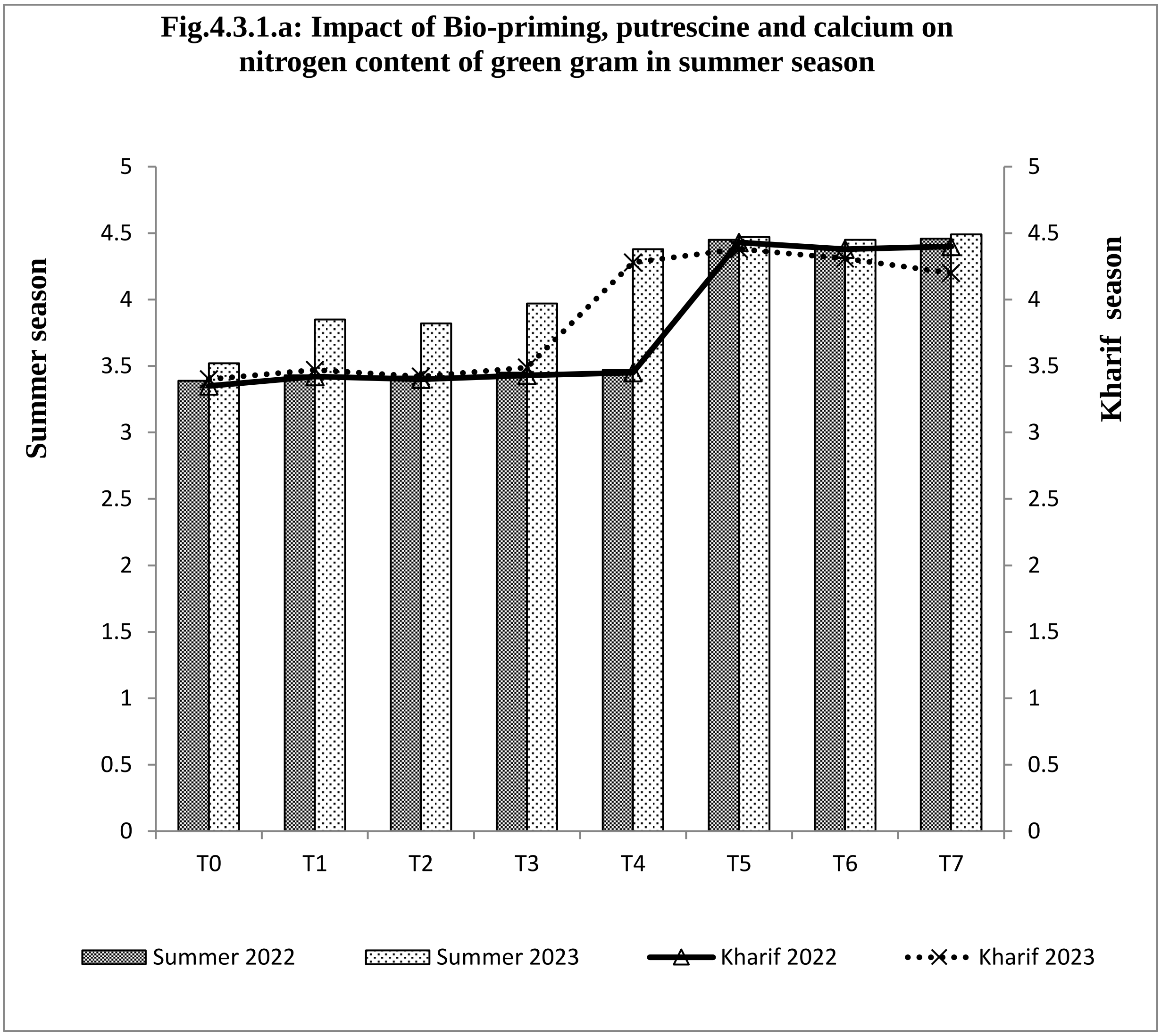


Table 4.3.1.b: Impact of the treatments on phosphorus content (%) of green gram in *Summer* and *Kharif* season

Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	0.083	0.079	0.076	0.078
Calcium	0.085	0.082	0.081	0.081
Bio-priming with rhizobium	0.082	0.08	0.079	0.08
Putrescine	0.087	0.085	0.082	0.083
Calcium + Bio-priming with rhizobium	0.09	0.089	0.085	0.085
Calcium + Putrescine	0.093	0.092	0.09	0.089
Bio-priming with rhizobium + Putrescine	0.091	0.09	0.087	0.087
Calcium + Bio priming with rhizobium + Putrescine	0.096	0.094	0.091	0.09

Note: Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

Fig. 4.3.1.b: Impact of the treatments on phosphorus content (%) of green gram in *Summer* and *Kharif* season

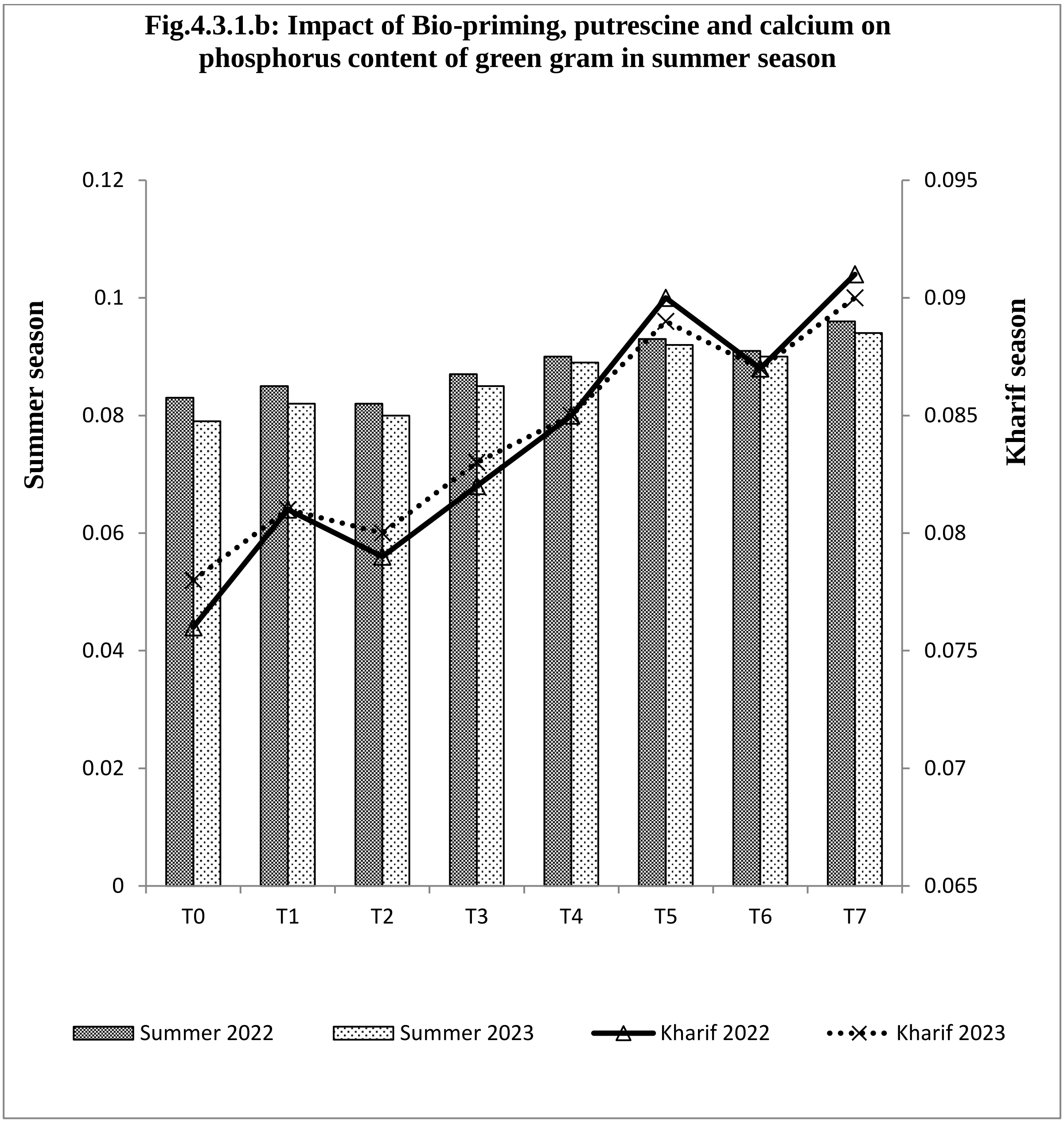
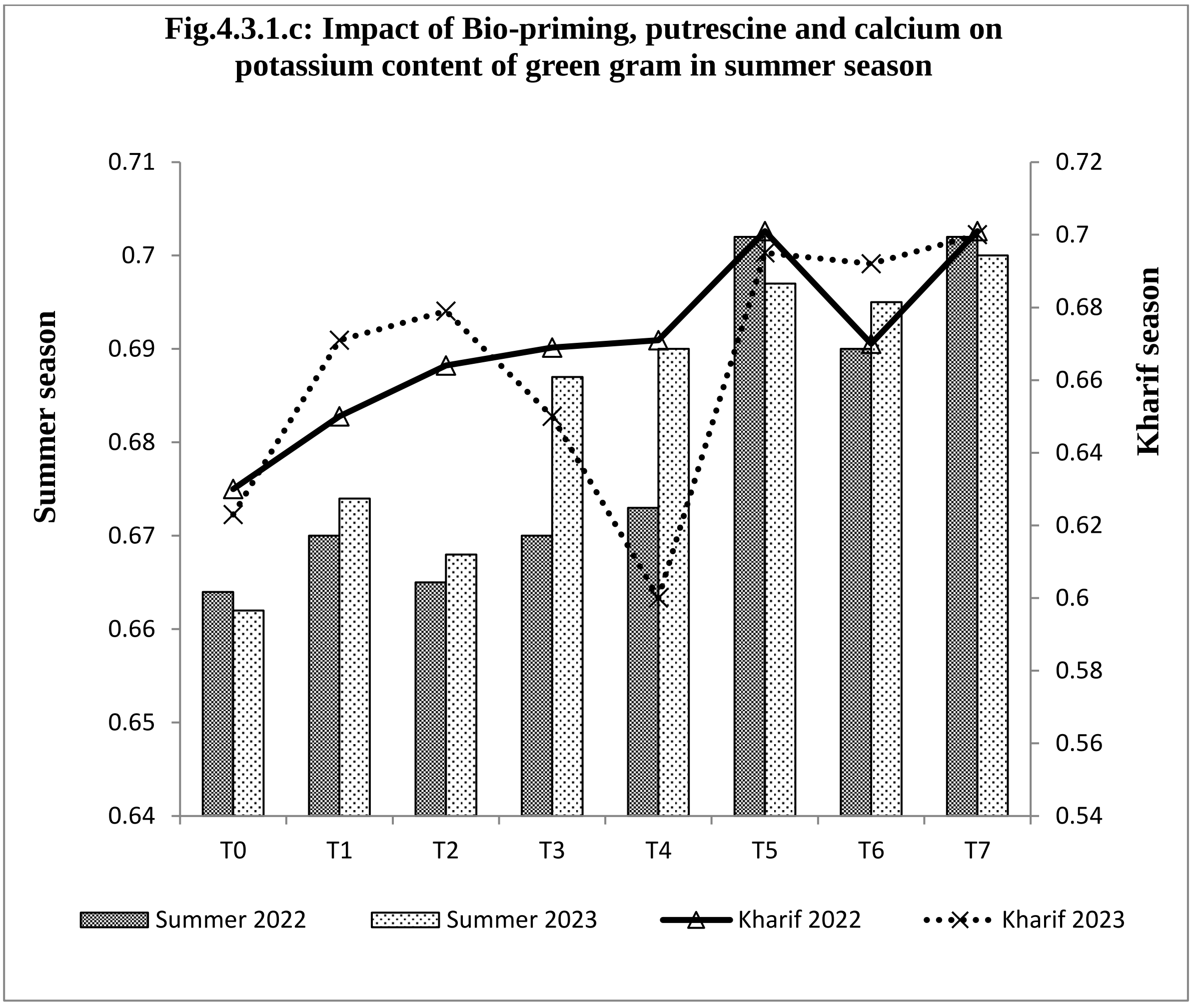


Table-4.3.1.c: Impact of the treatments on potassium content (%) of green gram in *Summer* and *Kharif* season

Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	0.664	0.662	0.630	0.623
Calcium	0.670	0.674	0.650	0.671
Bio-priming with rhizobium	0.665	0.668	0.664	0.679
Putrescine	0.670	0.687	0.669	0.650
Calcium + Bio-priming with rhizobium	0.673	0.690	0.671	0.600
Calcium + Putrescine	0.702	0.697	0.701	0.695
Bio-priming with rhizobium + Putrescine	0.690	0.695	0.670	0.692
Calcium + Bio priming with rhizobium + Putrescine	0.702	0.700	0.701	0.700

Note: Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

Fig. 4.3.1.c: Impact of the treatments on potassium content (%) of green gram in *Summer* and *Kharif* season



4.3.2 Arginine content in seed

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons. Arginine content was measured with detailed results in Table 4.3.2 and illustrated in Figure 4.3.2.

In the *Summer* seasons of 2022 and 2023, treatment T_7 significantly increased arginine content, showing improvements of 4.53% and 3.73% compared to the control. Treatment T_5 decreased arginine content by 3.96% and 3.54%, while treatment T_6 showed 2.61% and 3.16% reductions. The lowest arginine content was observed in treatment T_1, with 1.62% and 1.21% reductions.

During the *Kharif* season of 2022 and 2023, treatment T_7 again demonstrated a notable increase in arginine content with 4.55% and 3.77% improvements, followed by treatment T_5 decreased by 4.17% and 3.39%. Conversely, treatment T_6 decreased by 2.82% and 3.00% and treatment T_1 recorded the lowest arginine content with 1.83% and 1.62% reductions.

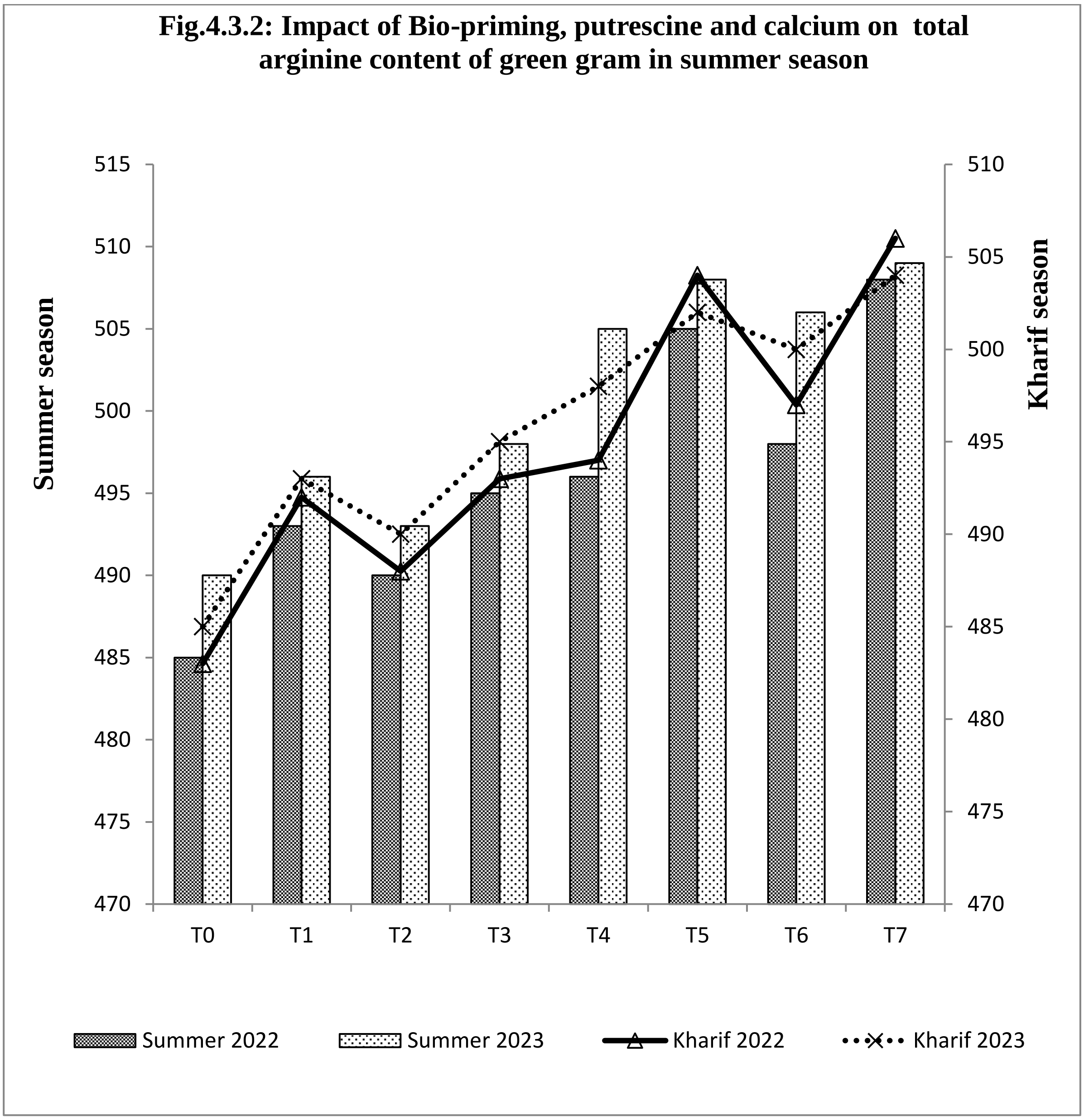
Arginine is a crucial amino acid in protein synthesis, plant metabolism, and stress responses. Calcium is critical in various plant physiological processes, including enzyme activation and cell signalling (White and Broadley, 2004). The increase in arginine content may be linked to calcium's role in enhancing overall plant health and metabolic activity. Calcium is known to influence nitrogen metabolism and uptake. Putrescine can improve arginine synthesis by affecting the enzymatic pathways involved in its production and alleviating stress, which can otherwise deplete amino acid reserves. The findings align with recent studies by Zhao *et al.*,.(2023), which demonstrated that polyamine application, including putrescine, increases arginine content by enhancing nitrogen assimilation. The positive effect of Rhizobium on arginine content can be attributed to improved nitrogen supply, which facilitates the production of amino acids and supports overall plant growth. Recent research by Ahmed *et al.*, (2023) confirms that Rhizobium inoculation improves nitrogen uptake and enhances legumes' amino acid profile, including arginine.

Table 4.3.2: Impact of the treatments on arginine (mg g^{-1}) content in seed of green gram in *Summer* and *Kharif* season

Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	485	490	483	485
Calcium	493	496	492	490
Bio-priming with rhizobium	490	493	488	490
Putrescine	495	498	493	495
Calcium + Bio-priming with rhizobium	496	505	494	498
Calcium + Putrescine	505	508	504	502
Bio-priming with rhizobium + Putrescine	498	506	497	500
Calcium + Bio priming with rhizobium + Putrescine	508	509	506	504

Note: Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

Fig. 4.3.2: Impact of the treatments on arginine (mg g^{-1}) content in seed of green gram in *Summer* and *Kharif* season



4.3.3 Seed protein

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons. Seed protein was measured with detailed results in Table 4.3.3 and illustrated in Figure 4.3.3

In the *Summer* seasons of 2022 and 2023, treatment T_7 significantly increased seed protein, showing improvements of 3.07% and 4.93% compared to the control. Treatment T_5 decreased seed protein content by 2.81% and 4.50%, while treatment T_6 showed 2.60% and 0.65% reductions. The lowest seed protein content was observed in treatment T_1, with 2.26% and 0.35% reductions.

During the *Kharif* season of 2022 and 2023, treatment T_7 again demonstrated a notable increase in seed protein content with 2.73% and 4.29% improvements, followed by treatment T_5, which decreased by 2.30% and 4.08%. Conversely, treatment T_6 decreased by 2.13% and 3.95%, and treatment T_1 recorded the lowest seed protein content at 1.92% and 0.23% reductions.

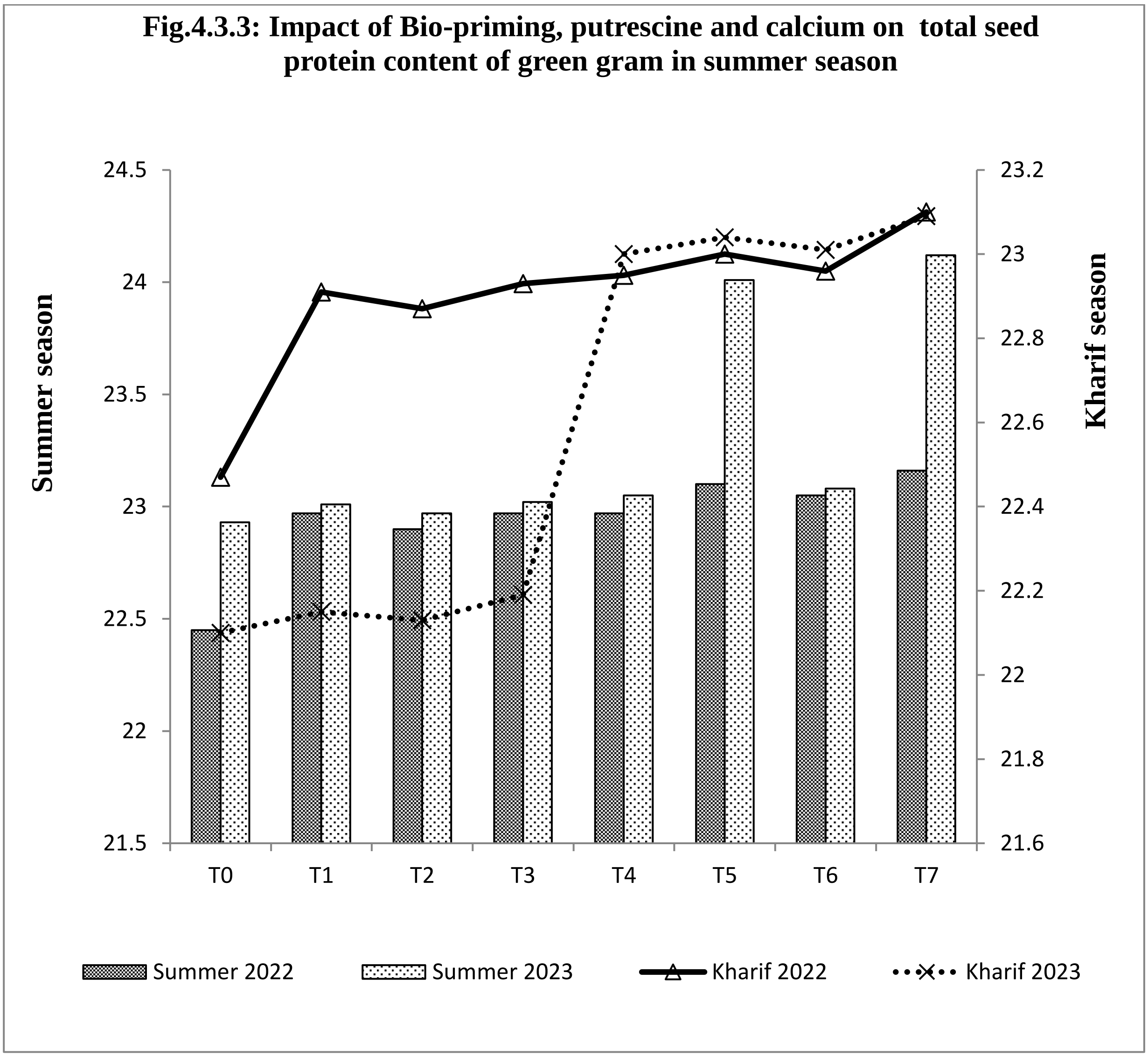
Seed protein content is a crucial quality parameter for leguminous crops, impacting nutritional value and marketability. Calcium's role in protein content is linked to its effects on nitrogen metabolism and assimilation, as calcium can enhance nitrogen utilisation efficiency, directly related to protein synthesis (Raddatz *et al.,* 2022). Recent studies, such as those by Ali *et al.,* (2023), have shown that calcium application can increase seed protein content by improving nitrogen uptake and utilisation. Putrescine, a polyamine involved in growth regulation and stress tolerance, also significantly affected seed protein content. Polyamines like putrescine influence protein synthesis and accumulation by modulating stress responses and metabolic processes (Hussain *et al.,* 2021). Putrescine enhances protein content by stabilising proteins and nucleic acids, improving stress tolerance, and promoting efficient nitrogen utilisation (Zhao *et al.,* 2023). The increased nitrogen availability due to Rhizobium inoculation directly contributes to higher seed protein content by supporting the biosynthesis of proteins in developing seeds. Recent studies, such as those by Prakash *et al.,* (2023), confirm that Rhizobium inoculation improves seed protein content by enhancing nitrogen fixation and ensuring a steady supply of nitrogen to the plant.

Table 4.3.3: Impact of the treatments on seed protein (g) content of green gram in *Summer* and *Kharif* season

Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	22.45	22.93	22.47	22.10
Calcium	22.97	23.01	22.91	22.15
Bio-priming with rhizobium	22.90	22.97	22.87	22.13
Putrescine	22.97	23.02	22.93	22.19
Calcium + Bio-priming with rhizobium	22.97	23.05	22.95	23.00
Calcium + Putrescine	23.10	24.01	23.00	23.04
Bio-priming with rhizobium + Putrescine	23.05	23.08	22.96	23.01
Calcium + Bio priming with rhizobium + Putrescine	23.16	24.12	23.10	23.09

Note: Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

Fig. 4.3.3: Impact of the treatments on seed protein (g) content of green gram in *Summer* and *Kharif* season



4.4 Yield attributing parameters

4.4.1 No. of pods plant^{-1}

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. No. of pods, plant^{-1} was measured with detailed results in Table 4.4.1 and illustrated in Figure 4.4.1.

In the *Summer* of 2022, treatment T_7 significantly increased the no. of pods plant^{-1}, showing improvements of 20.3% as compared to control followed by treatment T_5, which decreased no. of pods plant^{-1} by 18.9%, while treatment T_6 showed 17.3% reductions. The lowest no. of pods plant^{-1} was observed in treatment T_1, with 7.1% reductions. During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in pod plant-1, with 15.9% improvements, followed by treatment T_5, which decreased by 14.5%. Conversely, treatment T_6 decreased by 14.0%, and treatment T_1 recorded the lowest number of pods in plant^{-1}, with a 7.4% reduction.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest no. of pods plant^{-1}, with 17.0% increases. Treatment T_5 decreased by 7.9%, followed by treatment T6, which decreased by 8.0%, while the lowest no. of pods plant^{-1} were recorded for treatment T_1, with 4.3% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest no. of pods plant^{-1}, increasing 16.9%. Treatment T_5 followed with improvements of 15.7% and treatment T_6 with 13.2%. The minimum no. of pods plant^{-1} in the *Kharif* season was observed in treatment T_1, showing 4.0% reductions compared to the control.

The number of pods per plant is a crucial determinant of overall yield, as it integrates the effects of several factors, including pod formation, seed set, and plant health. The foliar application of calcium was found to have a significant positive impact on the number of pods per plant. Calcium is essential for various physiological processes in plants, including cell wall stabilisation, enzyme activation, and critical signalling pathways during reproductive development (Hepler and Winship, 2022). Enhanced calcium availability can improve the plant's health, leading to better pod formation and seed set. Putrescine can mitigate the effects of abiotic stresses, such as drought and heat, which often negatively impact seed sets (Zhao *et al.*, 2023). In this study, the application of putrescine resulted in a higher number of pods per plant, likely due to its role in enhancing stress tolerance and reproductive efficiency. Rhizobium's ability to improve root growth and nutrient uptake further supports the production of more pods per plant (Prakash *et al.*, 2023).

Table 4.4.1: Impact of the treatments on no. of pods plant^{-1}of green gram in *Summer* and *Kharif* season

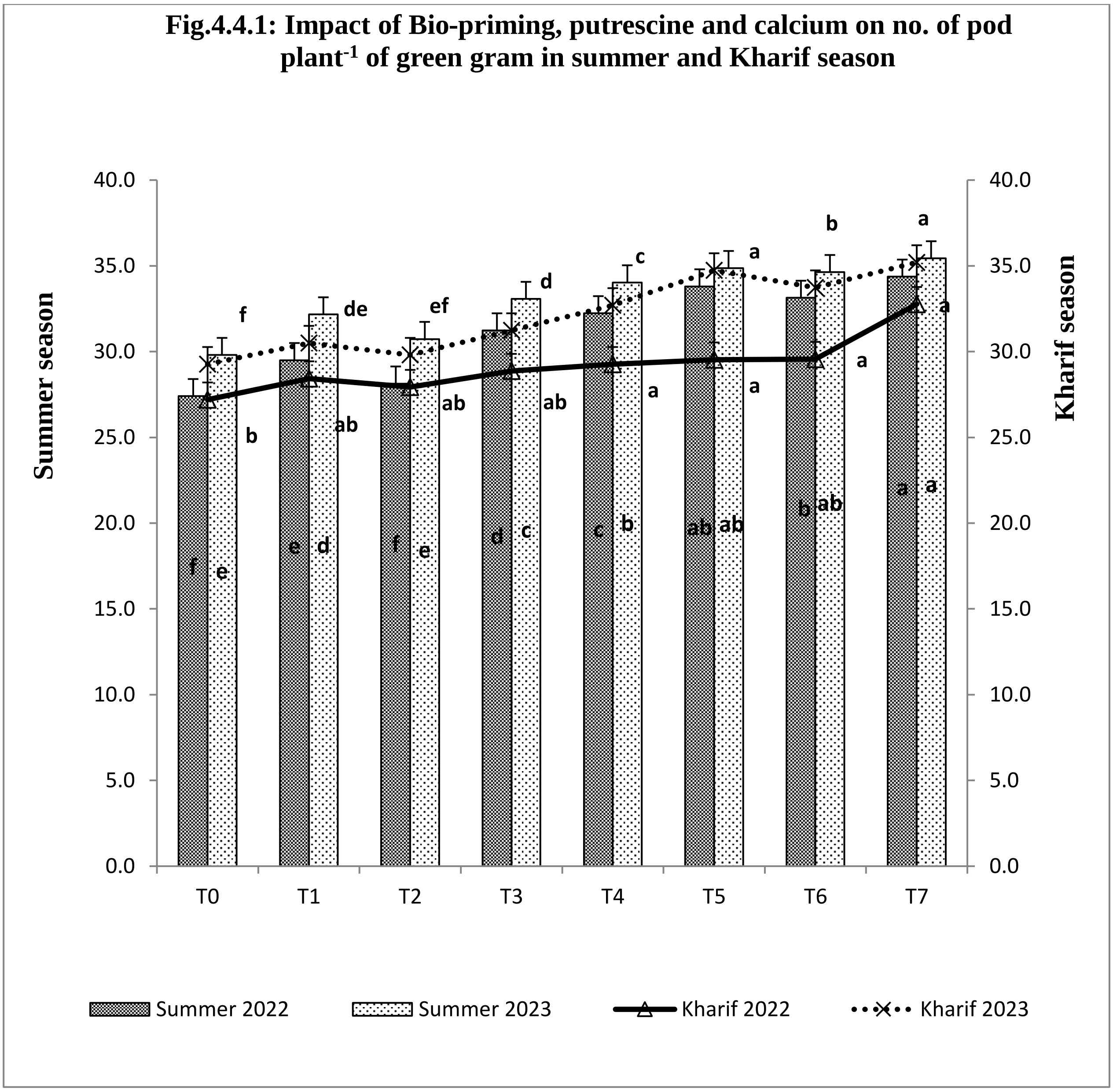
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	27.400^{f}±0.265	29.800^{e}±0.300	27.200^{b}±0.265	29.267^{f}±0.153
Calcium	29.500^{e}±0.300 [7.1]	32.167^{d}±0.603 [4.3]	28.433^{ab}±0.153 [7.4]	30.500^{de}±0.300 [4.0]
Bio-priming with rhizobium	28.133^{f}±0.503 [2.6]	30.733^{e}±0.321 [2.6]	27.933^{ab}±0.306 [3.0]	29.800^{ef}±0.200 [1.8]
Putrescine	31.233^{d}±0.252 [12.3]	33.067^{c}±0.451 [5.8]	28.867^{ab}±0.635 [9.9]	31.700^{d}±0.361 [6.3]
Calcium + Bio-priming with rhizobium	32.233^{c}±0.379 [15.0]	34.033^{b}±0.208 [7.1]	29.267^{a}±0.153 [12.4]	32.700^{c}±0.361 [10.5]
Calcium + Putrescine	33.800^{ab}±0.400 [18.9]	34.867^{ab}±0.702 [7.9]	29.533^{a}±0.351 [14.5]	34.733^{a}±0.503 [15.7]
Bio-priming with rhizobium + Putrescine	33.133^{b}±0.351 [17.3]	34.633^{ab}±0.451 [8.0]	29.567^{a}±0.306 [14.0]	33.733^{b}±0.777 [13.2]
Calcium + Bio priming with rhizobium + Putrescine	34.367^{a}±0.513 [20.3]	35.433^{a}±0.473 [17.0]	32.767^{a}±2.747 [15.9]	35.200^{a}±0.600 [16.9]
C.D at p=0.05	0.701	0.864	1.810	0.799

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.4.1: Impact of the treatments on no. of pods $plant^{-1}$ of green gram in *Summer* and *Kharif* season



4.4.2 No. of seeds pod^{-1}

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. No. of seeds, pod^{-1} was measured with detailed results in Table 4.4.2 and illustrated in Figure 4.4.2.

In the *Summer* of 2022, treatment T_7 significantly increased the number of seeds pod^{-1}, showing improvements of 17.7% compared to the control. Treatment T_5 decreased the number of seeds pod^{-1} by 15.9%, while treatment T_6 showed 14.3% reductions. The lowest number of seeds, pod^{-1}, was observed in treatment T_1, with 5.7% reductions. During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in no. of seeds pod^{-1} with 20.9% improvements, followed by treatment T_5 decreased by 20.1%. Conversely, treatment T_6 decreased by 19.4%, and treatment T1 recorded the lowest number of pod^{-1} seeds, with 10.7% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest no. of seeds pod^{-1}, with 17.6% increases. Treatment T_5 decreased by 15.6%, followed by treatment T_6, which decreased by 11.8%, while the lowest no. of seeds pod^{-1} was recorded for treatment T_1, with 3.5% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest no. of seeds pod^{-1}, increasing 17.5%. Treatment T_5 followed with improvements of 17.0% and treatment T_6 with 11.7%. The minimum number of pod^{-1} seeds in the *Kharif* season was observed in treatment T_1, showing 7.1% reductions compared to the control.

The number of seeds per pod is a crucial yield component, directly influencing the overall productivity of the crop. Calcium supplementation can enhance reproductive success by stabilising cellular processes during pod and seed development (Bashir *et al.,* 2021). Specifically, calcium's role in strengthening the pollen tube and improving fertilisation efficiency increases the number of seeds per pod. Polyamines like putrescine are known to enhance cell division, differentiation, and growth, which are critical during the formation of seeds within pods (Hussain *et al.,* 2021). The application of putrescine can mitigate the effects of environmental stress on reproductive development, leading to an increase in the number of seeds per pod.

Rhizobium's role in improving overall plant vigour and health contributes to better reproductive outcomes, as a well-nourished plant can produce more seeds per pod (Prakash *et al.,* 2023)

Table 4.4.2: Impact of the treatments on no. of seed pod^{-1} of green gram in *Summer* and *Kharif* season

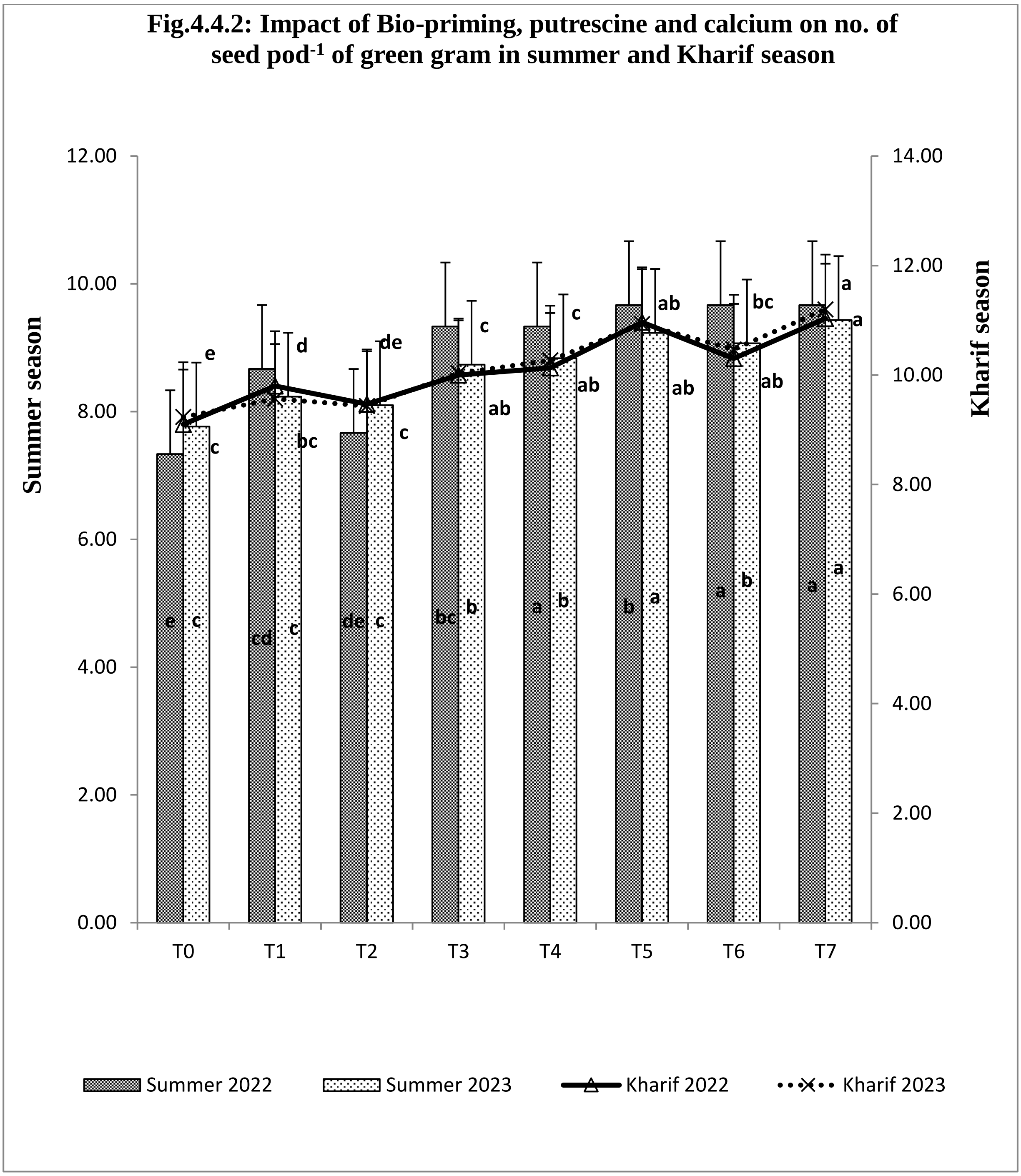
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	9.100^{e}±0.173	9.233^{c}±0.252	7.550^{c}±0.48	7.767^{e}±0.252
Calcium	9.800^{cd}±0.436 [5.7]	9.567^{c}±0.252 [3.5]	8.450^{bc}±0.18 [10.7]	8.233^{d}±0.252 [7.1]
Bio-priming with rhizobium	9.467^{de}±0.503 [4.1]	9.433^{c}±0.208 [2.1]	7.880^{c}±0.68 [4.2]	8.100^{de}±0.173 [3.9]
Putrescine	10.000^{bc}±0.200 [11.1]	10.033^{b}±0.208 [8.0]	9.030^{ab}±0.86 [16.4]	8.733^{c}±0.208 [9.0]
Calcium + Bio-priming with rhizobium	10.133^{a}±0.231 [12.1]	10.267^{b}±0.252 [10.1]	9.080^{ab}±0.68 [16.9]	8.833^{c}±0.208 [10.2]
Calcium + Putrescine	10.967^{b}±0.153 [15.9]	10.933^{a}±0.379 [15.6]	9.450^{ab}±0.33 [20.1]	9.233^{ab}±0.252 [17.0]
Bio-priming with rhizobium + Putrescine	10.300^{a}±0.200 [14.3]	10.467^{b}±0.153 [11.8]	9.370^{ab}±0.76 [19.4]	9.067^{bc}±0.058 [11.7]
Calcium + Bio priming with rhizobium + Putrescine	11.033^{a}±0.153 [17.7]	11.200^{a}±0.300 [17.6]	9.550^{a}±0.22 [20.9]	9.433^{a}±0.153 [17.5]
C.D at p=0.05	0.423	0.464	0.963	0.326

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.4.2: Impact of the treatments on no. of seed pod^{-1} of green gram in *Summer* and *Kharif* season



4.4.3 Seed weight pod^{-1}

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Seed weight pod^{-1} was measured with detailed results in Table 4.4.3 and illustrated in Figure 4.4.3.

In the *Summer* of 2022, treatment T_7 significantly increased seed weight pod^{-1}, showing improvements of 30.8% as compared to control. Treatment T_5 decreased seed weight pod^{-1} by 28.3%, while treatment T_6 showed 19.9% reductions. The lowest seed weight, pod^{-1}, was observed in treatment T_1, with 9.4% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in seed weight pod^{-1} with 30.1% improvements, followed by treatment T_5, which decreased by 26.7%. Conversely, treatment T_6 decreased by 25.6%, and treatment T_1 recorded the lowest seed weight pod^{-1} with 5.4% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest seed weight pod^{-1}, with 26.0% increases. Treatment T_5 decreased by 21.2%, treatment T_6 decreased by 19.9%, while the lowest seed weight, pod^{-1}, was recorded for treatment T_1, with 9.4% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest seed weight pod^{-1}, increasing by 32.5%. Treatment T_5 followed with improvements of 29.8% and treatment T_6 with 27.6%. The minimum seed weight pod^{-1} in the *Kharif* season was observed in treatment T_1, showing 11.8% reductions compared to the control.

Seed weight per pod is a critical yield component, as it directly influences the crop's overall productivity and market value. Calcium is pivotal in cell wall formation, membrane stability, and enzymatic activities, which are crucial for developing seeds (Hepler and Winship, 2022). Adequate calcium supply ensures seeds develop fully and reach their optimal weight, contributing to a higher seed weight per pod. Putrescine has been shown to improve the plant's overall metabolic activity, leading to more efficient synthesis and accumulation of storage compounds in seeds (Shabbir *et al.*, 2022). Recent research has demonstrated that putrescine application can mitigate the adverse effects of environmental stresses, ensuring that seeds develop fully and reach their potential weight (Zhao *et al.*, 2023). Bio-priming with Rhizobium significantly increased seed weight per pod. Rhizobium inoculation enhances nitrogen fixation, providing the plant with a steady supply of nitrogen, which is essential for protein synthesis and seed development (Udvardi and Poole, 2020). The increased nitrogen availability supports accumulating storage proteins in seeds, contributing to increased seed weight.

Table 4.4.3: Impact of the treatments on seed weight pod^{-1} (g) of green gram in *Summer* and *Kharif* season

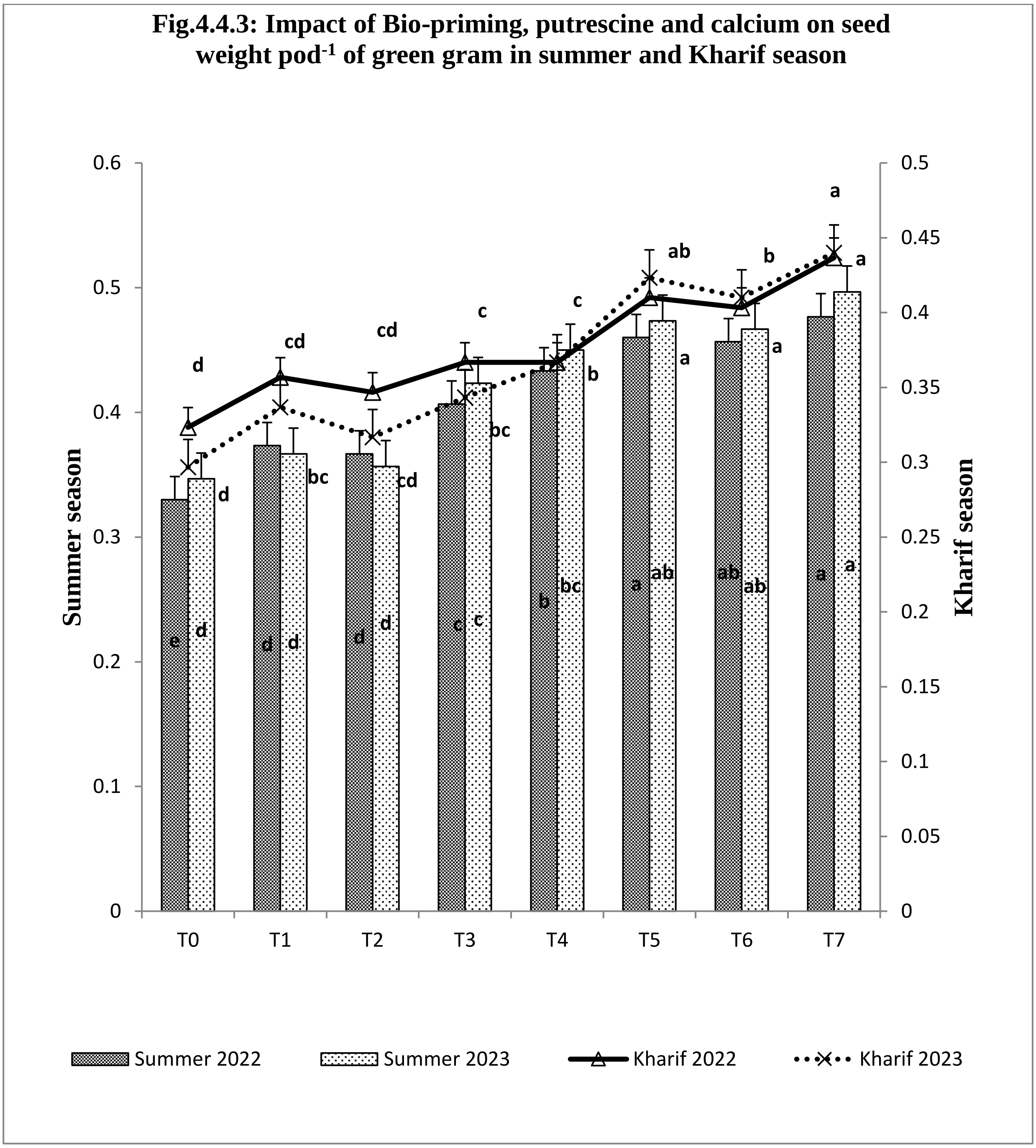
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	0.330^{e}±0.020	0.347^{d}±0.006	0.323^{d}±0.012	0.297^{d}±0.015
Calcium	0.373^{d}±0.015 [11.6]	0.367^{d}±0.015 [9.4]	0.357^{cd}±0.006 [5.4]	0.337^{bc}±0.015 [11.8]
Bio-priming with rhizobium	0.367^{d}±0.015 [10.0]	0.357^{d}±0.006 [6.8]	0.347^{cd}±0.015 [2.7]	0.317^{cd}±0.021 [6.2]
Putrescine	0.407^{c}±0.015 [18.9]	0.423^{c}±0.025 [11.9]	0.367^{c}±0.006 [18.0]	0.343^{bc}±0.015 [13.5]
Calcium + Bio-priming with rhizobium	0.433$_{b}$±0.015 [23.8]	0.450^{bc}±0.020 [11.9]	0.367^{c}±0.015 [22.9]	0.367^{b}±0.015 [19.0]
Calcium + Putrescine	0.460^{a}±0.026 [28.3]	0.473^{ab}±0.015 [21.2]	0.410^{ab}±0.026 [26.7]	0.423^{a}±0.015 [29.8]
Bio-priming with rhizobium + Putrescine	0.457^{ab}±0.012 [27.7]	0.467^{ab}±0.015 [19.9]	0.403^{b}±0.021 [25.6]	0.410^{a}±0.020 [27.6]
Calcium + Bio priming with rhizobium + Putrescine	0.477^{a}±0.015 [30.8]	0.497^{a}±0.012 [26.0]	0.437^{a}±0.015 [30.1]	0.440^{a}±0.020 [32.5]
C.D at p=0.05	0.022	0.028	0.029	0.032

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.4.3: Impact of the treatments on seed weight pod^{-1} (g) of green gram in *Summer* and *Kharif* season



4.4.4 Seed weight plant^{-1}

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Seed weight plant^{-1} was measured with detailed results in Table 4.4.4 and illustrated in Figure 4.4.4.

In the *Summer* of 2022, treatment T_7 significantly increased seed weight plant^{-1}, showing improvements of 44.8% as compared to control. Treatment T_5 decreased seed weight plant^{-1} by 41.8%, while treatment T_6 showed 40.2% reductions. The lowest seed weight plant^{-1}, was observed in treatment T_1, with 17.9% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in seed weight plant^{-1} with 41.3% improvements, followed by treatment T_5 decreased by 37.4%. Conversely, treatment T_6 decreased by 36.1%, and treatment T_1 recorded the lowest seed weight in plant^{-1}, with a 12.4% reduction.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest seed weight plant^{-1}, with 41.4% increases. Treatment T_5 decreased by 27.4%, treatment T_6 decreased by 26.2%, while the lowest seed weight, plant^{-1}, was recorded for treatment T_1, with 13.3% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest seed weight plant^{-1}, increasing by 38.2%. Treatment T_5 followed with improvements of 36.2% and treatment T_6 with 33.5%. The minimum seed weight plant^{-1} in the *Kharif* season was observed in treatment T_1, showing 16.9% reductions compared to the control.

Seed weight per plant is a crucial determinant of crop yield, directly affecting the crop's overall productivity and seed value. Calcium plays a vital role in plant development, particularly in strengthening cell walls, enhancing enzyme activity, and facilitating nutrient transport, which is essential during the seed-filling stage. Putrescine has also been reported to enhance the plant’s stress tolerance, which can prevent yield losses under adverse environmental conditions. By stabilising cellular structures and improving nutrient assimilation, putrescine ensures that more energy and resources are allocated to seed development, thereby increasing seed weight per plant (Shabbir *et al.,* 2022). The positive effect of Rhizobium on seed weight per plant observed in this study is consistent with recent research, which highlights the role of Rhizobium in enhancing seed yield through improved nitrogen availability and overall plant health (Ahmed *et al.,* 2023).

Table 4.4.4: Impact of the treatments on seed weight plant^{-1}(g) of green gram in *Summer* and *Kharif* season

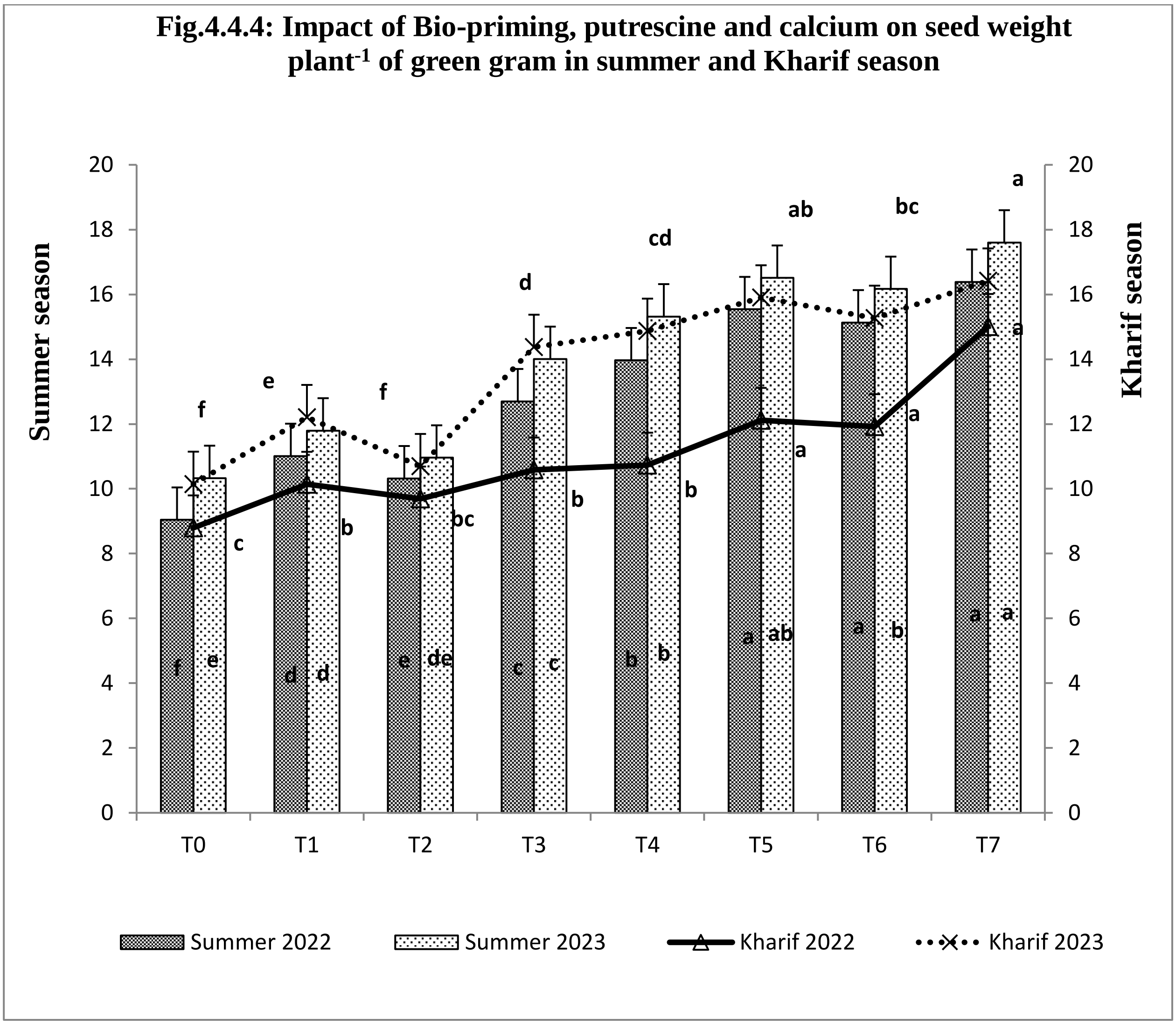
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	9.043^{f}±0.574	10.331^{e}±0.202	8.796^{c}±0.375	10.147^{f}±0.428
Calcium	11.010^{d}±0.340 [17.9]	11.797^{d}±0.621 [13.3]	10.142^{b}±0.208 [12.4]	12.207^{e}±0.561 [16.9]
Bio-priming with rhizobium	10.321^{e}±0.612 [12.4]	10.960^{de}±0.068 [9.2]	9.687^{bc}±0.531 [5.7]	10.693^{f}±0.562 [5.1]
Putrescine	12.699^{c}±0.376 [28.8]	14.006^{c}±1.021 [16.9]	10.583^{b}±0.064 [26.2]	14.373^{d}±0.484 [29.4]
Calcium + Bio-priming with rhizobium	13.964^{b}±0.331 [35.2]	15.318^{b}±0.770 [18.0]	10.732^{b}±0.491 [32.6]	14.870^{cd}±0.056 [31.8]
Calcium + Putrescine	15.541^{a}±0.727 [41.8]	16.511^{ab}±0.866 [27.4]	12.115^{a}±0.925 [37.4]	15.900^{ab}±0.178 [36.2]
Bio-priming with rhizobium + Putrescine	15.133^{a}±0.534 [40.2]	16.167^{b}±0.734 [26.2]	11.921^{a}±0.495 [36.1]	15.270^{bc}±0.324 [33.5]
Calcium + Bio priming with rhizobium + Putrescine	16.387^{a}±0.767 [44.8]	17.600a±0.530 [41.4]	15.014^{a}±0.869 [41.3]	16.420^{a}±0.375 [38.2]
C.D at p=0.05	0.677	1.247	1.055	0.755

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.4.4: Impact of the treatments on seed weight plant^{-1}(g) of green gram in *Summer* and *Kharif* season



4.4.5 Test weight

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Test weight was measured with detailed results in Table 4.4.5 and illustrated in Figure 4.4.5

In the *Summer* of 2022, treatment T_7 significantly increased test weight, showing improvements of 24.4% compared to the control. Treatment T_5 decreased test weight by 23.3%, while treatment T_6 showed 18.8% reductions. The lowest test weight was observed in treatment T_1, with 9.8% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in test weight with 32.8% improvements, followed by treatment T_5, which decreased by 30.1%. Conversely, treatment T_6 decreased by 26.4%, and treatment T_1 recorded the lowest test weight with a 7.8% reduction.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest test weight, with a 24.6% increase. Treatment T_5 decreased by 22.6%, treatment T_6 decreased by 18.3%, while the lowest test weight was recorded for treatment T_1, with a 10.4% reduction. During the *Kharif* season 2023, treatment T_7 again recorded the highest test weight, increasing 23.0%. Treatment T_5 followed with improvements of 20.5% and treatment T_6 with 19.0%. The minimum test weight in the *Kharif* season was observed in treatment T_1, showing 10.8% reductions compared to the control.

Test weight is a critical yield parameter, reflecting seed size and density, essential for the crop's quality and market value. The enhancement in test weight observed in this study can be attributed to calcium’s role in optimising cellular functions, including nutrient uptake and assimilation, which are essential for seed filling and development. Putrescine’s ability to modulate stress responses and promote nutrient uptake can lead to better seed filling and increased test weight (Shabbir *et al.,* 2022). Recent research by Zhao *et al.,* (2023) demonstrated that putrescine application can improve seed weight by enhancing metabolic activities during seed development, leading to more extensive and denser seeds. This aligns with the current study's findings, where putrescine treatment significantly increased test weight. Bio-priming with Rhizobium had a notable effect on the test weight of green gram seeds. Rhizobium inoculation enhances nitrogen fixation, providing a steady supply of nitrogen, which is critical for seed development and filling (Udvardi and Poole, 2020).

Table 4.4.5: Impact of the treatments on test weight (g) of green gram in *Summer* and *Kharif* season

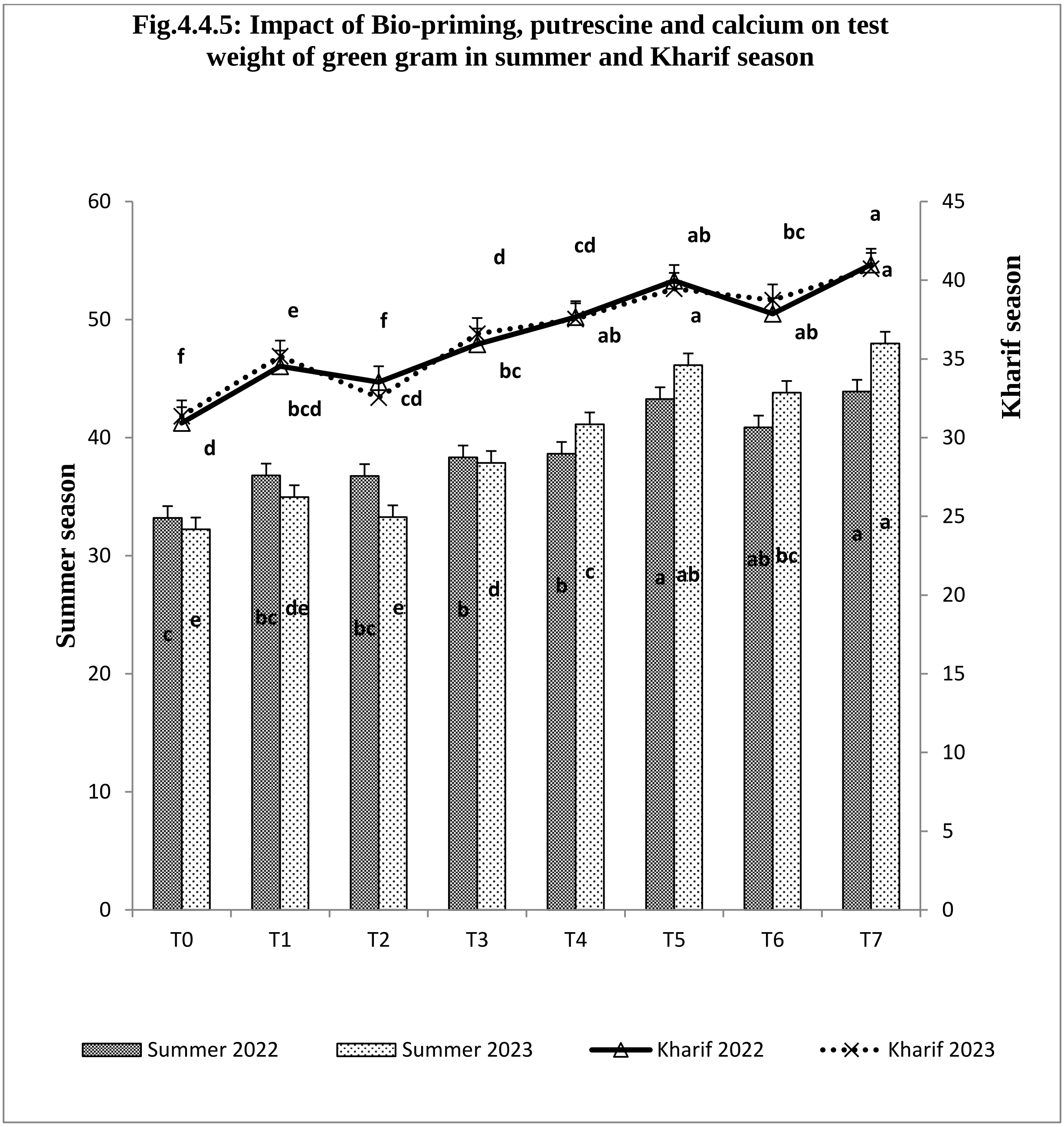
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	33.20^{c}±1.57	30.93^{e}±0.59	32.23^{d}±1.78	31.37^{f}±0.67
Calcium	36.80^{bc}±1.54 [9.8]	34.53^{de}±1.52 [10.4]	34.97^{bcd}±2.22 [7.8]	35.17^{e}±0.60 [10.8]
Bio-priming with rhizobium	36.76^{bc}±3.01 [9.7]	33.53^{e}±1.95 [7.8]	33.27^{cd}±1.57 [3.1]	32.53^{f}±1.06 [3.6]
Putrescine	38.33^{b}±2.15 [13.4]	35.93^{d}±2.48 [13.9]	37.87^{bc}±2.03 [14.9]	36.60^{d}±0.56 [14.3]
Calcium + Bio-priming with rhizobium	38.63^{b}±2.01 [14.1]	37.67^{c}±1.83 [17.9]	41.13^{ab}±1.46 [21.6]	37.53^{cd}±0.70 [16.4]
Calcium + Putrescine	43.27^{a}±2.03 [23.3]	39.97^{ab}±1.29 [22.6]	46.13^{a}±0.49 [30.1]	39.47^{ab}±0.75 [20.5]
Bio-priming with rhizobium + Putrescine	40.87^{ab}±1.21 [18.8]	37.87^{bc}±1.95 [18.3]	43.80^{ab}±1.41 [26.4]	38.73^{bc}±0.78 [19.0]
Calcium + Bio priming with rhizobium + Putrescine	43.90^{a}±2.14 [24.4]	41.00^{a}±1.64 [24.6]	47.97^{a}±1.93 [32.8]	40.73^{a}±0.68 [23.0]
C.D at p=0.05	3.77	3.03	3.22	1.32

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.4.5: Impact of the treatments on test weight (g) of green gram in *Summer* and *Kharif* season



4.5 Yield

4.5.1 Biological yield

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Biological yield was measured with detailed results in Table 4.5.1 and illustrated in Figure 4.5.1

In the *Summer* of 2022, treatment T_7 significantly increased biological yield, showing improvements of 17.6% compared to the control. Treatment T_5 decreased biological yield by 16.7%, while treatment T_6 showed 14.5% reductions. The lowest biological yield was observed in treatment T_1, with 6.9% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in biological yield with 18.4% improvements, followed by treatment T_5, which decreased by 16.2%. Conversely, treatment T_6 decreased by 14.0%, and treatment T_1 recorded the lowest biological yield with 6.2% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest biological yield, with a 19.1% increase. Treatment T_5 decreased by 16.8%, followed by treatment T_6 decreased by 14.4%, while the lowest biological yield was recorded for treatment T_1, with a 6.1% reduction. During the *Kharif* season 2023, treatment T_7 again recorded the highest biological yield, an increase of 19.5%. Treatment T_5 followed with improvements of 17.3% and treatment T_6 with 14.9%. The minimum biological yield in the *Kharif* season was observed in treatment T_1, showing 6.5% reductions compared to the control.

Biological yield, representing the total biomass produced by a crop, is a critical measure of crop productivity and overall health. Calcium contributes to the stability of cell walls, regulates enzyme activities, and facilitates nutrient uptake, all of which are essential for robust plant growth and biomass accumulation (Hepler and Winship, 2022). Putrescine is critical in regulating cell division, development, and stress responses and vital for maximising plant biomass (Hussain *et al.*, 2021). The application of putrescine enhances these processes, leading to improved plant growth and a corresponding increase in biological yield. Rhizobium inoculation improves nitrogen fixation, providing the plant with a continuous supply of nitrogen, which is crucial for protein synthesis and overall growth (Udvardi and Poole, 2020). Enhanced nitrogen availability supports the development of reproductive structures and vigorous vegetative growth, leading to higher biomass production.

Table 4.5.1: Impact of the treatments on biological yield (kg ha^{-1}) of green gram in *Summer* and *Kharif* season

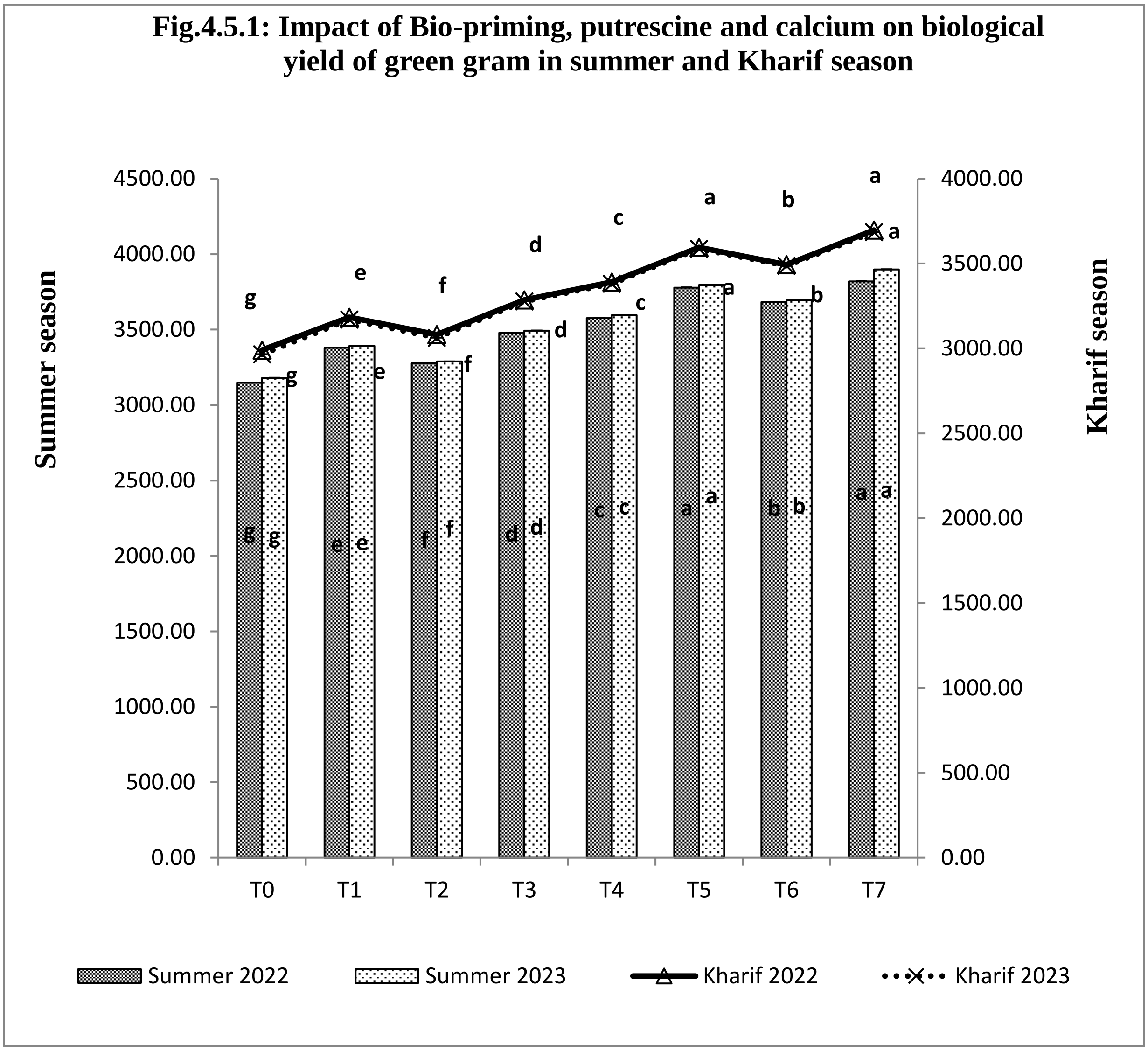
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	3147.47^{g}±6.60	3179.67^{g}±0.66	2990.57^{g}±3.65	2964.83^{g}±8.14
Calcium	3379.47^{e}±6.38 [6.9]	3390.73^{e}±1.66 [6.1]	3183.63^{e}±0.51 [6.2]	3169.87^{e}±4.92 [6.5]
Bio-priming with rhizobium	3277.77^{f}±4.71 [4.0]	3287.77^{f}±3.25 [2.9]	3081.37^{f}±0.81 [3.3]	3062.57^{f}±1.067 [3.2]
Putrescine	3478.60^{d}±2.10 [9.5]	3492.63^{d}±1.06 [9.0]	3285.87^{d}±1.52 [9.0]	3276.77^{d}±4.27 [9.5]
Calcium + Bio-priming with rhizobium	3575.47^{c}±8.64 [12.0]	3594.33^{c}±2.42 [11.8]	3390.90^{c}±0.82 [11.5]	3380.80^{c}±3.86 [12.3]
Calcium + Putrescine	3778.40^{a}±7.17 [16.7]	3796.03^{a}±1.27 [16.8]	3594.40^{a}±0.85 [16.2]	3586.57^{a}±4.56 [17.3]
Bio-priming with rhizobium + Putrescine	3681.87^{b}±1.56 [14.5]	3795.47^{b}±0.91 [14.4]	3492.50^{b}±0.53 [14.0]	3485.47^{b}±4.27 [14.9]
Calcium + Bio priming with rhizobium + Putrescine	3818.97^{a}±5.98 [17.6]	3898.57^{a}±0.95 [19.1]	3697.30^{a}±1.57 [18.4]	3685.13^{a}±4.72 [19.5]
C.D at p=0.05	9.80	2.668	2.89	8.77

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.5.1: Impact of the treatments on biological yield (kg ha^{-1}) of green gram in *Summer* and *Kharif* season



4.5.2 Seed yield

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Seed yield was measured with detailed results in Table 4.5.2 and illustrated in Figure 4.5.2

In the *Summer* of 2022, treatment T_7 significantly increased seed yield, showing improvements of 28.7% compared to the control. Treatment T_5 decreased seed yield by 24.8%, while treatment T_6 showed 14.6% reductions. The lowest seed yield was observed in treatment T_1, with 7.7% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in seed yield with 28.6% improvements, followed by treatment T_5, which decreased by 25.2%. Conversely, treatment T_6 decreased by 14.5%, and treatment T_1 recorded the lowest seed yield with a 6.1% reduction.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest seed yield, with a 28.6% increase. Treatment T_5 decreased by 25.2%, followed by treatment T_6 decreased by 14.5%, while the lowest seed yield was recorded for treatment T_1, with a 6.1% reduction. During the *Kharif* season 2023, treatment T_7 again recorded the highest seed yield, increasing by 28.9%. Treatment T_5 followed with improvements of 20.1% and treatment T_6 with 12.3%. The minimum seed yield in the *Kharif* season was observed in treatment T_1, showing 6.0% reductions compared to the control.

Seed yield refers to the portion of the biological yield that is harvested and sold, directly influencing the profitability of the crop. The foliar application of calcium has been found to increase green gram's seed yield significantly. This increase is primarily due to calcium's ability to enhance overall plant health, leading to improved pod formation and seed development, critical components of seed yield (Hepler and Winship, 2022). Putrescine is known to enhance cell division, growth, and stress tolerance, which is essential for maximising marketable produce yield (Hussain *et al.*, 2021). Applying putrescine leads to improved seed filling and development, resulting in higher seed weight and number, directly contributing to increased seed yield. The positive effect of Rhizobium Bio-priming on seed yield observed in this study is consistent with recent research, which emphasises the role of Rhizobium in improving legume yield and quality through enhanced nitrogen fixation and overall plant health (Ahmed *et al.*, 2023). The findings of this study confirm that the combination of calcium, putrescine, and Rhizobium treatments leads to substantial improvements in seed yield, suggesting that these treatments work synergistically to enhance the marketable yield of green gram.

Table 4.5.2: Impact of the treatments on seed yield (kg ha^{-1}) of green gram in *Summer* and *Kharif* season

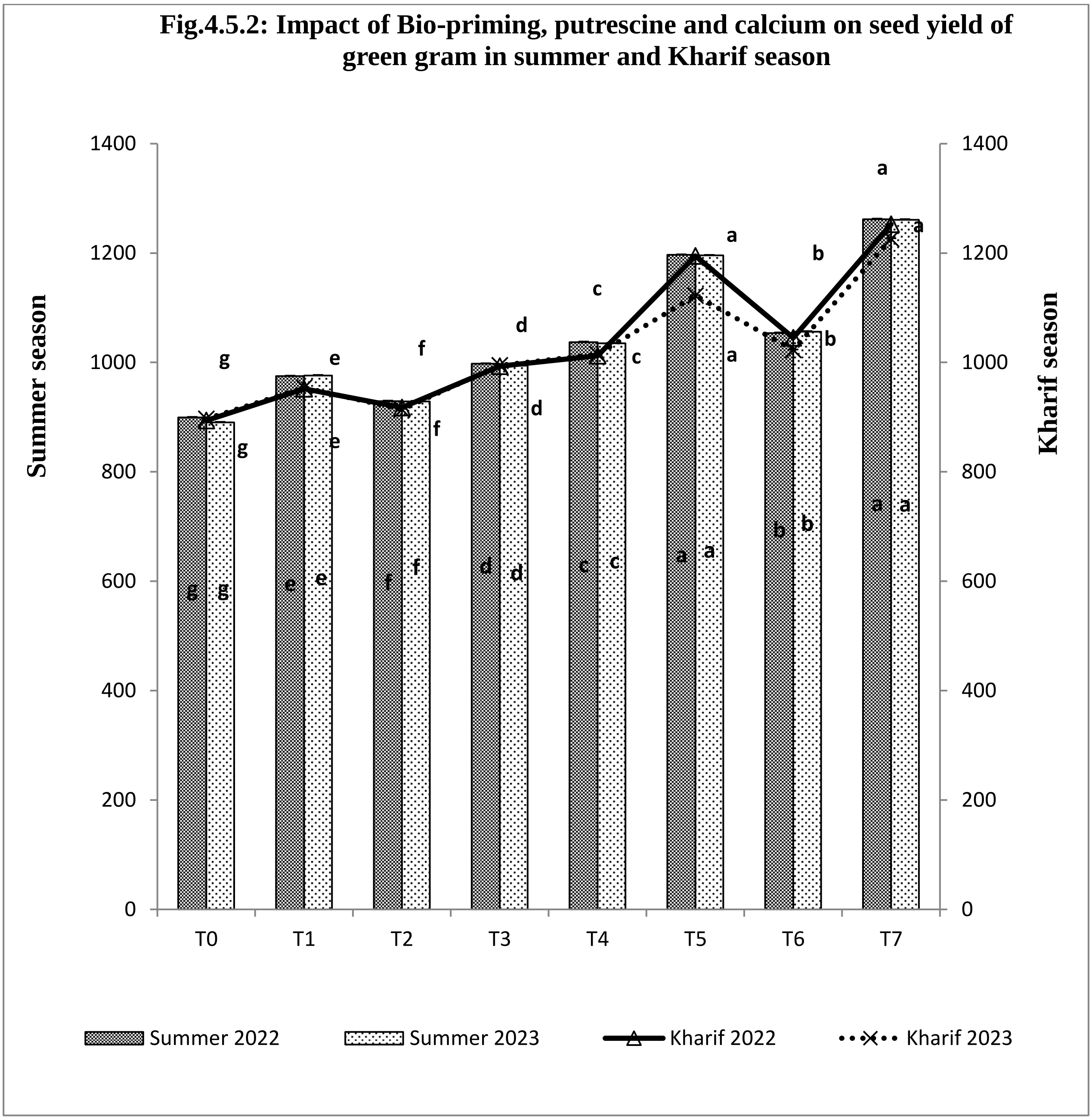
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	899.43^{g}±0.80	890.40^{g}±4.35	893.80^{g}±2.26	896.80^{g}±3.26
Calcium	974.80^{e}±4.20 [7.7]	976.17^{e}±2.94 [6.1]	952.03^{e}±2.02 [8.8]	953.70^{e}±4.74 [6.0]
Bio-priming with rhizobium	929.20^{f}±0.69 [3.2]	928.83^{f}±1.00 [2.6]	917.73^{f}±2.27 [4.1]	914.10^{f}±3.44 [1.9]
Putrescine	997.50^{d}±2.25 [9.8]	994.23^{d}±10.09 [10.0]	993.23^{d}±2.15 [10.4]	994.80^{d}±3.16 [9.9]
Calcium + Bio-priming with rhizobium	1037.20^{c}±4.20 [13.3]	1034.87^{c}±2.10 [11.7]	1012.50^{c}±4.31 [14.0]	1014.77^{c}±4.86 [11.6]
Calcium + Putrescine	1196.63^{a}±4.00 [24.8]	1195.63^{a}±2.95 [25.2]	1194.73^{a}±4.12 [25.5]	1122.10^{a}±3.87 [20.1]
Bio-priming with rhizobium + Putrescine	1053.80^{b}±3.60 [14.6]	1056.33^{b}±1.10 [14.5]	1045.03^{b}±3.65 [15.7]	1022.70^{b}±4.74 [12.3]
Calcium + Bio priming with rhizobium + Putrescine	1262.00^{a}±9.19 [28.7]	1261.00^{a}±1.31 [28.6]	1252.53^{a}±5.18 [29.4]	1226.07^{a}±3.25 [26.9]
C.D at p=0.05	7.66	7.62	6.35	7.33

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.5.2: Impact of the treatments on seed yield (kg ha^{-1}) of green gram in *Summer* and *Kharif* season



4.5.3 Stover yield

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. Stover yield was measured with detailed results in Table 4.5.3 and illustrated in Figure 4.5.3

In the *Summer* of 2022, treatment T_6 significantly increased stover yield, showing improvements of 13.3% compared to the control. Treatment T_7 decreased stover yield by 13.2%, while treatment T_6 showed 12.0% reductions. The lowest stover yield was observed in treatment T_1, with 5.2% reductions.

During the *Kharif* season of 2022, treatment T_6 again demonstrated a notable increase in stover yield with 15.5% improvements, followed by treatment T_7, which decreased by 15.4%. Conversely, treatment T_5 decreased by 15.5%, and treatment T_1 recorded the lowest stover yield with a 6.1% reduction.

In 2023, during the *Summer* season, it was revealed that treatment T_6 achieved the highest stover yield, with a 14.5% increase. Treatment T_7 decreased by 12.1%, followed by treatment T_5 decreased by 12.9%, while the lowest stover yield was recorded for treatment T_1, with a 6.5% reduction. During the *Kharif* season 2023, treatment T_6 again recorded the highest stover yield, increasing by 14.3%. Treatment T_7 followed with improvements of 14.2% and treatment T_5 with 12.6%. The minimum stover yield in the *Kharif* season was observed in treatment T_1, showing 6.0% reductions compared to the control.

The foliar application of calcium has been found to increase green gram's stover yield significantly. This increase is primarily due to calcium's ability to enhance overall plant health, leading to improved pod formation and seed development, critical components of yield (Hepler and Winship, 2022). Putrescine is known to enhance cell division, growth, and stress tolerance, which is essential for maximising marketable produce yield (Hussain *et al.*, 2021). Applying putrescine leads to improved seed filling and development, resulting in higher seed weight and number, directly contributing to increased seed and stover yield. The positive effect of Rhizobium Bio-priming on seed yield observed in this study is consistent with recent research, which emphasises the role of Rhizobium in improving legume yield and quality through enhanced nitrogen fixation and overall plant health (Ahmed *et al.,* 2023). The findings of this study confirm that the combination of calcium, putrescine, and Rhizobium treatments leads to substantial improvements in seed yield, suggesting that these treatments work synergistically to enhance the marketable yield of green gram.

Table 4.5.3: Impact of the treatments on Stover yield (kg ha^{-1}) of green gram in *Summer* and *Kharif* season

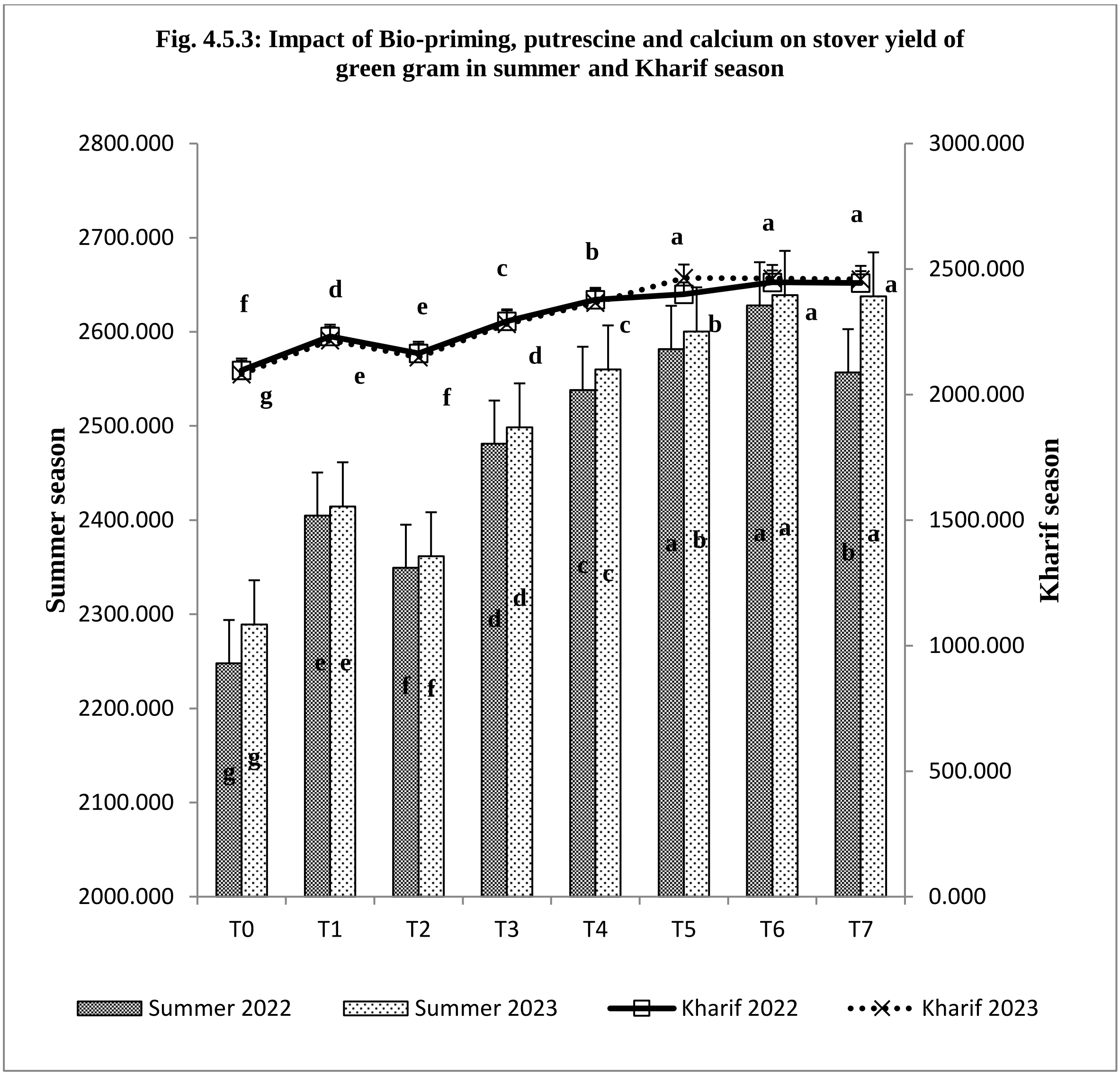
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	2248.03±6.9	2289.27±4.1	2096.77±2.4	2081.37±33.9
Calcium	2404.67±8.8 [5.2]	2414.57±3.0 [6.5]	2231.60±1.5 [6.1]	2216.17±0.9 [6.0]
Bio-priming with rhizobium	2349.33±4.5 [3.1]	2361.00±3.6 [4.3]	2163.63±3.0 [3.1]	2148.47±4.4 [3.1]
Putrescine	2481.10±1.4 [8.4]	2498.40±9.1 [9.4]	2292.63±3.6 [8.8]	2281.97±7.0 [8.5]
Calcium + Bio-priming with rhizobium	2538.27±12.1 [10.6]	2559.97±1.5 [11.4]	2378.40±5.1 [12.0]	2366.03±1.4 [11.8]
Calcium + Putrescine	2581.77±4.7 [12.0]	2600.40±3.1 [12.9]	2399.67±5.0 [15.5]	2464.47±5.6 [12.6]
Bio-priming with rhizobium + Putrescine	2628.07±4.5 [13.3]	2639.13±0.7 [14.5]	2447.47±3.6 [15.5]	2462.77±5.8 [14.3]
Calcium + Bio priming with rhizobium + Putrescine	2556.97±6.4 [13.2]	2637.57±1.1 [12.1]	2444.77±3.9 [15.4]	2459.07±7.7 [14.2]
C.D at p=0.05	11.83	6.79	7.36	23.25

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.5.3: Impact of the treatments on Stover yield (kg ha^{-1}) of green gram in *Summer* and *Kharif* season



4.5.4 Harvest index

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. The Harvest index was measured with detailed results in Table 4.5.4 and illustrated in Figure 4.5.4

In the *Summer* of 2022, treatment T_7 significantly increased the harvest index, showing improvements of 13.5% compared to the control. This was followed by treatment T_5, which decreased the harvest index by 9.8%, while treatment T_6 showed 0.2% reductions. The lowest harvest index was observed in treatment T_1, with 0.9% reductions.

During the *Kharif* season of 2022, treatment T_7 again demonstrated a notable increase in harvest index with 13.4% improvements, followed by treatment T_5, which decreased by 11.1%. Conversely, treatment T_6 decreased by 2.0%, and treatment T_1 recorded the lowest harvest index with 2.7% reductions.

In 2023, during the *Summer* season, it was revealed that treatment T_7 achieved the highest harvest index, with an 11.8% increase. Treatment T_5 decreased by 10.1%, followed by treatment T_6 decreased by 0.1%, while the lowest harvest index was recorded for treatment T_1, with 0.1% reductions. During the *Kharif* season 2023, treatment T_7 again recorded the highest harvest index, increasing by 9.1%. Treatment T_5 followed with improvements of 3.3% and treatment T_6 with 3.1%. The minimum harvest index in the *Kharif* season was observed in treatment T_1, showing 0.5% reductions compared to the control.

The harvest index (HI) is a crucial parameter in agronomy, representing the ratio of seed yield (such as seeds) to the total biological yield (including stems, leaves, and roots). It reflects the efficiency with which a plant converts biomass into the part of the crop that is harvested. The foliar application of calcium in green gram has been shown to improve the harvest index by enhancing pod development and seed filling, leading to a higher proportion of the total biomass allocated to the seeds (Hepler and Winship, 2022). The increased nitrogen availability supports both vegetative and reproductive growth, but its significant impact on reproductive growth, particularly seed development, leads to a higher harvest index (Udvardi and Poole, 2020). In green gram, foliar application of putrescine has been associated with enhanced seed filling, increased seed weight, and improved pod development, all of which contribute to a higher harvest index. The combination of calcium, putrescine, and Rhizobium treatments resulted in the highest harvest index observed in this study. This synergistic effect is likely due to the complementary roles of these treatments in enhancing both vegetative and reproductive growth while ensuring that a more significant proportion of the total biomass is allocated to the seed yield.

Table 4.5.4: Impact of the treatments on harvest index (%) of green gram in *Summer* and *Kharif* season

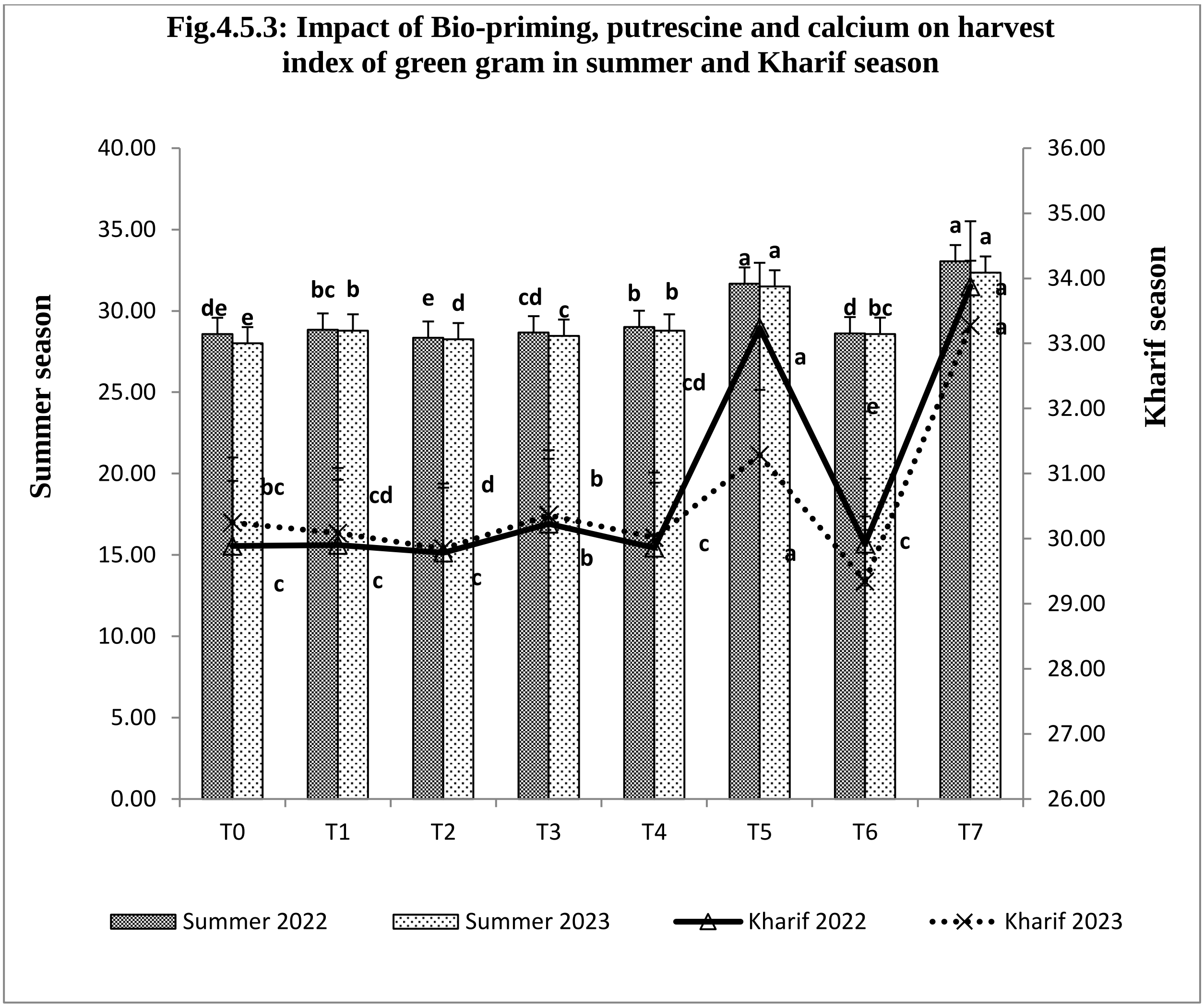
Treatments	*Summer* 2022	*Summer* 2023	*Kharif* 2022	*Kharif* 2023
Control	28.57^{de}±0.07	28.00^{e}±0.13	29.88^{c}±0.05	30.25^{bc}±0.18
Calcium	28.85^{bc}±0.15 [0.9]	28.78^{b}±0.08 [0.1]	29.90^{c}±0.06 [2.7]	30.09^{cd}±0.10 [0.5]
Bio-priming with rhizobium	28.35^{e}±0.06 [0.8]	28.25^{d}±0.02 [0.3]	29.78^{c}±0.08 [0.9]	29.85^{d}±0.12 [1.3]
Putrescine	28.67^{cd}±0.05 [0.3]	28.47^{c}±0.28 [1.1]	30.23^{b}±0.08 [1.6]	30.36^{b}±0.13 [0.4]
Calcium + Bio-priming with rhizobium	29.01^{b}±0.17 [1.5]	28.78^{b}±0.04 [0.1]	29.86^{c}±0.13 [2.7]	30.01^{cd}±0.11 [0.8]
Calcium + Putrescine	31.67^{a}±0.07 [9.8]	31.5^{a}±0.07 [10.1]	33.24^{a}±0.12 [11.1]	31.29^{a}±0.11 [3.3]
Bio-priming with rhizobium + Putrescine	28.62^{d}±0.10 [0.2]	28.58^{bc}±0.02 [0.1]	29.92^{c}±0.10 [2.0]	29.34^{e}±0.13 [3.1]
Calcium + Bio priming with rhizobium + Putrescine	33.05^{a}±0.21 [13.5]	32.34^{a}±0.03 [11.8]	33.87^{a}±0.13 [13.4]	33.27^{a}±0.13 [9.1]
C.D at p=0.05	0.21	0.22	0.18	0.24

Note:

1-Calcium, Putrescine and Rhizobium were used in [10mM, 3mM and 10 ml kg^{-1} of seeds]

2-Data in parenthesis represent the per cent increase /decrease over the control

Fig. 4.5.4: Impact of the treatments on harvest index (%) of green gram in *Summer* and *Kharif* season



4.6 Economics

The effects of Bio-priming, putrescine, and calcium on green gram growth, yield, and quality were assessed during the *Summer and Kharif* seasons of 2022 and 2023. The economics were calculated, with detailed results in Tables 4.6.a, 4.6.b, 4.6.c, and 4.6.d and appendix-ii.

In the *Summer* of 2022, treatment T_7 showed the maximum cost of cultivation, Rs.38364, gross return, Rs.91810.5, and net return, Rs.53446. This was followed by treatment T_5, with a price of cultivation of Rs. 37,584, gross return Rs. 87055.08, and a net return of Rs.49470. The minimum was calculated in treatment control, with a cost of cultivation of Rs.31187, gross return of Rs.65433.78, and net return of Rs.28246.

In 2023, during the *Summer* season, it was revealed that treatment T_7 showed the maximum cost of cultivation, Rs.39864, gross return, Rs.97790.55, and net return, Rs.57926. This was followed by treatment T_5, with cost of cultivation Rs.39084, gross return Rs.92721, and net return Rs.53637. The minimum was calculated in treatment control, with a price of cultivation of Rs.38687, a gross return of Rs.69050.52, and a net return of Rs.3.363.52.

During the *Kharif* of 2022, treatment T_7 showed the maximum cost of cultivation, Rs.36364, gross return, Rs.95081.73, and net return, Rs.58717.73. This was followed by treatment T_5 with the cost of cultivation, Rs.35584, gross return Rs.87018.86, and net return, Rs. 51434.85. The minimum was calculated in treatment control, with a price of cultivation of Rs.35187, gross return of Rs.69546.84, and net return of Rs.34359.84.

During the *Kharif* season 2023, treatment T_7 shows the maximum cost of cultivation of Rs.36364, a gross return of Rs.95081.47, and net return of Rs.58717.73, followed by treatment T_5 with the cost of cultivation Rs.35584, gross return Rs.87018.86, and net return Rs.51434.85. The minimum was calculated in treatment control with cost of cultivation Rs.35187, gross return Rs.69546, and net return Rs.3435984.

4.6.a Economics of *Summer* season 2022

Treatment	COC	GROSS RETURN	NET RETURN	B:C RATIO
Control	37,187	65433.53	28246.53	1.76
Calcium	37,577	70916.70	33339.70	1.89
Bio-priming with rhizobium	37,967	67599.30	29632.30	1.78
Putrescine	37,194	72568.13	35374.13	1.95
Calcium + Bio-priming with rhizobium	38,357	75456.30	37099.30	1.97
Calcium + Putrescine	37,584	87054.83	49470.83	2.32
Bio-priming with rhizobium + Putrescine	37,974	76663.95	38689.95	2.02
Calcium + Bio priming with rhizobium + Putrescine	38,364	91810.50	53446.50	2.39

4.6.b Economics of *Summer* season 2023

Treatment	COC	GROSS RETURN	NET RETURN	B:C RATIO
Control	38687	69050.52	30363.52	1.78
Calcium	39077	75701.98	36624.98	1.94
Bio-priming with rhizobium	39467	72030.77	32563.77	1.83
Putrescine	38694	77102.54	38408.54	1.99
Calcium + Bio-priming with rhizobium	39857	80254.17	40397.17	2.01
Calcium + Putrescine	39084	92721.11	53637.11	2.37
Bio-priming with rhizobium + Putrescine	39474	81918.39	42444.39	2.08
Calcium + Bio priming with rhizobium + Putrescine	39864	97790.55	57926.55	2.45

4.6.c Economics of *Kharif* season 2022

Treatment	COC	GROSS RETURN	NET RETURN	B:C RATIO
Control	35187	69546.84	34359.84	1.98
Calcium	35577	73959.44	38382.44	2.08
Bio-priming with rhizobium	35967	70888.46	34921.46	1.97
Putrescine	35194	77146.74	41952.74	2.19
Calcium + Bio-priming with rhizobium	36357	78695.41	42338.41	2.16
Calcium + Putrescine	35584	87018.86	51434.85	2.45
Bio-priming with rhizobium + Putrescine	35974	79310.39	43336.39	2.20
Calcium + Bio priming with rhizobium + Putrescine	36364	95081.73	58717.73	2.61

4.6.d Economics of *Kharif* season 2023

Treatment	COC	GROSS RETURN	NET RETURN	B:C RATIO
Control	35187	69546.84	34359.84	1.98
Calcium	35577	73959.44	38382.44	2.08
Bio-priming with rhizobium	35967	70888.46	34921.46	1.97
Putrescine	35194	77146.74	41952.74	2.19
Calcium + Bio-priming with rhizobium	36357	78695.41	42338.41	2.16
Calcium + Putrescine	35584	87018.86	51434.85	2.45
Bio-priming with rhizobium + Putrescine	35974	79310.39	43336.39	2.20
Calcium + Bio priming with rhizobium + Putrescine	36364	95081.73	58717.73	2.61

CHAPTER-5

SUMMARY AND CONCLUSION

The current part of the research study titled "Impact of Bio-priming, Putrescine and Calcium on the Growth, yield and Quality of Green Gram (*Vigna radiata*. L)" was carried out at the farm of Lovely Professional University, located in Phagwara, Punjab, during the summer and *Kharif* season of 2022-23. This experiment involves Bio-priming with rhizobium and foliar application with putrescine and calcium chloride at different growth stages. The effects of different treatments are clearly shown in the growth, biochemical, yield attributing characters and yield of green gram crop. Below is a concise overview of the materials and procedures employed in this research trial. Below is a summary of the materials and methods used for this research work. RBD (Randomized Block Design) was used in the field for this experiment. In this experiment, eight treatments (T_0-T_7) are chosen, each replicated three times. The treatments are represented as T_0= control, T_1= calcium chloride (10mM), T_2= bio priming with rhizobium (10ml), T_3= putrescine (3mM), T_4= calcium chloride (10mM) + bio priming with rhizobium (10ml), T_5= calcium chloride(10mM) + putrescine (3mM), T_6= bio priming with rhizobium (10ml)+ putrescine (3mM), T_7= calcium chloride (10mM) + bio priming with rhizobium (10ml)+ putrescine (3mM) with replications R1, R2 and R3. The variety of green gram used is SML-668 for both seasons. Fertilizer application is done at the time of sowing. Observations related to morphological and biochemical studies are recorded at 30 and 60DAS, whereas the parameters related to yield are recorded at harvest.

The result of the present study can be summarized as follows:

- The above observations show that the maximum plant height (cm) was found in the treatment T_7= calcium chloride + bio priming with rhizobium + putrescine with 46.5, 66.5 at 30DAS and 60DAS in summer 2022, 46.6, 65.8cm at 30DAS and 60DAS in summer 2023 and 43.0, 63.1cm at 30DAS and 60DAS in *Kharif* 2022, 44.3, 64.1cm at 30DAS and 60DAS in *Kharif* 2022.

- The maximum no. of branches was found in the treatment T_7 with in summer 2022, 3.63, 5.37 and 2023, 3.73, 5.33 and in *Kharif* 2022, 3.63, 4.70 and in 2023, 3.30, 5.07.
- The total no. of leaves was found highest in the treatment T_7 with calcium chloride + bio priming with rhizobium + putrescine with 22.90 and 32.10 in summer 2022, 21.90 and 32.00 in summer 2023, 20.30 and 30.10 in *Kharif* 2022, 19.60, 29.70 in *Kharif* 2023 at 30 and 60DAS.
- The leaf area (cm^2) and leaf area index was found maximum in the treatment T_7 with calcium chloride + bio priming with rhizobium + putrescine with (340.22, 769.17) and (9.51, 3.42) in summer 2022, (322.97, 750.60) and (1.44, 3.34) in summer 2023, (303.43, 749.73) and (1.35, 3.33) in *Kharif* 2022, (302.87, 733.47) and (1.35, 3.26) in *Kharif* 2023 at 30 and 60DAS.
- In summer and *Kharif* 2022, the leaf area duration (days) was maximum at 18.8 and 16.9, and in summer and *Kharif* 2023, with 15.5 and 15.6 in treatment T_7.
- The minimum days to 50% flowering and physiological maturity was found in the treatment T_7, with (36.33, 36.67) and (60.67, 61.00) in summer 2022 and 2023, whereas in *Kharif* 2022 and 2023, with (36.67, 37.2) and (66.00, 60.3).
- In the summer and *Kharif* of 2022 and 2023, treatment T_7 significantly increased root length (cm), showing improvements with 15.3 and 20.97, 15.1 and 21.2, 14.1 and 17.1, 14.4 and 18.7 cm at 30 and 60DAS.
- In the summer and *Kharif* seasons of 2022 and 2023, treatment T_7 significantly increased the number of nodules, with 71.67 and 87.00, 79.00 and 90.67, 77.67 and 94.33, 75.33 and 92.00 at 30 and 60DAS.
- The maximum SPAD index was found in the treatment T_7 with 40.7, 59.5 in summer 2022, 39.2, 65.8 in summer 2023 and 37.2, 54.2 in *Kharif* 2022, 41.4, 65.2 in *Kharif* 2023 at 30 and 60DAS.
- The chlorophyll content (mg g^{-1}) was found maximum in the treatment T_7 with 1.02, 2.32 in summer 2022, 1.01, 2.58 in summer 2023 and 1.08, 2.11 in *Kharif* 2022, 0.95, 2.11 in *Kharif* 2023 at 30 and 60DAS.

- Total protein content (mg g^{-1}) was highest in treatment T7, with 23.02 and 21.33, 22.25 and 20.54 at 30 and 60DAS in summer 2022 and 2023, whereas in *Kharif* 2022 and 2023, it was 22.81 and 20.59, 21.82 and 19.80 at 30 and 60DAS.
- In summer 2022 and 2023, the total carbohydrate content (mg g^{-1}) was highest in the treatment T7, with 8.11 and 7.50, 8.17 and 7.33, whereas in *Kharif* 2022 and 2023, it was 7.22 and 6.39, 7.11 and 6.22 at 30 and 60DAS.
- The maximum phenylalanine ammonia lyase activity (PAL) (μ^{-1} mg^{-1} $min.^{-1}$) was found in the treatment T_7= calcium chloride + bio priming with rhizobium + putrescine with 0.074, 0.078 at 30DAS and 60DAS in summer 2022, 0.07, 0.078 at 30DAS and 60DAS in summer 2023 and 0.072, 0.077 at 30DAS and 60DAS in *Kharif* 2022, 0.072, 0.077 at 30DAS and 60DAS in *Kharif* 2022.
- Total flavanol content (mg g^{-1}) was highest in the treatment T_7, with 0.499 and 0.562, 0.520 and 0.520 at 30 and 60DAS in summer 2022 and 2023, whereas in *Kharif* 2022 and 2023, it was 0.534 and 0.541, 0.477 and 0.557 at 30 and 60DAS.
- Total flavonoid content (mg g^{-1}) was found highest in the treatment T_7 with 0.658 and 0.735, 0.826 and 0.896 at 30 and 60DAS in summer 2022 and 2023 whereas in *Kharif* 2022 and 2023, it was 0.702 and 0.756, 0.739 and 0.771 at 30 and 60DAS.
- In summer 2022 and 2023, phenol content (mg g^{-1}) was found to be maximum in the treatment T_7 with 0.224 and 0.229, 0.225 and 0.231 whereas, in *Kharif* 2022 and 2023, it was 0.218 and 0.230, 0.220 and 0.220 at 30 and 60DAS.
- The nitrogen content was highest in the treatment T_7 with 4.46%, 4.49%, 4.4%, and 4.2% in summer 2022 and 2023 and *Kharif* 2022 and 2023.
- The phosphorus content was highest in the treatment T_7 with 0.096%, 0.094% and 0.091%, 0.09% in summer 2022 and 2023 and *Kharif* 2022 and 2023.
- The potassium content was highest in the treatment T_7 with 0.702%, 0.7% and 0.701%, 0.7% in summer 2022 and 2023 and *Kharif* 2022 and 2023.
- The arginine content (mg g^{-1}) was highest in the treatment T_7 with 508, 509 and 506, 504 in summer 2022 and 2023 and *Kharif* in 2022 and 2023.

- The total seed protein (g) content was highest in the treatment T_7 with 23.16, 24.12 and 23.10, 23.09 in summer 2022 and 2023 and *Kharif* in 2022 and 2023.
- The highest test weight (g) was found in the treatment T_7, with 43.90 in summer 2022, 41.00 in summer 2023, 47.97 in *Kharif* 2022 and 40.73 in *Kharif* 2023.
- The maximum number of pod^{-1} seeds was found in treatment T_7, with 11.03 in summer 2022, 11.20 in summer 2023, 9.55 in *Kharif* 2022, and 9.43 in *Kharif* 2023.
- The highest seed weight pod^{-1} (g) was found in the treatment T_7 with 0.47 in summer 2022, 0.49 in summer 2023, 0.43 in *Kharif* 2022 and 0.44 in *Kharif* 2023.
- The highest seed weight plant^{-1} (g) was found in the treatment T_7 with 16.38 in summer 2022, 17.60 in summer 2023, 15.01 in *Kharif* 2022 and 16.42 in *Kharif* 2023.
- The maximum no. of pods plant^{-1} was found in the treatment T_7 with 34.36 in summer 2022, 35.43 in summer 2023, 32.76 in *Kharif* 2022 and 35.20 in *Kharif* 2023.
- Yield parameters like biological yield (kg ha^{-1}), seed yield (kg ha^{-1}) and harvest index (%) were found to be maximum in the treatment T_7 with calcium chloride + bio priming with rhizobium + putrescine: 3818.96, 1262.0 and 33.04 in summer 2022, 3898.56, 1261.0 and 32.34 in summer 2023, 3697.30, 1252.5 and 33.8 in *Kharif* 2022 and 3685.13, 1226.0 and 33.27 in *Kharif* 2023.

The results of this experiment entitled **"Impact of Bio-priming, Putrescine and Calcium on the growth, yield and quality of green gram (*Vigna radiata*. L)"** concluded that

- As per objective no. 1 the effect of rhizobium, putrescine and calcium on morpho-physiological growth, yield and quality of green gram was observed. The treatment, combined with Bio-priming with rhizobium and foliar application with putrescine and calcium chloride, gives the best results in response to the growth and development of the crop.

- As per objective no.2, the impact of different treatments on biochemical reposes on green gram was measured. Applying 10ml of rhizobium as Bio-priming 10mM of calcium and 3mM of putrescine exhibited statistically significant results over the control compared to all other treatments.
- As per objective no. 3, Arginine, which is also the precursor of putrescine, is found to be the maximum in the treatment with the combination of Bio-priming with rhizobium and foliar application with putrescine and calcium chloride.
- As per objective no. 4, the results showed that the yield and yield attributing parameters provide the best results in the combination of treatments and are economically feasible.

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APPENDIX-i

Meteorological data of year 2022

Date	Temperature (Max.) ^0C	Temperature (Min.) ^0C	Relative humidity (Max.) %	Relative humidity (Min.) %	Wind speed (km/hr)	Rain fall (mm)
01-01-2022-08-01-2022	15.75	8.13	74.13	63.13	1.25	10.19
09-01-2022-15-01-2022	15.57	11.14	76.57	62.86	3.14	3.43
16-01-2022-22-01-2022	16.86	11.57	74.29	59.71	2.29	0.29
23-01-2022-29-01-2022	14.00	11.29	77.43	63.71	1.71	0.00
30-012022-5-02-2022	16.14	8.43	77.43	64.57	3.33	0.00
6-02-2022-12-02-2022	12.71	8.43	78.14	62.57	3.50	0.00
13-02-2022-19-02-2022	17.00	8.57	74.86	60.00	3.00	0.00
20-02-2022-26-02-2022	19.43	10.29	67.00	51.29	3.60	2.29
27-02-2022-05-02-2022	18.00	9.57	60.29	48.14	2.29	0.00
06-03-2022-12-03-2022	20.86	15.43	54.43	43.29	1.43	0.00
13-03-2022-19-03-2022	28.86	20.14	55.14	42.29	2.00	0.00
20-03-2022-26-03-2022	31.71	21.57	52.14	42.00	4.00	0.00

27-03-2022- 2-04-2022	31.71	21.43	51.29	42.71	3.14	0.00
03-04-2022- 09-04-2022	34.14	24.71	46.14	38.14	3.14	0.00
10-04-2022- 16-04-2022	41.29	27.14	44.57	33.57	4.00	0.00
17-04-2022- 23-04-2022	39.43	28.86	40.71	28.71	12.29	0.00
24-04-2022- 30-04-2022	42.00	31.43	33.71	22.14	10.29	0.00
01-05-2022- 07-05-2022	40.00	29.71	36.43	21.00	10.29	0.00
8-05-2022- 14-05-2022	39.43	31.00	36.57	27.43	18.57	0.00
15-05-2022- 21-05-2022	40.57	31.86	32.43	28.29	16.00	0.00
22-05-2022- 28-05-2022	40.14	28.14	35.29	36.43	6.29	1.60
29-05-2022- 04-06-2022	37.86	31.29	39.00	38.43	12.86	0.00
05-06-2022- 11-06-2022	43.00	32.29	33.43	41.86	9.57	0.00
12-06-2022- 18-06-2022	39.29	32.29	43.00	44.86	10.86	0.00
19-06-2022- 25-06-2022	40.00	28.86	43.14	47.86	13.00	10.09
26-06-2022- 02-07-2022	40.71	32.00	53.00	56.14	16.86	0.00
03-07-2022- 09-07-2022	43.14	31.57	60.57	63.57	6.29	5.63
10-07-2022-	36.57	31.43	59.57	60.29	12.00	0.89

16-07-2022						
17-07-2022-23-07-2022	34.43	29.29	69.00	65.14	10.29	29.39
24-07-2022-30-07-2022	34.43	26.43	72.29	64.86	10.00	0.34
31-08-2022-06-08-2022	34.86	25.43	77.29	65.29	7.57	0.00
07-08-2022-13-08-2022	35.29	26.14	73.57	62.43	7.71	1.19
14-08-2022-20-08-2022	33.43	26.29	74.86	64.86	8.71	1.29
21-08-2022-27-08-2022	33.86	25.57	75.43	64.86	7.00	0.67
28-08-2022-03-09-2022	35.43	26.00	75.71	63.43	7.57	0.61
04-09-2022-10-09-2022	37.71	25.43	70.14	62.29	8.71	0.00
11-09-2022-17-09-2022	34.71	24.00	72.14	65.57	11.43	0.57
18-09-2022-24-09-2022	35.86	24.14	73.14	62.86	12.57	0.30
25-09-2022-01-10-2022	34.00	22.14	69.14	60.57	10.00	0.34
02-10-2022-08-10-2022	35.00	21.86	54.71	43.14	9.57	0.00
09-10-2022-15-10-2022	32.71	21.57	57.43	46.86	3.86	0.00
16-10-2022-22-10-2022	31.71	20.29	53.71	43.43	2.14	0.00
23-10-2022-29-10-2022	29.57	18.29	54.29	43.71	3.29	0.00

30-10-2022- 5-11-2022	28.71	18.43	53.14	44.29	2.57	0.00
6-11-2022- 12-11-2022	27.86	15.00	53.43	44.14	2.00	0.00
13-11-2022- 19-11-2022	26.57	13.71	56.86	47.29	2.43	0.00
20-11-2022- 26-11-2022	23.57	13.14	56.71	46.14	1.86	0.00
27-11-2022- 03-12-2022	23.29	11.29	67.86	56.43	1.00	0.00
04-12-2022- 10-12-2022	27.29	10.86	88.00	59.57	4.00	0.00
11-12-2022- 17-12-2022	26.57	10.86	89.14	70.43	7.71	0.00
18-12-2022- 24-12-2022	24.00	9.43	91.57	75.43	4.57	0.00
25-12-2022- 31-12-2022	21.43	8.86	93.71	80.00	7.57	0.29

Meteorological data of year 2023

Date	**Temperature (Max.) ^{0}C**	**Temperature (Min.) ^{0}C**	**Relative humidity (Max.) %**	**Relative humidity (Min.) %**	**Wind speed (km/hr)**	**Rain fall (mm)**
01-01-2023- 07-01-2023	14.4	5.5	94.1	81.9	6.6	0.0
08-01-2023- 14-01-2023	13.5	8.4	95.6	82.7	8.9	0.2
15-01-2023- 21-01-2023	14.4	5.9	91.4	76.6	6.7	0.0

22-01-2023- 28-01-2023	18.7	7.6	84.1	63.6	7.7	4.9
39-01-2023- 04-02-2023	20.1	8.7	94.6	76.0	11.4	1.8
05-02-2023- 11-02-2023	23.7	11.8	80.3	58.1	10.1	0.0
12-02-2023- 18-02-2023	26.0	10.8	63.0	41.4	9.6	0.0
19-02-2023- 25-02-2023	28.1	14.3	92.3	56.3	9.9	0.0
26-02-2023- 04-02-2023	27.4	14.0	66.3	47.9	9.1	0.0
05-03-2023- 11-03-2023	29.4	14.0	46.4	37.3	8.7	0.0
12-03-2023- 18-03-2023	27.8	16.7	74.4	45.1	10.6	2.1
19-03-2023- 25-03-2023	24.7	13.9	93.3	59.7	1.8	5.5
26-03-2023- 01-04-2023	27.6	15.7	88.7	50.6	10.1	0.3
02-04-2023- 08-04-2023	27.8	14.2	65.1	37.7	2.9	0.3
09-04-2023- 15-04-2023	35.5	16.1	75.7	24.0	3.4	0.0
16-04-2023- 22-04-2023	35.3	17.2	79.6	29.3	4.9	1.3
23-04-2023- 29-04-2023	35.0	18.2	76.9	30.9	9.4	0.0
30-05-2023- 06-05-2023	34.0	20.4	70.0	41.4	14.0	0.7
07-05-2023-	40.5	23.2	49.6	38.6	10.9	0.0

13-05-2023						
14-05-2023-20-05-2023	42.9	25.4	65.3	40.0	9.0	1.1
21-05-2023-27-05-2023	37.0	22.2	79.4	35.6	10.3	2.0
28-05-2023-03-06-2023	31.9	20.5	85.6	48.4	5.9	5.6
04-06-2023-10-06-2023	37.6	22.1	79.4	34.6	5.3	3.3
11-06-2023-17-06-2023	36.3	23.7	84.4	45.9	3.6	5.9
18-06-2023-24-06-2023	37.9	27.6	80.9	51.6	2.6	2.3
25-06-2023-01-07-2023	35.7	27.0	82.7	56.1	2.6	0.3
02-07-2023-08-07-2023	33.6	25.3	86.7	65.6	3.0	21.9
09-07-2023-15-07-2023	33.6	25.9	88.4	66.1	7.0	1.6
16-07-2023-22-07-2023	34.6	27.4	86.4	70.0	5.7	7.1
23-07-2023-29-07-2023	33.6	27.0	90.9	72.7	4.3	8.6
30-08-2023-05-08-2023	35.4	27.6	90.9	68.7	5.6	8.7
06-08-2023-12-08-2023	34.3	26.9	90.3	73.0	4.3	0.8
13-08-2023-19-08-2023	34.7	27.3	90.6	72.1	5.6	0.3
20-08-2023-26-08-2023	34.7	26.9	92.0	70.0	4.4	0.0

27-08-2023- 02-09-2023	33.6	26.5	91.7	64.3	5.2	1.6
03-09-2023- 09-09-2023	34.9	24.7	92.0	60.9	5.2	0.0
10-09-2023- 16-09-2023	34.2	26.0	91.0	70.0	4.3	0.6
17-09-2023- 23-09-2023	31.6	23.9	92.0	71.1	4.0	2.3
24-09-2023- 30-10-2023	33.8	21.2	93.0	60.2	4.4	0.3
01-10-2023- 07-10-2023	33.8	17.8	92.5	48.5	5.8	0.0
08-10-2023- 14-10-2023	32.3	18.7	91.9	53.2	4.5	0.9
15-10-2023- 21-10-2023	28.2	15.5	92.0	52.2	4.9	0.3
22-10-2023- 28-10-2023	30.5	15.7	93.6	50.9	2.2	0.6
29-10-2023- 04-11-2023	30.3	13.7	92.6	47.1	5.4	0.0
05-11-2023- 11-11-2023	30.2	13.5	93.5	45.2	4.0	0.0
12-11-2023- 18-11-2023	27.5	9.7	93.7	48.6	4.2	0.0
19-11-2023- 25-11-2023	25.7	10.3	92.9	47.6	4.9	0.0
26-11-2023- 02-12-2023	23.5	10.9	91.8	61.5	4.4	0.9
03-12-2023- 09-12-2023	23.2	7.5	94.7	50.3	5.5	0.0
10-12-2023-	21.9	4.5	93.9	51.5	3.4	0.0

16-12-2023						
17-12-2023-23-12-2023	21.5	4.3	94.2	57.3	4.0	0.0
24-12-2023-30-12-2023	17.3	7.7	94.3	71.1	2.5	0.0

Appendix-ii

ECONOMICS OF SUMMER 2022

Fixed cost of *Summer* 2022

S.no	Operations	Name	Quantity	Cost per quantity	Total (₹)
1.	Land preparation (Tractor ploughing and bunds)	10		500	5000
2.	Layout preparation	10 labourers		500 per day	5000
3.	Seed	37.5kg/ha		160 per kg	6000
4.	Sowing and basal dose of fertilizer application	10labours*1day		500 per day	5000
5.	Fertilizer	Urea SSP	12.5 kg/ha 40 kg/ha	268 per 50kg/bag 362 per 50kg/bag	67 320
6.	Intercultural operations	Hand weeding	5 labours*3day	400 per day	6000
		Spray	2 Labour	500 per day	1000
7.	Herbicide	Herbicide	1 bottle	350	350

8.	Plant protection chemicals	Pesticide	1bottle	450	450
9.	Irrigation	2 Labour*3 times		500 per day	3000
10.	Harvesting	6labours		500 per day	3000
11.	Land lease and miscellaneous for cropping season				2000
TOTAL					37,187

Treatment wise cost of *Summer* 2022

Treatment	**Dose**	**Dose cost**	**Total**
Control	0	0	0
Calcium	10mM	160rs/250g	390rs/ha
Bio-priming with rhizobium	10ml	120rs/250ml	780rs/ha
Putrescine	3mM	100rs/1kg	7rs/ha
Calcium + Bio-priming with rhizobium	10mM+10ml	160rs/250g+120rs/250ml	1170rs/ha
Calcium + Putrescine	10mM+3mM	160rs/250g+100rs/1kg	397rs/ha
Bio-priming with rhizobium + Putrescine	10ml+3mM	120rs/250ml+100rs/1kg	787rs/ha

Calcium + Bio priming with rhizobium + Putrescine	10mM+10ml+3mM	160rs/250g+120rs/250ml+ 100rs/1kg	1177rs/ha

Cost of cultivation *Summer* 2022

Treatment	Fixed cost (₹)	Variable cost (₹)	Total (₹)
Control	37,187	0	37,187
Calcium	37,187	390	37,577
Bio-priming with rhizobium	37,187	780	37,967
Putrescine	37,187	7	37,194
Calcium + Bio-priming with rhizobium	37,187	1170	38,357
Calcium + Putrescine	37,187	397	37,584
Bio-priming with rhizobium + Putrescine	37,187	787	37,974
Calcium + Bio priming with rhizobium + Putrescine	37,187	1177	38,364

ECONOMICS OF SUMMER 2023

Fixed cost of *Summer* 2023

S.no	Operations	Name	Quantity	Cost per quantity	Total (₹)
1.	Land preparation (Tractor ploughing and bunds)	10		500	5000
2.	Layout preparation	10 labourers		500 per day	5000
3.	Seed	37.5kg/ha		160 per kg	6000
4.	Sowing and basal dose of fertilizer application	10labours*1day		500 per day	5000
5.	Fertilizer	Urea SSP	12.5 kg/ha 40 kg/ha	268 per 50kg/bag 362 per 50kg/bag	67 320
6.	Intercultural operations	Hand weeding	5 labours*3day	500 per day	7500
		Spray	2 Labour	500 per day	1000
7.	Herbicide	Herbicide	1 bottle	350	350
8.	Plant protection chemicals	Pesticide	1bottle	450	450
9.	Irrigation	2 Labour*3 times		500 per day	3000
10.	Harvesting	6labours		500 per day	3000
11.	Land lease and miscellaneous for cropping season				2000
TOTAL					38,687

Treatment wise cost of *Summer* 2023

Treatment	**Dose**	**Dose cost**	**Total**
Control	0	0	0
Calcium	10mM	160rs/250g	390rs/ha
Bio-priming with rhizobium	10ml	120rs/250ml	780rs/ha
Putrescine	3mM	100rs/1kg	7rs/ha
Calcium + Bio-priming with rhizobium	10mM+10ml	160rs/250g+120rs /250ml	1170rs/ha
Calcium + Putrescine	10mM+3mM	160rs/250g+100rs /1kg	397rs/ha
Bio-priming with rhizobium + Putrescine	10ml+3mM	120rs/250ml+100 rs/1kg	787rs/ha
Calcium + Bio priming with rhizobium + Putrescine	10mM+10ml+3mM	160rs/250g+120rs /250ml+ 100rs/1kg	1177rs/ha

Cost of cultivation of *Summer* 2023

Treatment	**Fixed cost (₹)**	**Variable cost (₹)**	**Total (₹)**
Control	38687	0	38687
Calcium	38687	390	39077
Bio-priming with rhizobium	38687	780	39467
Putrescine	38687	7	38694
Calcium + Bio-priming with rhizobium	38687	1170	39857
Calcium + Putrescine	38687	397	39084
Bio-priming with rhizobium + Putrescine	38687	787	39474
Calcium + Bio priming with rhizobium + Putrescine	38687	1177	39864

ECONOMICS OF *KHARIF* 2022

Fixed cost of *Kharif* 2022

S.no	Operations	Name	Quantity	Cost per quantity	Total (₹)
1.	Land preparation (Tractor ploughing and bunds)	10		500	5000
2.	Layout preparation	10 labourers		500 per day	5000
3.	Seed	37.5kg/ha		160 per kg	6000
4.	Sowing and basal dose of fertilizer application	10labours*1day		500 per day	5000
5.	Fertilizer	Urea SSP	12.5 kg/ha 40 kg/ha	268 per 50kg/bag 362 per 50kg/bag	67 320
6.	Intercultural operations	Hand weeding	5 labours*3day	400 per day	6000
		Spray	2 Labour	500 per day	1000
7.	Herbicide	Herbicide	1 bottle	350	350
8.	Plant protection chemicals	Pesticide	1bottle	450	450
9.	Irrigation	2 Labour*2 times		500 per day	2000
10.	Harvesting	6labours		500 per day	3000
11.	Land lease and miscellaneous for cropping season				2000
TOTAL					36,187

Treatment wise cost of *Kharif* 2022

Treatment	**Dose**	**Dose cost**	**Total**
Control	0	0	0
Calcium	10mM	160rs/250g	390rs/ha
Bio-priming with rhizobium	10ml	120rs/250ml	780rs/ha
Putrescine	3mM	100rs/1kg	7rs/ha
Calcium + Bio-priming with rhizobium	10mM+10ml	160rs/250g+120rs/250ml	1170rs/ha
Calcium + Putrescine	10mM+3mM	160rs/250g+100rs/1kg	397rs/ha
Bio-priming with rhizobium + Putrescine	10ml+3mM	120rs/250ml+100rs/1kg	787rs/ha
Calcium + Bio priming with rhizobium + Putrescine	10mM+10ml+3mM	160rs/250g+120rs/250ml+ 100rs/1kg	1177rs/ha

Cost of cultivation of *Kharif* 2022

Treatment	**Fixed cost (₹)**	**Variable cost (₹)**	**Total (₹)**
Control	36,187	0	37,187
Calcium	36,187	390	37,577
Bio-priming with rhizobium	36,187	780	37,967
Putrescine	36,187	7	37,194
Calcium + Bio-priming with rhizobium	36,187	1170	38,357
Calcium + Putrescine	36,187	397	37,584
Bio-priming with rhizobium + Putrescine	36,187	787	37,974
Calcium + Bio priming with rhizobium + Putrescine	36,187	1177	38,364

ECONOMICS OF *KHARIF* 2023

Fixed cost of *Kharif* 2023

S.no	Operations	Name	Quantity	Cost per quantity	Total (₹)
1.	Land preparation (Tractor ploughing and bunds)	10		500	5000
2.	Layout preparation	9 labourers		500 per day	4500
3.	Seed	37.5kg/ha		160 per kg	6000
4.	Sowing and basal dose of fertilizer application	10labours*1day		500 per day	5000
5.	Fertilizer	Urea SSP	12.5 kg/ha 40 kg/ha	268 per 50kg/bag 362 per 50kg/bag	67 320
6.	Intercultural operations	Hand weeding	5 labours*3day	400 per day	6000
		Spray	2 Labour	500 per day	1000
7.	Herbicide	Herbicide	1 bottle	350	350
8.	Plant protection chemicals	Pesticide	1bottle	450	450
9.	Irrigation	2 Labour*2 times		500 per day	2000
10.	Harvesting	5labours		500 per day	2500
11.	Land lease and miscellaneous for cropping season				2000
TOTAL					35,187

Treatment wise cost of *Kharif* 2023

Treatment	**dose**	**Dose cost**	**Total**
Control	0	0	0
Calcium	10mM	160rs/250g	390rs/ha
Bio-priming with rhizobium	10ml	120rs/250ml	780rs/ha
Putrescine	3mM	100rs/1kg	7rs/ha
Calcium + Bio-priming with rhizobium	10mM+10ml	160rs/250g+120rs/250ml	1170rs/ha
Calcium + Putrescine	10mM+3mM	160rs/250g+100rs/1kg	397rs/ha
Bio-priming with rhizobium + Putrescine	10ml+3mM	120rs/250ml+100rs/1kg	787rs/ha
Calcium + Bio priming with rhizobium + Putrescine	10mM+10ml+3mM	160rs/250g+120rs/250ml+ 100rs/1kg	1177rs/ha

Cost of cultivation of *Kharif* 2023

Treatment	Fixed cost (₹)	Variable cost (₹)	Total (₹)
Control	35,187	0	35187
Calcium	35,187	390	35577
Bio-priming with rhizobium	35,187	780	35967
Putrescine	35,187	7	35194
Calcium + Bio-priming with rhizobium	35,187	1170	36357
Calcium + Putrescine	35,187	397	35584
Bio-priming with rhizobium + Putrescine	35,187	787	35974
Calcium + Bio priming with rhizobium + Putrescine	35,187	1177	36364