

STUDIES ON THE EFFECT OF POLYAMINES ON THE GROWTH, YIELD AND QUALITY IN BLACK GRAM

Vigna mungo (L.) Hepper

Thesis Submitted for the Award of the Degree of

DOCTOR OF PHILOSOPHY
in Agronomy

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DECLARATION

I, hereby declared that the presented work in the thesis entitled STUDIES ON THE EFFECT OF POLYAMINES ON THE GROWTH, YIELD AND QUALITY IN BLACK GRAM Vigna mungo (L.) Hepper 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 the general practice of reporting scientific observations, due acknowledgements have been made whenever work described here has been based on findings of other investigators. 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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ABSTRACT

The present research study, entitled "Studies on the Effect of Polyamines on Growth, Yield, and Quality of Black Gram *Vigna mungo* (L.) Hepper" was conducted to examine the individual and combined effects of foliar-applied polyamines, including Putrescine and Cadaverine, on selected growth, physiological, biochemical, yield, quality, and economic parameters of black gram. As an essential leguminous crop that is grown across the globe for its nutritional aspects and its ability to bind atmospheric nitrogen to the soil for enhancement, there is a need to understand innovative ways of increasing productivity and grain quality in black gram. Polyamines are known to have some plant growth-regulating properties. They are being investigated for their ability to stabilize growth and drought tolerance, so we assessed their impact under field conditions. The research analysed different growth characteristics together with biochemical markers that included plant height, branch count, leaf area, root length, nodule count, and chlorophyll content, total protein, carbohydrate content, phenolic compounds, and tryptophan concentration. During research, yield and yield characteristics were measured, including the number of pods per plant, the number of seeds in each pod, and the test weight and seed weight of both pods and entire plants. The research evaluated seed nutrient levels through nitrogen, phosphorus, and potassium analysis and conducted economic assessments of various polyamine treatment approaches to understand black gram production. Research findings demonstrated that a combined treatment of Putrescine at 1500 μ M and Cadaverine at 1000 μ M (T3) yielded better growth results than either treatment alone or control conditions. The addition of T3 treatment identified substantial growth enhancement along with increased photosynthetic pigment concentration and seed nutrient accumulation, and tryptophan content, leading to higher seed yields and improved test weight. The economic evaluation shows integrated polyamine treatments deliver superior financial returns, which make them practical solutions for farmers who want to boost black gram yield and quality in an environmentally friendly way. The research demonstrates that foliar-applied polyamines, especially the combined treatment of Putrescine and Cadaverine, function as an efficient agronomic method for enhancing black gram development and physiological outcomes, biochemical parameters, and yield characteristics and seed quality, which leads to both increased crop profitability and sustainability.

Key words: Polyamines, Putrescine, Cadaverine, SPAD index, Biochemical

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With a heart full of thankfulness,

Place: Jalandhar

Priyanka Aley

Date:

ABBREVIATIONS

Abbreviation and symbol	Full form
%	Percent
&	And
μl	Microliter
μm	Micrometer
°C	Degree Celsius
B	Blank
B:C	Benefit cost ratio
Chl.	Chlorophyll
Cm	Centimeter
DAS	Days after sowing
EC	Electrical conductivity
Cad	Cadaverine
<i>et al.,</i>	And others
FCR	Folin-Ciocalteu reagent
H ₂ O ₂	Hydrogen peroxide
HCl	Hydrochloric acid
hr.	Hour
HPLC	High-performance liquid chromatography
<i>i.e.,</i>	That is
IBA	Indole butyric acid
K	Potassium
kg/ha	Kilo gram per hectare
LAD	Leaf area duration
LAI	Leaf area index

L	Liter
Mg	Milligram
ml	Milliliter
mm	Millimeter
mm	Millimole
N	Nitrogen
Na ₂ CO ₃	Sodium carbonate
NaOH	Sodium hydroxide
Nm	Nanometer
the number	Number
P	Phosphorus
PAL	Phenylalanine ammonia lyase
PAs.	Polyamine
PGPR	Plant growth-promoting rhizobacteria
pH	Negative logarithm of hydrogen
Ppm	Parts per million
RBD	Randomized block design
RDF	Recommended dose of fertilizer
Rs.	Rupees
SA	Salicylic acid
SDS	Sodium dodecyl sulphate
SEM	Scanning electron microscope
sp.	Species
SPAD	Soil plant analysis development
T	Treatment
TEM	Transmission electron microscope
V	Volume
W	Weight
WP	Water potential
wt.	Weight
XRD	X-ray diffraction
Zn	Zinc

INTRODUCTION

Pulses cover an essential portion of world agriculture, not only because they are nutritionally valuable, but also because they are a necessary contributor to sustainable agriculture systems and can provide an economic impact. Pulse crops are part of the Fabaceae family and have been a part of agriculture for thousands of years. Pulses refer to many edible seeds such as chickpea (*Cicer arietinum*), lentil (*Lens culinaris*), pea (*Pisum sativum*), pigeon pea (*Cajanus cajan*), black gram (*Vigna mungo* L.), and many more. Pulses are a staple food source for hundreds of millions of people worldwide, especially plant protein, which is a prominent protein source in their diets. Pulses are nutritionally valuable, protein sources (20% to 25% protein), a source of complex carbohydrates, a source of dietary fibre, trace minerals (e.g., iron and magnesium), and folate vitamins. In India, pulse production has been trending upwards in a big way. India produced 260.48 lakh tons of pulses in 2022–2023 as opposed to 230.25 lakh tonnes in 2019–20. Government programs like the National Food Security Mission (NFSM) and PM-AASHA programs, which aim to boost domestic output and give farmers Minimum Support Prices (MSPs), have helped with all of this. Import and export trends also tell the same tale; whereas import was approximately 24.96 lakh tonnes during 2022 and 2023, India exported 7.62 lakh tonnes between the same two years. Out of pulse crops, black gram or urad bean is essential because it has nutritional, economic, and agricultural importance. Indigenous to India, black gram has been planted for thousands of years and is now a significant crop for food and soil conditioning. This is a major pulse crop of the family of legumes, the black gram, commonly called *Vigna mungo*, or urad bean or black lentil. This pulse crop is farmed chiefly in South and Southeast Asia. It was domesticated about 4,000 years ago in India, where it is thought to originate. The reasons why black gram is a significant crop for traditional agriculture include its nutritional value, adaptability, and associative ability for fixing nitrogen. It is a vital source of protein in vegetarian diets, especially in India, besides being nutritionally and economically very beneficial. The seeds yield a vibrant source of diet for most of the masses by contributing 18-25% protein, carbohydrates, and essential amino acids. It thrives exceedingly well in warm, semi-arid regions and is agronomically suited to a wide range of environments. Due to its low input requirements and relatively short growth cycle, black gram can be produced using a variety of systems: as a pure crop, as an intercrop, or in a crop rotation system, particularly in rice fallows. This versatility makes it an essential aspect of sustainable agricultural systems. One of the most excellent services to farming systems provided by black gram is its ability to

biologically fix atmospheric nitrogen in the soil through a symbiotic relationship with root-nodule-forming *Rhizobium*. This improves soil fertility. In 2024, global black gram production was 3.80 million tons, with nearly 70% of that production coming from India.

However, while biologically and nutritionally this crop is important, production of black gram can be hindered by environmental stressors and nutrient deficiency, which can impact yields. There are extensive areas devoted to the cultivation of black gram, even though it spans several agroclimatic zones. Black gram yields about 10.25% of India's total pulse production and occupies close to 29% of our country's area growing pulses. Further reduction in yield has been documented, implying that black gram's productivity suffers from a variety of factors.

In the *Kharif* season of 2024–2025, for example, black gram production plummeted to 1.2 million tonnes, down from 1.6 million tonnes the previous year, representing a reduction of 25%. Such an extreme reduction highlights the urgent need to rethink innovative methods for boosting productivity and enhancing resilience in black gram production. India remains both the largest producer and consumer of black gram, with India producing roughly 70% of the world's total production. In 2024, there was a total of 3.80 million tonnes of black gram produced worldwide, with India accounting for a substantial contribution of the area harvested. An uncertainty with production in India demonstrated a considerable volatility caused by various coexisting biotic and abiotic stresses. The 25% reduction in output during the 2024–2025 *Kharif* season points to the importance of creating new solutions to increase the yield of crops and strengthen resilience from agricultural impacts. Black gram production, although it is a high-yield crop, also suffers from challenges. The crop faces multiple sources of stress, including nutrient-poor soils, extreme weather conditions, and a combination of drought, salinity, pests, and pathogens. The stressors contribute to poor overall crop yield, including poor growth and crop yields, poor photosynthetic efficiency, and poor seed germination, among other issues. Research has focused on evidence-based agronomic and biochemical practices that can help the plant grow better under stress and even increase productivity. One possibility is to use plant growing regulators and/or biochemical materials that can help to improve the growth and productivity of black gram during adversity.

There is increasing interest in polyamines due to their role in plant growth, development, and stress response. Polyamines are naturally occurring organic compounds that are necessary for many physiological and biochemical processes, including stress response, flowering and fruiting, as well as cell division. Since polyamines can modulate critical metabolic pathways,

they can be instrumental in promoting crop resilience. Polyamines, such as putrescine and cadaverine, are well known in plant physiology. Polyamines can help achieve optimal metabolic processes, assist in nutrient uptake, and promote tolerance to environmental stress.

Putrescine is formed from ornithine or arginine, while cadaverine is derived from lysine. These two polyamines have essential roles in stabilizing cell membranes, promoting antioxidant defenses, and maintaining osmotic balance when a plant is under stress. Implementation of putrescine and cadaverine into black gram cultivation appears to be a viable option for improving the growth, stress tolerance, and yield of plant production. Overall, these polyamines reduced oxidative stress, improved nitrogen metabolism, and increased a plant's defence against factors of stress. However, more studies are needed to understand how putrescine and cadaverine affect black gram as well as the overall crop performance. This study aims to investigate the effects of polyamines, putrescine and cadaverine, on the growth, yield, and physiological aspects of black gram. Putrescine is formed from the amino acids ornithine or arginine, while cadaverine is formed from lysine. These polyamines stimulate plant stress tolerance through balancing osmotic pressure, enhancing antioxidant physiological defence, and stabilizing cell membranes. The use of putrescine and cadaverine provides valuable insights into their application in improving the growth and yield of black gram. This knowledge can inform efforts to improve plant growth, yield, and tolerance to stress. Their beneficial effects on nitrogen metabolism, reducing oxidative stress, and enhancing environmental stress in plants have been attributed to polyamines as biostimulators; however, there is a need for much more research, particularly on cadaverine and putrescine in black gram, which should be considered for exploring improved crop performance.

OBJECTIVES

- 1- To study the effect of Putrescine & Cadaverine on the growth, yield, and quality of *Vigna mungo*.
- 2- To study the impact of different treatments on the physiological responses of *Vigna mungo*.
- 3- To study the evaluation of different treatments on Tryptophan analysis in grain.
- 4- To study the assessment of economics in *Vigna mungo*.

REVIEW OF LITERATURE

Black gram is a popular pulse crop cultivated in India and other countries that is known for its high nutritional value, nitrogen-fixing ability, and adaptability to a vast range of agroclimatic conditions. Considerable research has been conducted in many areas of black gram production for agronomy, disease resistance, management of soil health, management of stress, and genetic improvement. This chapter provides a synthesis of previous black gram research, which will serve as a basis for future work in the discipline.

Various Research conducted on *Vigna mungo*:

The technique of seed priming has been implemented on black gram to enhance crop productivity, flowering, and germination. Verma and Singh (2014) studied hydropriming and Osmo priming using various solutions and found that potassium nitrate (KNO_3), with 2% priming, was the most effective. Their research revealed that increased early-season seed vigor, root-shoot length, and percentage of germination were all improved with seed priming. Their study demonstrated that the importance of seed treatments to crop production for a range of environmental conditions cannot be overstated.

Organic additions are another avenue that can improve black gram productivity by enhancing nutrient availability, microbial activity, and soil structure. Evidence presented by Tiwari *et al.* (2018) details the long-term impact of compost and vermicompost treatments, with regular organic inputs demonstrating greater soil moisture retention, root development, and improved seed yield. Evidence has shown that organic farming has the potential to sustain soil fertility and enhance it over time.

Symbiotic nitrogen fixation is vital for enhancing soil fertility and increasing the yield of the black gram. In their work, Dubey *et al.* (2019) investigated the influence of inoculation by the Rhizobium bacteria. The study confirmed that soaking the seeds in Rhizobium culture, compared to other treatments, led to greater grain yield, nitrogen uptake, and nodulation. Their work indicated that microbial inoculants are essential to reduce the application of chemical fertilizers and enhance biological nitrogen fixation.

Water availability is another critical factor in the yield of black gram. In a study by Sharma *et al.* (2019), different irrigation schedules were examined. Irrigating during periods of importance in plant development, such as at flowering and pod development, yielded

improvements in terms of increasing biomass, seed yield, and water-use efficiency. Their evidence also demonstrated that deficit irrigation may reduce productivity, thus highlighting the importance of efficient water management.

Weeds are a significant problem in black gram production, because they can lower yield substantially. Kumar *et al.* (2019) looked at several weed control methods; their results indicated that using a pre-emergent herbicide and weeding once 25 days after planting provided the best weed control and yields. They also demonstrated that integrated weed management is crucial to maintaining production.

Mulching also provides benefits as it enhances the soil microbial environment and weed pressure while inhibiting moisture losses. Patel *et al.* (2019) studied polyculture plastic mulch, and a study of organic mulch suggested straw mulch had the most effective influence on pod development and moisture content in soil. Furthermore, they posited that mulching is a viable method to increase black gram productivity in lower moisture conditions.

The importance of biofertilizers is in enhancing nodulation and nutrient supply to black gram. Kumar *et al.* (2020) found that treatments with *Rhizobium*, *Azotobacter*, and phosphate-solubilizing bacteria (PSB) increased plant height, root nodulation, and seed yield (especially when treated together). This study also showed some promise in reducing chemical fertilizer reliance while promoting more environmentally sustainable practices.

The time and methods of sowing black gram are essential for improving its production. Singh *et al.* (2020) observed differences in planting dates, finding that sowing in early June has greater potential for high yield than late July due to faster vegetative growth and pod development. They also found that line sowing of black gram received better light and a higher plant population than broadcasting, which aided the subsequent seed development better than the broadcasting method.

Drought stress similarly has adverse impacts on black gram growth, nodulation, and seed production. Reddy *et al.* (2020) examined the physiological response of black gram to water stress, finding reduced biomass accumulation and photosynthesis, with a substantial decrease in photosynthetic pigments (chlorophyll). They concluded that if water is not available and dry conditions are the norm, then it is essential to select drought-tolerant cultivars and conduct management practice that encourages moisture conservation.

Balanced fertilization improves the production and growth characteristics of black gram. Patil and Deshmukh (2021) conducted a study of integrated nutrient management. They found that application of organic manures combined with recommended levels of NPK improved soil fertility, seed protein quality, and nodulation in black gram relative to the treatments with no organic manures. The findings reinforced the need for studies of organic amendments to black gram as they help prevent losses to weeds; improve growth and root nodulation; and improve nutrient availability and nutrient uptake.

Intercropping black gram with suitable companion crops could increase resource utilization and increase black gram production. As shown in Mishra *et al.* (2021), evaluation of maize, pearl millet, and sesame intercropping with black gram, maize in combination with black gram produced the best economic return and LER. Results from their study pointed to increased production rates and weed suppression, as well as improved land use efficiency from intercropping.

It is essential to comprehend genetic variety to enhance crops. Pandey and Yadav (2021) found substantial genetic diversity in plant height, seed weight, and pod number after evaluating various black gram genotypes for yield and stress tolerance features. According to their research, breeding strategies can help create superior cultivars by choosing features with high heritability.

Effect of growth regulators on *Vigna mungo*:

In the last few years, plant growth regulators (PGRs) have received significant praise for their considerable effect on the growth, development, and productivity of pulse crops, including black gram. There will be a wide variety of studies on the impact of several regulators, and their effects on PGRs on black gram performance, including salicylic acid, gibberellic acid (GA₃), naphthalene acetic acid (NAA), and brassinosteroids.

In the beginning, many studies suggested that using growth regulators would help crops grow and develop more quickly. An example of this was Deshmukh and Waghmare (2012) whose research showed that using ethrel would synchronize flowering and also reduce flowering and growing times of crops and would be of great advantage for crops having short growing seasons. Verma and Singh (2014) supported Deshmukh and Waghmare's findings by suggesting that using ethrel as a foliar treatment (i.e., spray on the leaves of the crop) at 100 ppm also synchronized flowering and enhanced plant maturity, thus benefiting short duration cultivars, especially those sown late in the season.

Numerous investigations have concentrated on the impact of growth regulators on increasing physiological activity and enhancing various yield component aspects. Thakur and Rajput demonstrated that the addition of triacontanol (TRIA) positively impacted the length of time needed to accumulate dry weight, photo-synthesise (produce starch), and increase yield components such as the total number of seeds per pod for black gram. In a subsequent study, Sharma et al. also showed that gibberellic acid and naphthalene acetic acid resulted in increased flowering and sped up the pod's development; when used together, these hormones improved both grains' weight and biological yield.

In addition to this, several researchers have looked at growth retardants to enhance the structure and productivity of plants. Yadav and Meena reported that spraying cycocel (CCC) at 250 ppm on the leaves resulted in less than normal plant height but improved branch and produced a higher harvest index and seed yield per unit of land under production. Similarly, Rani and Sivasubramaniam found that foliar NAA applied at 40 ppm during flower development significantly improved pod formation while decreasing flower drop; therefore, increasing the number of pods per plant and grain yield.

There has been a growing amount of evidence supporting gibberellic acid's influence positively on growth rates and yield characteristics. Kumar et al. (2018) found that applying GA₃ to the plants as a foliar spray at a rate of 50 ppm would produce higher seed yields (approximately 15–20% greater than untreated plants) through increasing plant height, number of branches, and biomass accumulation. Similarly, Mishra et al. (2018) concluded that GA₃ applied as a foliar spray improved germination percentage and seedling vigour and accelerated the early growth of black gram seedlings. On the other hand, results from earlier studies conducted by Sarkar and Roy (2013) demonstrate that NAA could effectively reduce flower drop and enhance pod set, indicating that regulators may affect crop productivity differently based on the physiological mechanisms involved.

The use of plant growth regulators in a stressed environment has also been researched. The authors of this paper have conducted research to show that the use of Kinetin has shown to improve plant physiological parameters including relative water content and membrane stability, ultimately enhancing the plants' ability to tolerate water limitation (Drought Stress). In addition, Das and Barman (2019) have demonstrated that salicylic acid (1.0 mM) can

improve chlorophyll content, as well as the efficiency of photosynthesis and yield characteristics under moisture stress, thus further demonstrating salicylic acid's potential for use as a plant growth regulator in mitigating stress.

Recent studies have also evaluated the effects of combined use of growth regulators on crop production. Jadhav et al. (2020) observed that when GA₃ and NAA were applied together through foliar spraying at critical points during growth, superior seed yield was achieved compared to either regulator alone. Similar findings were reported by Patel et al. (2020) who also demonstrated that application of Brassinosteroids at the vegetative and flowering phases increased growth rate and pod growth; in the end, Brassinosteroid application yielded approximately 12% more seed than untreated plants.

Several physiological advantages of utilizing signalling molecules have also been researched in black gram production. According to Nayak et al. (2021), the use of jasmonic acid improved both resistance to biotic stress and certain physiological traits (such as leaf area index and relative water content) and seed protein content. Similarly, Kavitha et al. (2022) demonstrated that when IRR seeds were primed with ascorbic acid, improvements were observed with respect to germination, early seedling vigour, pod yield, and seed quality traits, when in a semi-arid environment.

Effect of putrescine on *Vigna mungo* morphology and yield

Haque and Bano (2012) applied 1.0 mM putrescine. They observed improved nitrate reductase and glutamine synthetase activity in chickpea and indicated that such effects could also be observed in black gram, enhancing nitrogen metabolism and growth.

Sharma *et al.* (2013) found that foliar application of 0.5 mM putrescine, under drought stress conditions, resulted in a significant increase in proline content (a key osmolyte, which is essential for osmotic balance and stabilizing cellular components from damage imposed by the stress).

Singh *et al.* (2014), using a topical application of 0.5 mM putrescine to black gram, noted a significant increase in plant height, branch number, and leaf area, and suggested that these results were caused by the effects of putrescine enhancing cell division and elongation processes, particularly in the vegetative phase.

Mishra and Pandey (2014) demonstrated that seeds treated with putrescine (0.5 mM)

gave rise to plants with greater seed weight, improved germination under stress, and elevated harvest index, affirming the application of polyamines for enhancing seed quality.

Seed priming with 0.5 mM putrescine was found to be an effective pre-treatment on beans by Iqbal and Rehman (2014), in which observed increases in metabolic activity and ATP synthesis correlated with improved germination responses, as well as improved root and shoot development. The seeds receiving seed priming with putrescine produced more biomass as indicated by the increased root and shoot development

Anuradha *et al.* (2015) noted that foliar spray of putrescine (0.5 mM) during the flowering and development stages of pod retarded senescence of leaf, maintained the chlorophyll content, and enhanced seed weight. The work concluded that the reproductive stage was promoted by putrescine through maintaining the photosynthetic activity.

Malik and Begum (2015) reported that foliar spray with putrescine at 0.5 mM under salt stress enhanced growth, yield, and ion balance by improving the content of K⁺ and decreasing Na⁺ accumulation in black gram seedlings.

Ramesh and Prasad (2016) investigated varying concentrations (0.25, 0.5, and 1.0 mM) of putrescine given as a foliar spray at 30 and 45 days after sowing. The treatment with 0.5 mM led to maximum chlorophyll content and root dry weight, suggesting that intermediate concentrations of putrescine are best suited for growth stimulation under semi-arid conditions.

In a separate study, Jahan and Nasreen (2016) tested foliar-applied putrescine at 0.25 and 0.5 mM. They reported improvements in black gram growth in addition to the enhanced dry matter with the former. They improved photosynthetic rate and stomatal conductance with the latter, leading to greater biomass accumulation and pod yield according to both the relative growth rates and apparent photosynthetic performance.

Furthermore, Sinha and Tripathi (2017) highlight the potential molecular basis of putrescine action on internode elongation and root development mediated by an increased expression of genes associated with auxin biosynthesis in legumes after treatment with 1.0 mM putrescine.

Kumar and Verma (2017) have documented that putrescine foliar application at a concentration of 1.0 mM during pre-flowering and flowering increased the nodulation and nitrogen fixation in black gram. Increased nodule number and weight were observed, indicating a role for polyamines to enhance symbiotic efficiency.

Devi and Rani (2018) explored seed priming with putrescine at 0.25, 0.5, and 1.0 mM for 6 hours. The 0.5 mM concentration performed optimally in germination percentage, seedling vigor index, and membrane stability index in black gram, particularly under water stress.

Patil *et al.* (2018) also showed that foliar application of 0.5 mM putrescine improved pod growth and seed filling on low fertility soils. They indicated there was enhanced assimilate partitioning to reproductive parts.

Bhattacharya *et al.* (2018) exhibited synergistic action of 0.5 mM putrescine with micronutrients (Zn and B) on growth, flowering, and yield in black gram, establishing the potential of integrated foliar nutrition practices with polyamines.

Rajkumar *et al.* (2019) reported that foliar spray with 0.25- and 0.5-mM putrescine during pre-flowering prolonged the period of flowering, delayed senescence, and increased pod numbers and seed weight.

Mitra and Ghosh (2019) used putrescine at 0.25, 0.5, and 1.0 mM under drought stress. They observed that 0.5 mM was the most effective in enhancing relative water content, dry matter accumulation, and water-use efficiency in black gram, and its potential in drought mitigation.

Venkatesh and Kumari (2020) noted a greater leaf turgidity, a decreased electrolyte leakage, and increased relative water content in black gram for their plants receiving 0.5 mM putrescine in dry conditions. The effect was more dramatic when applied at the flowering stage.

Likewise, Thakur and Joshi (2020) found that flower retention in black gram was improved (with a substantial increase in the number of flowers and a reduced flower drop) through the application of 1.0 mM putrescine as foliar application, which they attributed to increased pollen viability under unfavourable weather and when endogenous hormones were controlled.

Reddy *et al.* (2021) also identified that foliar spraying of 0.5 mM putrescine increased the activity of peacekeeper antioxidant enzymes peroxidase (POD), catalase (CAT), and superoxide dismutase (SOD), which suggested an overall improvement in the antioxidant defense system to reduce the effects of stress.

Yadav and Singh (2022) concluded that foliar spray of 0.5 mM putrescine markedly suppressed lipid peroxidation and enhanced membrane stability, with the potential to improve

cellular integrity in heat and oxidative stress conditions in pulse crops.

Effect of cadaverine on *Vigna mungo* morphology and yield

Maheshwari and Sharma (2014) reported that in water-stressed legumes, cadaverine promoted growth and delayed leaf senescence by inhibiting ABA accumulation while simultaneously promoting auxin and cytokinin production.

Ahmed and Naz (2014) found that under saline stress conditions, 0.5 mM cadaverine lowers the contents of malondialdehyde (MDA) and increases antioxidative enzyme (SOD and CAT) activity, decreases oxidative damages, but stimulates growth.

Improved early seedling germination and emergence, root development, and vigor index were seen from cadaverine seed priming under drought stress, Ali *et al.* (2015), especially at 0.5 mM cadaverine concentration for 8 hours of application. The authors suggested that cadaverine could help with osmotic adjustment and antioxidant defense priming during the early growth period.

According to Reddy and Rani (2015), 0.5 mM cadaverine increased chlorophyll fluorescence attributes and helped support the photosystem II activity and photosynthetic efficiency during salinity.

Kumar and Roy (2016) reported field studies that used foliar application of 0.5 mM cadaverine during or shortly after early bloom, with the treatment improving flow of assimilates to reproductive sinks, harvest index, and seed yield.

The ability of cadaverine to improve black gram's overall vegetative growth has been demonstrated. Cadaverine applied at 0.5 mM at the flowering stage resulted in greater plant height, the leaf area index, and dry biomass accumulation due to its properties to stimulate protein performance and development (Sharma and Singh, 2016).

In addition, Bhardwaj *et al.* (2016) showed that cadaverine (0.5 mM) had a significant impact on pod-setting efficiency and led to improved seed maturity, grain fill, and decreased hollow seeds.

Jain and Sinha (2017) indicated that exogenous application of 0.5 mM cadaverine increased membrane stability, relative water content, and chlorophyll stability index for heat-stressed black gram plants, thereby ensuring better yield with stress tolerance.

Gupta *et al.* (2017) primed seeds with cadaverine (0.5 mM) and noted that cadaverine

improved the root/shoot ratio and seedling vigor when subjected to PEG-induced water stress, suggesting it can be used as a pre-sowing treatment.

Black gram plants with 1.0 mM due to the augmentation of nitrogenase activity and root nodule development increased growth and nitrogen uptake in the field (Tripathi and Varma, 2018).

Yadav *et al.* (2018) used 0.5 mM cadaverine to increase the level of proline and hydrogen peroxide in the leaves of water-stressed black gram, providing osmotic homeostasis and helping survival and yield during moisture stress.

Cadaverine significantly enhanced the activity of nitrate reductase and glutamate dehydrogenase in legumes, which triggered nitrogen metabolism and enhanced plant growth conditions during the early phase of development (Iqbal and Zaidi, 2018).

A foliar spray of cadaverine and boron at 0.5 mM increased the height of black gram plants, leaf area index, pod number, and seed yield synergistically (Srivastava *et al.* 2019).

According to Deshmukh *et al.* (2019), foliar application of 0.5 mM cadaverine spray at pod initiation reduced blossom drop and pod abortion in black gram. It was able to increase the number of filled pods per plant and ultimately seed yield.

Patel *et al.* (2020) also concluded that cadaverine affected seed quality and composition. When treating plants with 0.25-0.5 mM cadaverine during blooming and pod formation, seeds had higher protein and starch levels.

Like their findings, Ravichandran *et al.* (2021) were able to report that cadaverine treatment enhanced the levels of chlorophyll a and b, thereby enhancing photosynthetic efficiency. Their results were based on investigating cadaverine at its optimal dose, which was 0.5 mM cadaverine for improved biomass accumulation and carbon assimilation.

Chakraborty and Paul (2021) found that black gram treated with 0.5 mM cadaverine had a higher expression of drought-responsive genes, such as DREB and RD29. This is informative about the role of cadaverine in various molecular signaling networks.

Effect of combined application of putrescine and cadaverine on *Vigna mungo* morphology, biochemical, and yield

Reddy *et al.* (2015) noted that the usage of putrescine-cadaverine combination

increased the protein and oil percentages of seeds of black gram. Concerning the reproductive periods, the combination therapy increased the nutrient uptake and utilization.

Ali *et al.* (2016) noted that foliar application of cadaverine (0.5 mM) and putrescine (0.5 mM) noticeably promoted some of the growth features of black gram (*Phaseolus mungo*), including plant height, root length, and shoot biomass. The authors speculated that the increased growth characteristics were a result of increased protein synthesis and osmotic adjustment of the plant system.

According to Kumar and Meena (2016), the combined effects of putrescine and cadaverine increased the activity of antioxidant enzymes, particularly catalase (CAT) and superoxide dismutase (SOD), under salt stress, enabling black gram plants to tolerate saline conditions more effectively.

Gupta and Tiwari (2016) reported that using putrescine and cadaverine together stimulated the black gram plants' production of polyamines, which assisted the plant in tolerating stress and delaying senescence, thus prolonging the productive growth period of the crop.

Kumar *et al.* (2016) looked at the root architecture effects of a combined polyamine treatment. The researchers discovered that the therapy made deeper root penetration with greater surface area for water and nutrient uptake in black gram plants under both stressed growth and expected growth.

Bhardwaj and Saha (2016) reported an increase in photosynthetic pigments, chlorophyll a and b, with the use of putrescine and cadaverine. The increase in chlorophyll resulted in increases in light harvesting and ultimately productivity through the states of background stress or salinity.

Patel and Patel (2017) found that the application of 0.5 mM putrescine and 0.5 mM cadaverine, as foliar treatments on black gram leaves, improved chlorophyll concentration. This treatment led to better growth evaluation and photosynthetic efficiency compared to the control plants under every day and stressed growth environments.

Bhardwaj *et al.* (2017) reported that the combined application of putrescine and cadaverine as a foliar application improved both the black gram seed yield and quality. A higher 100-seed weight and seed germination resulted from the treatment compared to all the single therapies alone.

Patel *et al.* (2017) noted that black gram plants treated with putrescine and cadaverine (each at 0.5 mM) had improved efficiency of water usage and lower transpiration loss. Such gains were significant when plants were under water stress, as yield stability was aided.

Simultaneously, Sharma and Singh (2017) found that the simultaneous application of putrescine and cadaverine improved the nitrogen fixation efficiency of black gram legumes. The growth of the plants and the seed yield were promoted by the treatment with putrescine and cadaverine, along with other variables, with increased nitrogenase activity and nodulation characterizing the therapy.

A combination of putrescine and cadaverine treatment improved the quality of black gram seeds (Reddy *et al.* 2017). The increase in seed size, germination, and seedling vigor was most profound when treatment was applied before planting.

Gupta and Ghosh (2017) showed that black gram saw a substantial increase in biomass of roots and shoots following cadaverine and putrescine treatment. Polyamines play a significant role in promoting growth, particularly under adverse environmental conditions, such as drought, where their presence is crucial.

When Sharma and Gupta (2018) combined two polyamines, putrescine and cadaverine, to treat black gram seeds, they enhanced the seeds' metabolism of carbohydrates by increasing starch levels. These factors positively impacted seed development by providing more energy for seed development.

Yadav *et al.* (2018) found that applying both putrescine and cadaverine to black gram seeds improved seed germination and seedling development in the early phases of the study. According to their findings, the polyamines helped regulate genes that are sensitive to stress throughout the early stages of development.

Kaur and Singh (2018) demonstrated that the combination of both putrescine and cadaverine significantly improved the accumulation of proteins and amino acids in black gram. This enhanced nutritional quality was related to greater grain yield and overall productivity.

Joshi and Sharma (2018) in a similar study observed that the combined use of putrescine and cadaverine during the flowering stage affected black gram's flower retention as well as pod set, and thereby complementarily increased yield.

Basu and Sharma (2018) also found that utilizing a foliar application method for putting cadaverine and putrescine on the black gram at both 0.5 mM levels improved the antioxidant potential of black gram plants. Due to the high temperature stress, the SOD and CAT enzyme antioxidants increased in plants, which mitigated oxidative damage.

Bandyopadhyay and Ray (2018) showed how cadaverine and putrescine affected total soluble sugars and proline content. The increase in total soluble sugars and proline content aided in osmotic adjustments to increase plant tolerance to water loss in a very low percentage of available water under severe drought stress conditions.

The treatment of black gram with putrescine and cadaverine also significantly decreased membrane integrity, indicated by reduced electrolyte leakage (Kaur & Kaur, 2018). This was particularly beneficial under times of osmotic stress, where the plants had increased cell integrity and significantly less wilting.

Ravichandran *et al.* (2019) discovered that by enhancing reproductive performance, they recorded an increase in pods harvested for black gram with the subsequent use of putrescine and cadaverine applications. This treatment also increased seed per pod and decreased flower and pod drop.

Deshmukh *et al.* (2019) found that plants treated with a combination of putrescine and cadaverine showed elevated concentrations of chlorophyll a and b, which enhanced photosynthetic efficiency, thus improving total biomass accumulation, as well as plant development.

Finally, Tripathi and Verma (2019) reported that application of 0.5 mM putrescine and cadaverine decreased oxidative stress effects in black gram (and as indicated by decreased hydrogen peroxide and malondialdehyde (MDA) levels). This study concluded that interactions among polyamines can be an effective means of "containing" the plant's antioxidant defense mechanism.

Yadav *et al.* (2019) showed that the interaction of putrescine and cadaverine increased total dry matter accumulation and leaf area index (LAI) of black gram plants. The interaction

of polyamines was most pronounced when applied during the early juvenile vegetative growth stage.

Singh *et al.* (2019) found that black gram plants that received the combination of polyamines handled pod development better and significantly higher shoot biomass, especially when applied from flowering to podding, but also improved total plant growth due to increased nitrogen uptake.

Cadaverine and putrescine-treated black gram plants showed a greater reduction in leaf senescence according to work done by Chandra and Chauhan (2019). Not only did this treatment delay leaf senescence and enhance leaf photosynthesis ability, but it also increased overall yield in both drought and control conditions.

Kumari *et al.* (2019) assessed the effect of amine treatment on the quality of black gram seeds. The results showed that the treatment created a significant increase in seed vigor, germination percentage, and seedling growth with putrescine and cadaverine.

Sengupta *et al.* (2019) identified that polyamine-treated black gram plants had better chlorophyll content and photosynthetic rate, even under saline conditions. Enhanced photosynthetic rate further enhanced plant growth and reduced yield, allowing the plants to cope with later external stresses.

The use of polyamines in enhancing the physiological performance of black gram under heat stress was investigated by Soni *et al.* (2019), and it was concluded that cadaverine and putrescine preserved cell membrane competence, as well as restricted heat-induced oxidative damage.

Singh and Joshi (2019) identified that black gram treated with putrescine at 0.5 mM and cadaverine at 0.5 mM indicated an increase in photosynthetic pigments, i.e., chlorophyll a and b. This later resulted in enhanced light absorption and growth under mild water stress.

As per Yadav and Yadav (2019), co-treating black gram with putrescine and cadaverine led to a significant increase in yield and nutritional value due to enhanced activity of nitrogen-fixing bacteria, only available to the plant by improved nutrition.

Chauhan *et al.* (2019) stated that the black gram's stress tolerance to metal toxicities was enhanced with the addition of putrescine and cadaverine at 0.5 mM and 1.0 mM concentrations,

respectively. The polyamines limited the growth-inhibiting effects of heavy metals cadmium and lead.

Rathi *et al.* (2020) examined the effect of polyamine treatment on black gram yield. They found that foliar spraying putrescine and cadaverine improved the overall quality of the seed and protein content, making black gram more nutrient-rich and marketable.

Bose and Ghosh (2020) documented that black gram plants treated with putrescine and cadaverine showed increased tolerance to drought stress as a result of enhanced root hydraulic conductivity for enhanced water uptake. Enhanced water uptake later enhanced biomass and productivity.

Thakur *et al.* (2020) noted that putrescine and cadaverine-treated black gram result in enhanced root growth and better nutrient utilization, which means better overall plant growth rate. Furthermore, the application of polyamines enabled the plant to maintain physiological processes under stress.

Bandyopadhyay and Ray (2020) illustrated that the application of polyamines to black gram enhanced pollen viability and stigma receptivity, which was reflected in increased reproduction success. Seed set and seed weight were increased in polyamine-treated crops relative to control crops.

Singh and Reddy (2020) documented pod yield and nitrogen efficiency improvement of black gram when treated with 1.0 mM putrescine and cadaverine simultaneously. Polyamines enhanced nitrogen uptake, enhancing growth and yield through enhancement in nutrient uptake by modifying nitrogen assimilation.

Patel *et al.* (2020) explained that black gram plants treated with cadaverine and putrescine had increased antioxidant enzyme activity (catalase (CAT) and superoxide dismutase (SOD)), produced high tolerance to oxidative stress from high temperatures.

Sahu *et al.* (2020) researched the role of polyamine interaction in the cell structure of plant cells. They concluded that black gram plants treated with putrescine and cadaverine produced plants with thicker cell walls and increased cell division, which improved development and tolerance to stress.

According to Patel and Verma (2020), the use of 0.5 mM putrescine and 0.5 mM cadaverine enhanced the physiological performance of black gram under salt and drought

abiotic stress by enhancing cell stability and nutrient mobilization at key growth periods. The combination of putrescine and cadaverine improved black gram's physiological response to drought stress, resulting in increased photosynthesis and water retention potential under water-deficient conditions, which in turn improved growth and yield (Gupta *et al.* 2020).

Singh and Verma (2020) demonstrated that the proline and soluble sugar synthesis associated with putrescine-cadaverine application helped black gram with drought stress adjust osmotic balance, and positively increased plant yield and survival abilities.

The reciprocal interactions of putrescine and cadaverine under nutrient stress improved root and shoot ratios (Sinha *et al.* 2020). Combination treatment enhanced nutrient and water uptake by improving root and shoot ratios.

According to Jain and Sinha (2020), the combined application of putrescine and cadaverine at 0.5 mM each improved reproductive parameters like pod setting and seed filling as well as physiological characteristics like chlorophyll content and photosynthesis, ultimately leading to higher yields.

Jha and Tiwari (2020) found that cadaverine and putrescine as foliar spray contributed to black gram's nitrogen use efficiency. This was demonstrated by higher yield and increased growth rate when nutritional stress conditions were reported.

Patel and Desai (2020) reported the results of increasing black gram podding efficiency where black gram plants are stressed, joined treatment between salt and drought stress with putrescine and cadaverine. This treatment enabled a higher number of seeds, pods, and grains per plant, demonstrating the ability to manage water loss through polyamines.

Nayak *et al.* (2020) shared that combining putrescine and cadaverine had synergetic potential and enhanced protein synthesis and cell development in black gram by supporting cellular metabolism and stability of the cell membranes, which limited the impacts of salt stress.

Together, putrescine and cadaverine improved black gram resistance to abiotic stress such as drought or salt according to Pandey and Rai (2020). Compared to control plants, treated plants had reduced oxidative damage and improved ability to combat oxidative stress from abiotic pressures.

Co-application of 0.5 mM putrescine with cadaverine improved black gram reproductive efficiency by reducing flower drop and increasing pod set according to Singh *et*

al. (2020). This improved reproductive efficiency also increased pods per plant under both optimal and stressful conditions.

By controlling the hormonal balance in black gram, Ravichandran *et al.* (2021) demonstrated that the combined application of putrescine and cadaverine under drought stress conditions significantly improved plant survival, root, and shoot growth.

Research conducted by Chakraborty and Basu (2021) suggests that treating black gram with 0.5 mM putrescine and 0.5 mM cadaverine improved its uptake of nitrogen and phosphorus and, in turn, stimulated vegetative and reproductive growth in nutrient-poor soils.

Srivastava *et al.* (2021) assessed black gram plants. They found that the application of putrescine and cadaverine at 0.5 mM each to leaves reduced the incidence of disease and pests, which can be attributed to an increase in the synthesis of secondary metabolites that function as a protective barrier.

. Chauhan and Malik (2021) showed how the combination of putrescine and cadaverine influenced the growth hormone ratios of the black gram to increase the quantities of auxins and cytokinins. The greater height and yield of black gram were due to the impact of increased root and shoot development from growth hormone ratios.

The use of putrescine and cadaverine via ionic homeostasis was shown to work synergistically with black gram. Homeostasis of potassium and sodium was optimized by this application, which plots lessening salt resistance.

Singh *et al.* (2021) reported that black gram seeds exhibited significantly higher protein levels compared to the control treatments when supplemented with 0.5 mM of putrescine and cadaverine. This nutritional increase was attributed to superior nitrogen fixation, facilitating an improved uptake of nutrients in the seeds.

Nayak *et al.* (2021) noted that foliar-applied cadaverine and putrescine together increased protein content and growth in black gram. These polyamines promoted both nitrogen fixation and leaf chlorophyll, which led to improved photosynthesis efficiency and increased biomass production.

Sharma and Mehta (2021) mentioned that black gram exposed to putrescine and cadaverine had increased proline and soluble sugar, which contributed to less water loss due to

drought stress. The polyamines reduced dehydration through osmotic equilibrium, which improved plant survival.

Ravichandran *et al.* (2021) noted that when putrescine and cadaverine were applied in conjunction, there was a significant increase in black gram root biomass under drought conditions. The combined treatment increased root length and surface area, which ultimately helped the plants uptake more water and nutrients from the soil.

Kaur and Kaur (2021) reported that polyamine-treated black gram plants-maintained membrane stability, with reduced electrolyte leakage into the surrounding solution. This was more anticipated with black gram in higher saline conditions, with treated black gram showing enhanced tolerance and enhanced ion homeostasis.

Reddy *et al.* (2021) found that soluble protein content in black gram improved when putrescine and cadaverine were used in combination. Contributing factors were increased metabolic activity and improved nitrogen fixation, which enhanced both vegetative and reproductive growth.

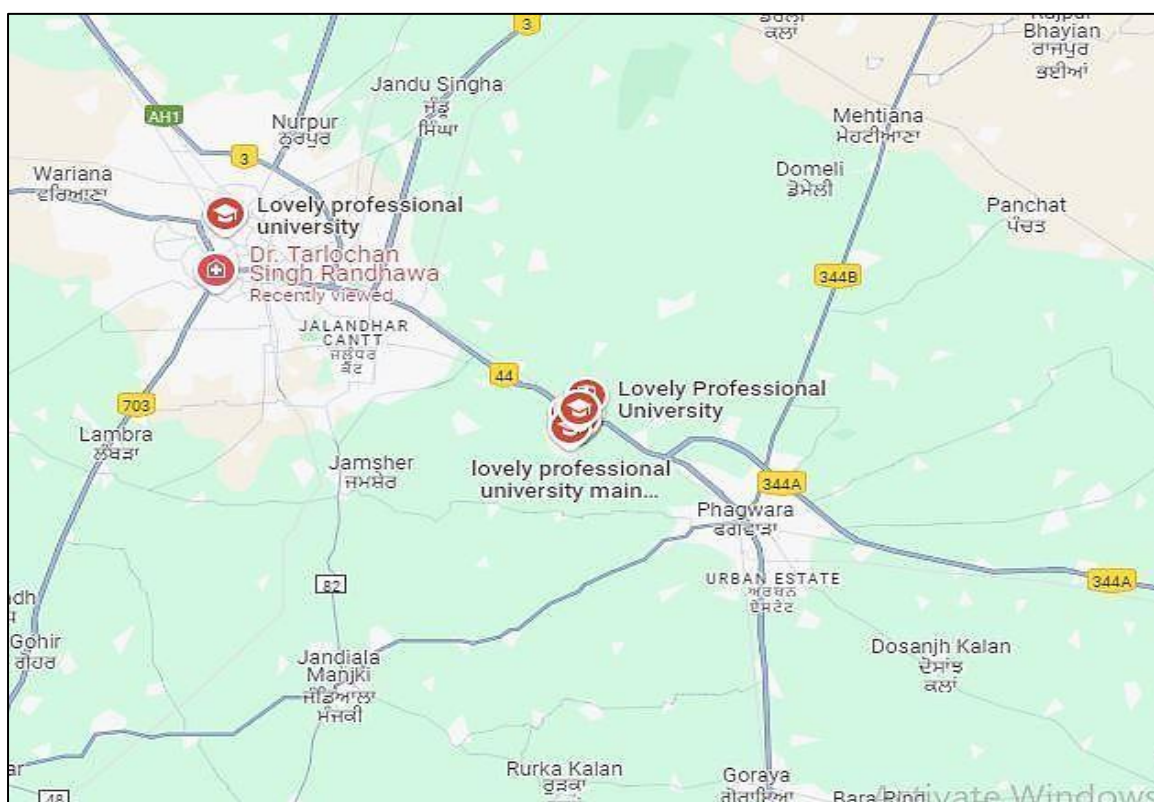
Ghosh *et al.* (2021) reported that putrescine and cadaverine enhanced black gram biotic stress tolerance (including a fungal disease), with polyamines improving plant performance by inducing activation of the plant defense system, which decreased the severity of the disease.

MATERIAL AND METHODS

3.1. LOCATION OF TRIAL SITE

The present research was conducted at the agriculture research farm of Lovely Professional University, Punjab, during the periods of April 2022-June 2022 and July 2022-September 2022 in summer and April 2023-June 2023 and July 2023-September 2023 *Kharif* season to evaluate the “**Studies on the effect of polyamines on growth, yield and quality of black gram *Vigna mungo* (L.) Hepper**”. The farm is situated at a latitude of 31°22'31.81" North and a longitude of 75°23'03.02" East, with an average altitude of 252 meters above the mean sea level. The distance between the Punjab and the Capital of India (Delhi) is about 350km. Punjab is situated in the sub-tropical region of the central plain of the state's agro-climatic zone. This chapter describes in detail the materials and methods employed during the current study.

Fig.3.1.1 Location of the field trial



3.2. WEATHER AND CLIMATIC CONDITIONS

The experimental site is located in an area in the subtropics. It is cool and warm during winter, and very little rainfall occurs during July, August, and September. Limited rainfall in the area is limited to the southwest monsoon. The temperature always remains above freezing in winter,

it was recorded that December and January are the coldest months of the year. The maximum temperature recorded in the summer months of April, May, and June was 47 °C. The monsoon rainfall typically begins in the last week of June until the end of September, but this is dependent on the southwest monsoon winds arriving on time. Most of the rainfall occurs, with the maximum amount of precipitation occurring in July. The maximum and minimum temperature, relative humidity, wind speed, and rainfall were all measured. The summer crop period spans from 20 March to 10 June and from 1 July to 20 September in both 2022 and 2023. The detailed meteorological data of the years 2022 and 2023 are recorded in Appendix 1 and Fig. 3.2.1.a, 3.2.1.b, 3.2.3.c and 3.2.4.d

Fig.3.2.1. Meteorological data of summer (2022)

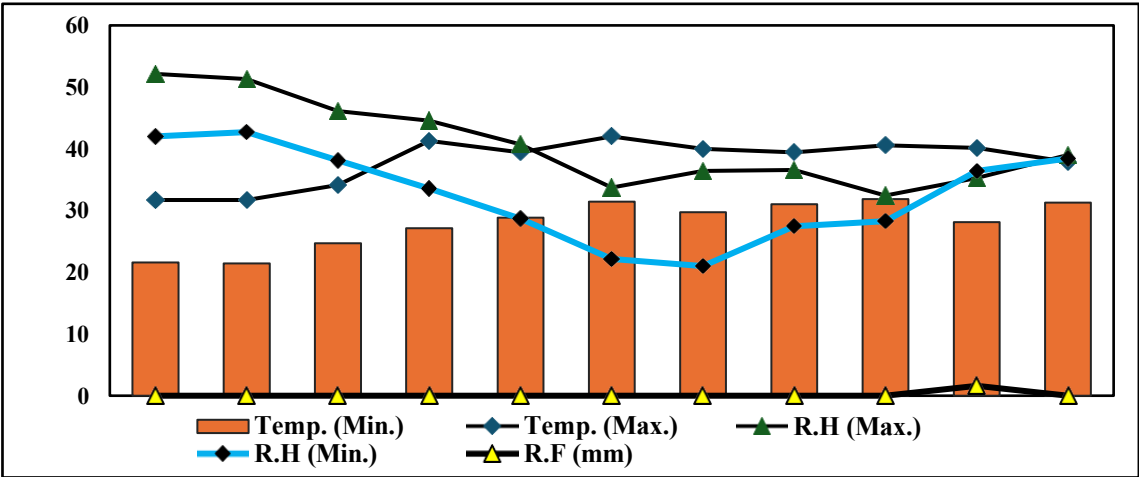


Fig.3.2.1. b Meteorological data of Kharif (2022)

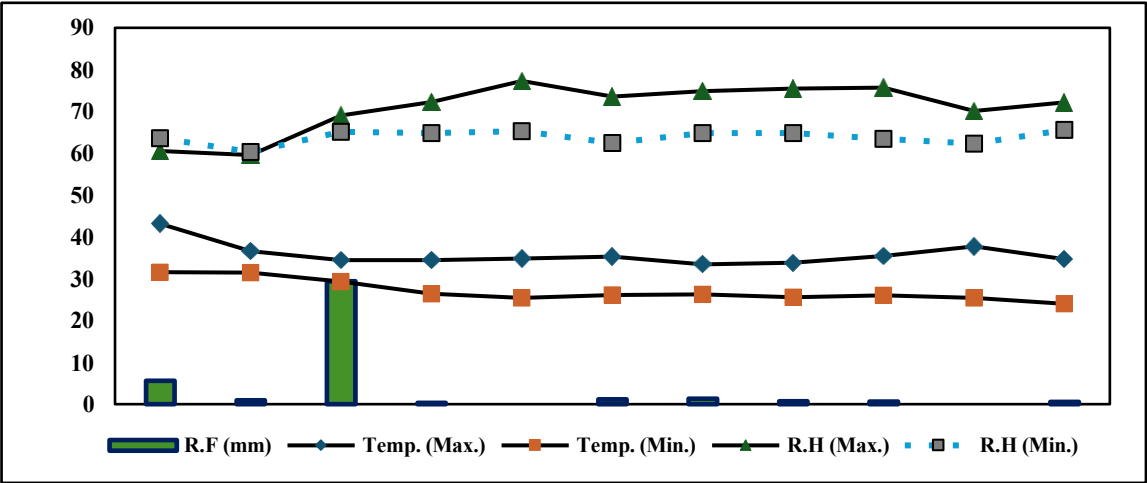


Fig.3.2.1.c Meteorological data of Summer (2023)

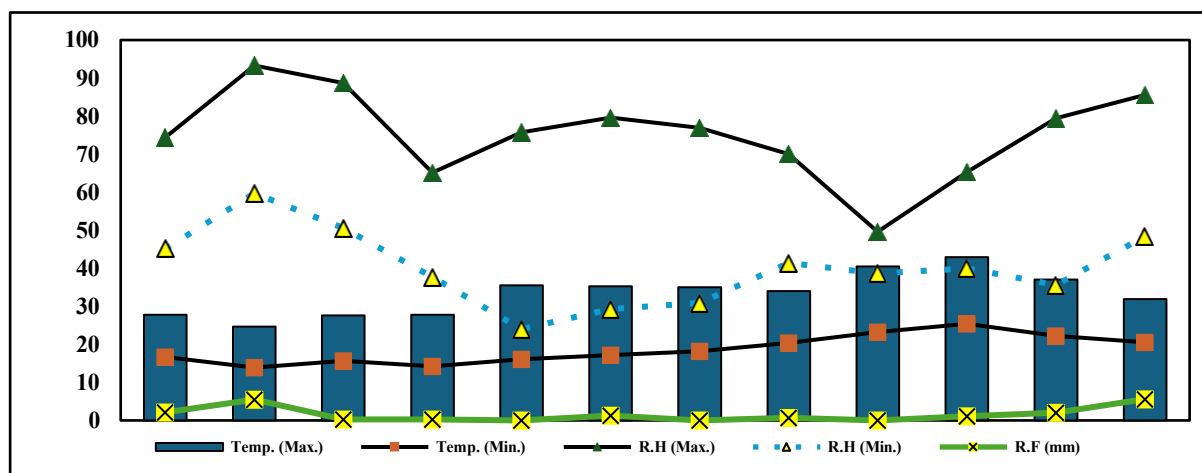
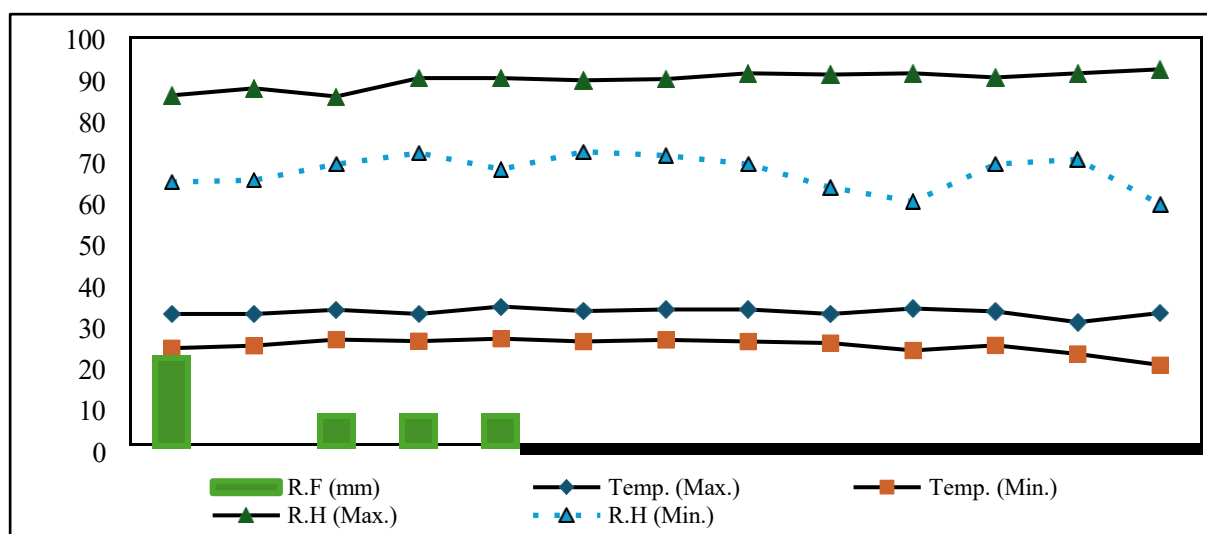


Fig.3.2.1. Meteorological data of Kharif (2023)



3.3. SOIL PARAMETERS

The study examines soil parameters, including total organic carbon, pH, electrical conductivity, and NPK levels. Using soil obtained before crop establishment and harvest, these characteristics were evaluated for the treatment plot of each replication. The estimation of these parameters is the subject of the discussion that follows.

3.3.1. Soil pH

Before planting, the soil pH was measured with the help of 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 had 25 milliliters of distilled water 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 EC of the soil was determined by using an EC meter. This required preparing a soil water suspension with a ratio of 1:5, and for 5 minutes the mixture was shaken vigorously in a shaker, then allowed to settle at room temperature. Next, the conductivity anode is immersed in 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 present in soil to ammonium sulphate. To conduct this analysis, 5g of soil was mixed with KMnO₄ (25mL) and NaOH (25mL). 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).

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

Whereas, 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 HPO₄²⁺ and HPO₄⁻ 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 NaHCO₃. 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.

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

3.3.5. Available Potassium

To measure the potassium content in soil, 5 grams of soil were extracted from the sample and mixed with 25 milliliters 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).

$$\mathbf{K\ (Kg\ ha^{-1}) = Reading\ x\ 2.24}$$

3.3.6. Organic Carbon

1. This procedure consists of dissolving 1 gram of soil in a solution consisting of 10 mL of potassium dichromate and 20 mL of sulfuric acid (H_2SO_4) within a 250-mL conical flask.
2. Subsequently, the mixture is cooled and added to 200 mL of distilled water, 10 mL of orthophosphoric acid, and four drops of diphenylamine.
3. 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).

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

Table 3.3.1 Soil analysis

S. No.	Particulars	Method used	Summer season		Kharif season	
			2022	2023	2022	2023
1.	Soil pH	Glass electrode	6.57	6.60	6.57	6.58
2.	Soil EC (dS m ⁻¹)	Electrical Conductivity	0.784	0.788	0.794	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 metho	145.67	146.5	147.53	147.65

3.4. EXPERIMENTAL DETAILS

A Randomized Block Design with eight treatments and three replications was used to set up the experiment during two consecutive seasons, *i.e.*, the *summer* and *Kharif* seasons in 2022 and 2023. To ensure that each treatment occurred once in each replication, the number of treatments was distributed at random across 24 plots. The treatments were designated T₀, T₁, T₂, T₃, T₄, T₅, T₆, and T₇, respectively, and the replications were designated R₁, R₂, and R₃. Below are the specifics displaying these three replications and eight treatments.

3.4.1.a TREATMENT DETAILS

T ₀	CONTROL
T ₁	Putrescine (1500μM)
T ₂	cadaverine (1000μM)
T ₃	Putrescine (1500μM) + Cadaverine (1000μM)
T ₄	Putrescine (3000μM) + Cadaverine (500μM)
T ₅	Putrescine (750μM) + Cadaverine (2000μM)
T ₆	Putrescine (750μM) + Cadaverine (500μM)
T ₇	Putrescine (3000μM) + Cadaverine (2000μM)

Notes:

T represents treatments; there are eight treatments ranging from (T₀ to T₇), T₀ = Control (only recommended dose of fertilizers is provided),

T₁= Putrescine (1500μM), T₂= Cadaverine (1000μM),

T₃= combined dose of putrescine (1500μM) + cadaverine (1000μM), T₄= Putrescine (3000μM) + cadaverine (500μM),

T₅= putrescine (750μM) + cadaverine (2000μM), T₆= putrescine (750μM) + cadaverine (500μM), T₇= putrescine (3000μM) + cadaverine (2000μM).

3.4.1.b Flow Sheet: Preparation and Foliar Application of Polyamines

Weigh the required quantity of Putrescine and/or Cadaverine (analytical grade)



Dissolve separately in a small volume of distilled water



Prepare stock solution



Dilute to the required concentration (μM) as per treatment



Mix thoroughly to ensure uniform concentration



Fill the hand-operated knapsack sprayer



Foliar application at specified growth stage (25 DAS and 50 DAS)



Uniform spraying during morning hours to avoid evaporation losses

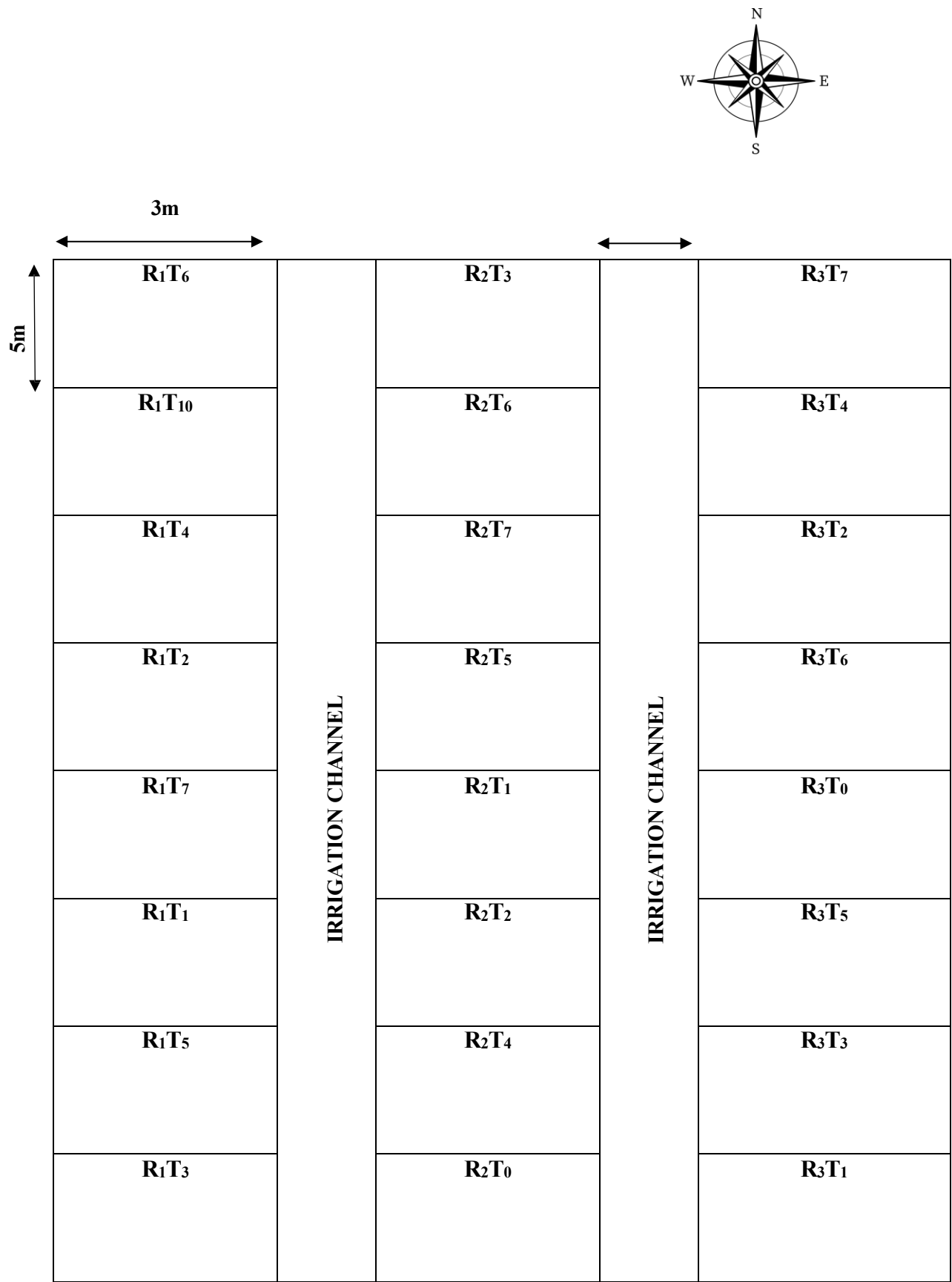


Ensure complete leaf surface coverage

3.4.1.b Table: Treatment details of polyamine concentrations and combinations used in the experiment

Treatment	Putrescine (μM)	Cadaverine (μM)	Type of Treatment
T ₀	–	–	Control (No spray)
T ₁	1500	–	Putrescine alone
T ₂	–	1000	Cadaverine alone
T ₃	1500	1000	Combination
T ₄	3000	500	Combination
T ₅	750	2000	Combination
T ₆	750	500	Combination
T ₇	3000	2000	Combination

3.4.2. Lay out 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

To get a consistent and beneficial tilth, initially the field was prepared with a disc plough, then harrowing and planking. Once the field was prepared, the experimental design was put out in the field according to the layout.

3.5.2. FERTILIZER APPLICATION

At the time of sowing, apply 25 kg N (55 kg urea), 50 kg P_2O_5 (300 kg single superphosphate), and 25 kg K_2O (40 kg muriate of potash) per hectare.

Table 3.5 Calendar showing the dates of the operation performed

Operations performed		Date on which operations were performed			
		Summer 2022	Summer 2023	<i>Kharif</i> 2022	<i>Kharif</i> 2023
1.	Field preparation				
	a. Plowing via disc plough	25 March	27 March	2 July	5 July
	b. Secondary tillage and planking via rotavator	26 March	28 March	3 July	6 July
	c. Seedbed preparation and sowing	27 March	29 March	4 July	7 July
	d. Fertilizer application	27 March	28 March	4 July	7 July
	e. Pre-emergence herbicide application	28 March	29 March	5 July	8 July
	f. Thinning	12 April	14 April	17 July	20 July
	g. Pesticide application	7 May	9 May	12 August	10 August
2.	Irrigation				
	a. First irrigation	27 March	29 March	2 July	5 July
	b. Second irrigation	19 April	21 April	25 July	27 July
	c. Third irrigation	5 May	6 May	14 August	12 August
	d. Fourth irrigation	19 May	17 May	27 August	29 August
3.	Weeding				
	a. First wedding	17 April	20 April	25 July	27 July
	b. Second wedding	1 May	5 May	8 August	10 August
4.	Treatment application				
	a. First application	12 April	14 April	18 July	21 July
	b. Second application	12 May	14 May	17 August	18 August
5.	Observation taken				
	First	27 April	29 April	2 August	4 August
	Second	17 May	19 May	22 August	23 August
5.	Harvesting	3 June	5 June	13 September	14 September
6.	Threshing and winnowing	8 June	7 June	17 September	20 September

3.5.3. VARIETAL DESCRIPTION OF MASH 1008 [Black gram]

Growth habit of black gram variety MASH 1008

- 1- Mash 1008 is a variety of black gram, *Vigna mungo* (L.) Hepper, which was released in 2011 by PAU, Ludhiana, for cultivation in Punjab and neighboring regions.
- 2- This variety has an erect and semi-determinate growth habit with medium plant height, which ranges between 40–50 cm.
- 3- It matures around 75 to 80 days, making it suitable for short-duration cropping systems, particularly in the kharif and summer seasons.
- 4- Its flowers in 35–40 days after sowing and has a yield potential of around 10–12 qt ha⁻¹ under optimal management conditions.

It has dark green, broad leaves and hairy stems, while the pods are medium-sized and contain black, medium-bold seeds.

Disease resistance/tolerance capacity

- 1- Mash 1008 shows moderate resistance to yellow mosaic virus and often shows disease tolerance for leaf spot and powdery mildew.
- 2- The variety also exhibits good lodging capacity and moderate drought tolerance, making it suitable for a range of field conditions.

Recommended agronomic practices

- 1- Mash 1008 contains around 24–26% protein and has excellent cooking qualities, with a short cooking time and texture.
- 2- Seed rate of 15–20 kg ha⁻¹, spacing of 30 cm X 10 cm between rows and plants, while application of 15–20 kg nitrogen and 40–50 kg phosphorus ha⁻¹.
- 3- Light irrigation at flowering and pod filling enhances productivity.

3.5.4. SEED RATE AND SOWING

20 kg of seed per acre for the *summer* season and 6-8 kg per acre for the *Kharif* 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. MASH 1008 was the chosen variety for this research.

3.5.5. IRRIGATION

From the date of sowing to the harvesting, crops require around three to four irrigations. The first irrigation was done on the day of sowing, just after the seeds were sown. The second irrigation was done when the crop was around 25days, the third irrigation was done at 40DAS.

And the last irrigation was done when the crop was at 60 days for high productivity.

3.5.6. THINNING AND INTERCULTURAL OPERATION

After 15 days of sowing, thinning was done to eliminate excess growth and preserve the target population. To prevent plant weed competition, the first weeding was completed 20 days after planting, and the second weeding was completed by hand by khurpi 35 days after sowing.

3.5.7. INSECT PEST MANAGEMENT

Aphids and caterpillars plagued the crop at the time of flowering. Aphids and caterpillars were managed by spraying 2 milliliters of chlorpyrifos per liter of water.

3.5.8. HARVESTING AND THRESHING

With the help of a quadrant, one square meter was harvested using a sickle. When the pod was 80% ripened, the crop was harvested. For around three to four days, the crops were left out in the field so that the pods would dry completely. The crops were spread out on the floor, and a stick was used to beat the plant to thresh it. Following threshing, the plants were washed, weighed, and measurements were taken.

3.6. GROWTH ATTRIBUTES

3.6.1. Plant height

Plant height was recorded at 25 and 50 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

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

3.6.3. Leaf number

The number of leaves was recorded after a few days of sowing; whole leaves of the plants were counted separately in each treatment, and the means were calculated.

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 unitless plant parameter that indicates an occupied canopy. It is obtained by dividing the plant leaf area by the plant land (Watson, 1947).

$$\text{LAI} = \text{Total leaf area} / \text{Total ground area}$$

3.6.6. Leaf Area Duration (LAD)

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

$$\text{LAD} = (\text{L}_1 + \text{L}_2 / 2) \times t_2 - t_1$$

Whereas,

LAI₁ is the leaf area index at the time interval T₁, LAI₂ is the leaf area index at time interval T₂
T₁ and T₂ 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 plants bear flowers, 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 present in the root of the plant at 25 and 50 DAS.

3.6.11 Chlorophyll index (SPAD meter)

Leaf chlorophyll was measured with an SPAD meter. The selected and tagged plants were used for chlorophyll index measurement. A chlorophyll index was considered a reading from the same leaf in different locations. At least 5 readings were taken, and the average was considered

one reading. Likewise, readings were taken from different tested leaves of 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

- 1- Measure 20 milliliters of 80% acetone to extract chlorophyll from 100 milligrams of leaf material.
- 2- The sample was centrifuged for 10 minutes at 5000 rpm, then the supernatant was transferred to the volumetric flask.
- 3- The residue was subjected to repeated extractions until it lost its colour. A total of one hundred milliliters of 80% acetone was added to the flask.
- 4- Spectrophotometer readings were taken at 645 and 663 nm with an 80% acetone blank to determine the extract's absorbance.
- 5- The chlorophyll content was quantified using the methodology provided below.

$$\text{Total chlorophyll (mg/g Fresh Weight)} = 20.2(A_{645}) + 8.02(A_{663}) * V1000 * W$$

V = Final volume of the extract.

W = Fresh weight of the leaves. An absorbance at the designated wavelength. The value is denoted as mg/g of fresh weight.

3.6.13 Total soluble protein (Lowry, 1951):

Principle:

Folin-Ciocalteu reagent (FCR) reacts with protein 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 and 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 ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$).
- 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 the Folin-Ciocalteu 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, 200 μg 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

- i- Grind 0.5 g of the plant material in a mortar and pestle with an appropriate solvent solution (buffer or water).
- ii- 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 5 mL of solution C, mix carefully, and permit incubation at ambient temperature for 10 minutes.
- 4- Incorporate 0.5 mL of FCR, mix thoroughly without delay, and allow it to incubate at room temperature in a dark setting for 30 minutes.
- 5- Assess the absorbance at 660 nm, using 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} , $\text{mg}/100\text{g}$ 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 utilizing trichloroacetic acid (TCA). Upon separation, dilute it in 2N NaOH and proceed with the protein quantification procedure.

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

Total carbohydrates were measured with the phenol-sulfuric acid method

Requirements:

- 1- 2.5N HCl (25mL Conc. HCl +91mL of distilled water)
- 2- 5% phenol solution (5 mL in 95 mL of distilled water) stored in an amber-colored bottle
- 3- 96% H₂SO₄ (96 m in 4 mL of distilled water)
- 4- Na₂CO₃

Procedure:

- 1- Crush 0.1g of the plant sample with a little HCl.
- 2- Add 5 mL of 2.5N HCl and place in a boiling water bath for 3 hours, and bring to room temperature.
- 3- Add Na₂CO₃ pinch by pinch (up to bubble formation stops) and centrifuge at 5000 rpm 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 H₂SO₄, place at room temperature for 30 minutes, and record the absorbance at 490nm.

3.6.15 Total Flavanol content

The flavanol content was determined using the methodology of Akkol et al. in 2008.

Principle: The basic principle of the Aluminum chloride colorimetric method is that Aluminum chloride forms stable complexes with the C-4 keto group, and either the C-3 or C- 5 hydroxyl group of flavonoids, which are resistant to acid.

Reagents: Methanol 80%, Sodium Acetate, Aluminum Chloride

Procedure

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

3.6.16 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:

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

3.6.17. Tryptophan content

Tryptophan content in black gram seeds is estimated using HPLC after alkaline hydrolysis. About 100 mg of defatted seed powder is hydrolyzed with 4 mL of 5 N NaOH at 110°C for 18–24 hours, then neutralized with HCl. HPLC is performed using a C18 column with a mobile phase of water and acetonitrile (both with 0.1% formic acid), using gradient elution at 1.0 mL/min. Detection is done at 280 nm. A tryptophan standard is used for calibration and quantification.

3.6.18. Test weight

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

3.6.19 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 to better understand the total number of seeds per pod.

3.6.20 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.

3.6.21 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.

3.6.22 Biological yield (kg ha⁻¹)

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

3.6.23 Seed yield (kg ha⁻¹)

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

3.6.24 Stover yield (kg ha⁻¹)

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

3.6.25 Harvest index

The harvest index is determined by calculating the economic yield (kg ha⁻¹) by the biological yield (kg ha⁻¹) and multiplying the result by 100.

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

3.6.26 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

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

STATISTICAL ANALYSIS

All data related to the study of the crop were collected and statistically analyzed by using OPSTAT. OPSTAT was developed and is maintained by Prof. O.P. Sheoran, from the Department of Mathematics and Statistics, CCS HAU, Hisar

PICTURE GALLERY

PLATE 1: Preparation of the research field for the sowing of black gram



PLATE 2: Demarcation of research field for the sowing of black gram



PLATE 3: First irrigation of the research field after sowing black gram



PLATE 4: Seed germination and seedlings emergence over the research field in black gram



PLATE 5: Emergence of a 10-day-old seedling over the research field in black gram



PLATE 6: Foliar application of putrescine and cadaverin as per the research plan in black gram



PLATE 7: Hand weeding over the research plot in black gram



PLATE 8: Morphological observation from the research plot in black gram

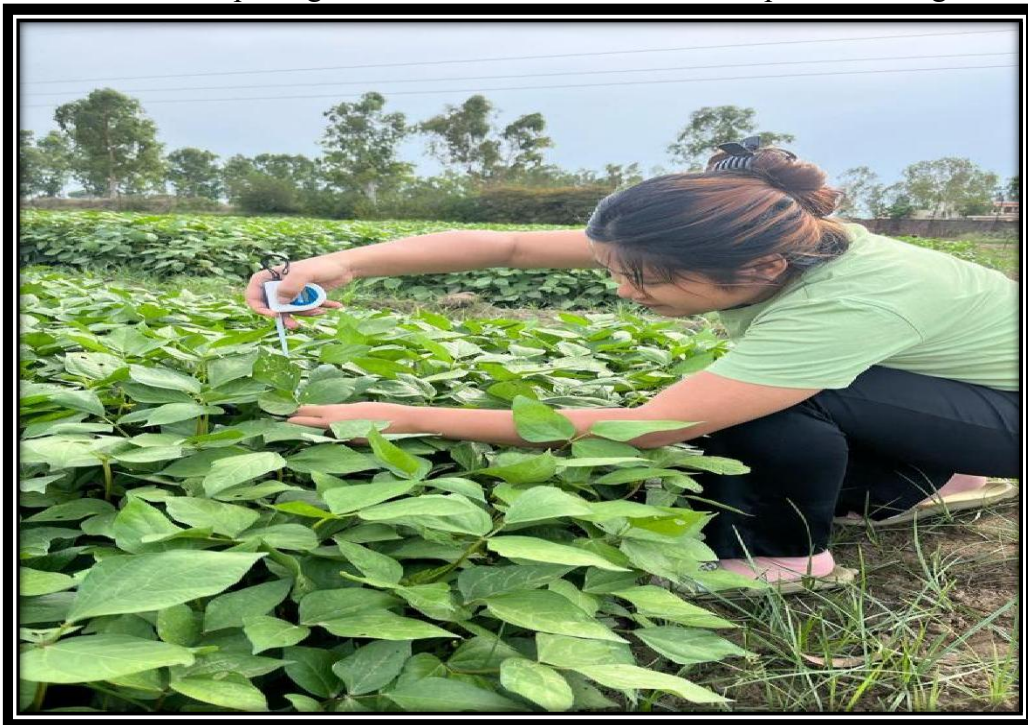


PLATE 9: Flowering stage over the research plot in black gram



PLATE 10: Measurement of leaf area using the leaf area meter of black gram

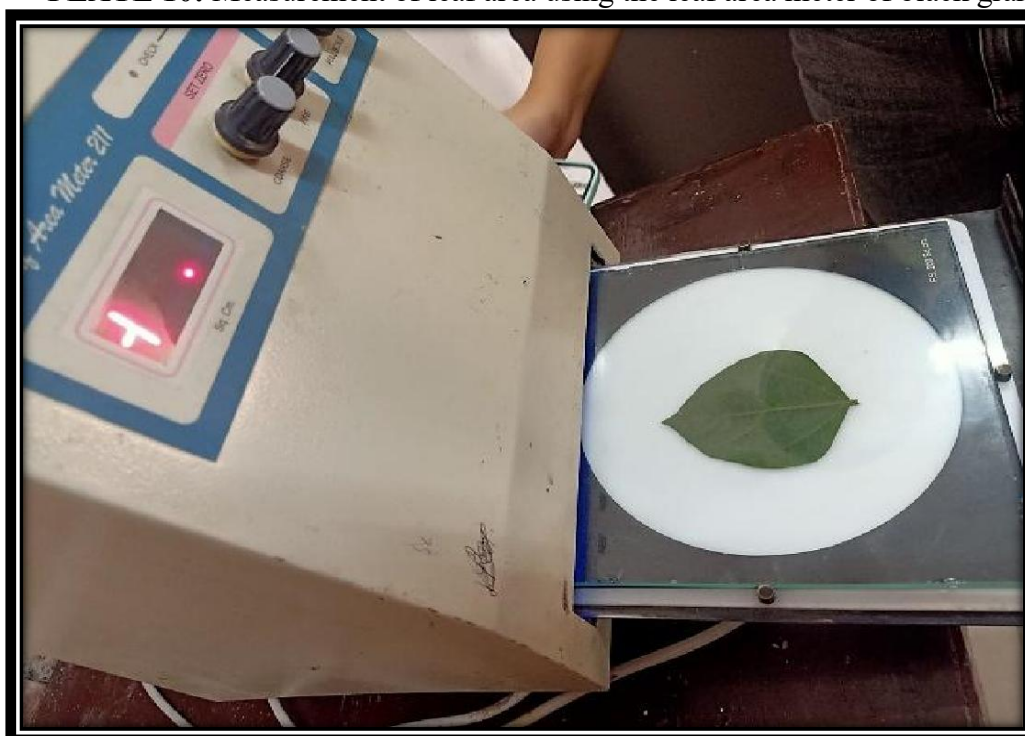


PLATE 11: Harvesting of crop using a quadrat over the research plot in black gram



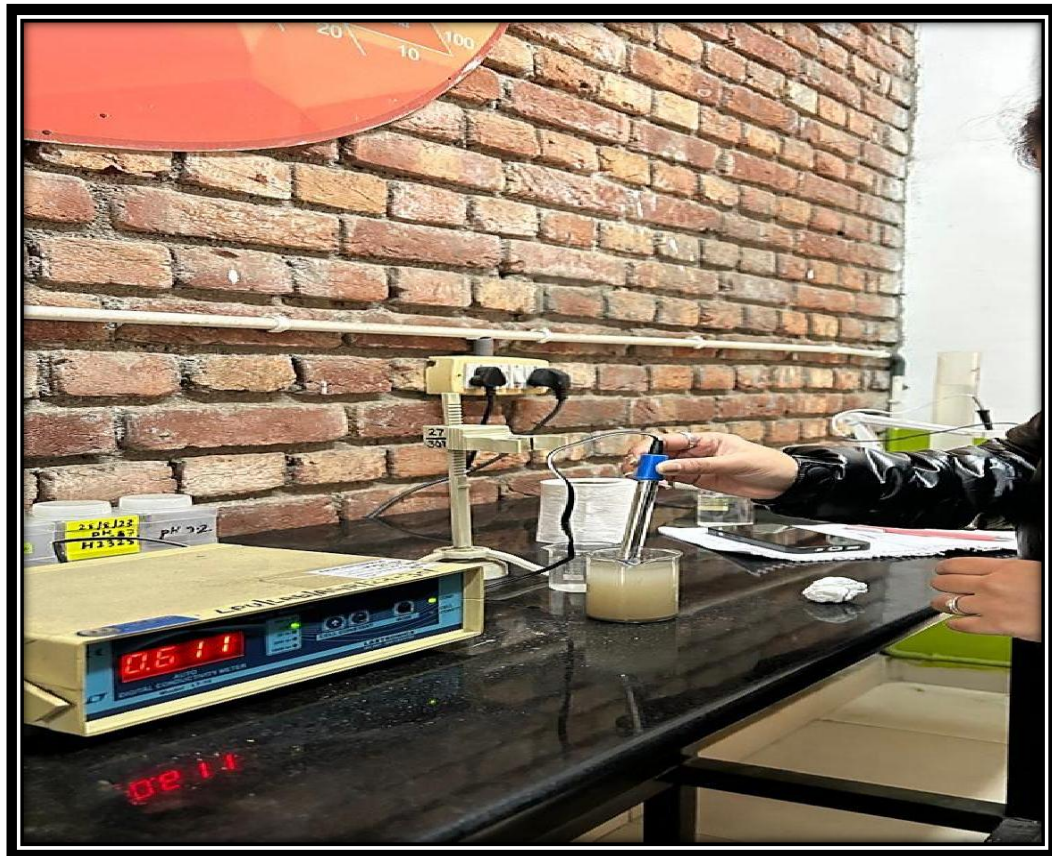
PLATE 12: SPAD reading using SPAD meter over the research plot in black gram



PLATE 13: Performing biochemical analysis on the leaf of black gram



PLATE 14: Performing soil analysis in the lab



RESULTS AND DISCUSSIONS

The current study, "**Effect of Polyamines on Growth and Yield of Black gram *Vigna mungo* (L.) Hepper,**" was carried out as a field experiment at the Agriculture Farm, Department of Agronomy, School of Agriculture, Lovely Professional University, Phagwara, during the summer and *Kharif* seasons of 2022 and 2023. The study was conducted to observe the effect of the individual and combined effects of putrescine and cadaverine on the growth, biochemical, and yield of black gram. For the trial, MASH 1008 variety was used. This chapter was divided into 4 parts, the first to assess the morphological parameters at 25 and 50 days after sowing. The study's second section examined the crop's biochemical reactions to various treatment scenarios using polyamines individually and in combination. The study's last section centered on seed quality and traits, whereas the third section examined yield and yield-related factors. The previous chapter provided a detailed description of the technique and procedures. In this chapter, we have discussed the treatment's effect in detail. The study spanned two years (2022 and 2023) across both seasons, summer and *Kharif*. It was observed that the summer season crop showed promising results as the MASH 1008 variety performed well in summer and was also preferred for summer by farmers.

4.1. Growth and Developmental Studies

4.1.1. Plant height

The effects of foliar application of polyamines on black gram growth, yield, and quality were assessed during two seasons (*Summer* and *Kharif*) of 2022 and 2023. Plant height was measured at 25 and 50 days after sowing (DAS), as shown in Table 4.1.1.a and 4.1.1.b and illustrated in Figures 4.1.1.a and 4.1.1.b.

For the Summer of 2022, treatment T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M) had a maximum plant height and significantly increased plant height in comparison to the control. Other treatments responded favorably to plant height increases. T3 produced significant increases in plant height of 27.07% and 24.21% at 25 and 50 DAS, respectively. The increase over control (10.41 cm at 25 DAS and 15.68 cm at 50 DAS) was much higher than the CD values (2.221 cm and 2.888 cm), confirming statistical significance. Treatments T5 (37.02 cm and 62.82 cm) and T6 (36.87 cm and 62.53 cm) were statistically at par with T3 at 50 DAS but significantly superior to control and other lower treatments. Treatment T2 (29.19 cm and 51.53 cm) showed the least improvement, and the increases over

control (1.16 cm and 2.45 cm) were marginal, though they exceeded CD slightly at 50 DAS.

In the Summer of 2023, T3 again produced the tallest plants with increases in height of 30.11% and 23.92% at 25 DAS and 50 DAS, respectively, followed by T5 and T4 with gains of increases in plant height of 28.36–28.79% and 21.50–22.40%, respectively. The absolute differences (11.07 cm and 15.02 cm) were substantially greater than CD values (1.749 cm and 3.464 cm), indicating strong statistical superiority. Treatments T5 and T6 were statistically comparable to T3 at 25 DAS but slightly lower at 50 DAS, although still significantly superior to control. Treatment T2 again recorded the minimum values (27.86 cm and 50.53 cm), and the differences over control were comparatively small but exceeded CD at 25 DAS only.

In the *Kharif* season of 2022, the increase in plant height observed in T3 from the control was 26.05% at 25 days after seeding (DAS) and 27.62% at 50 DAS, indicating a statistically significant effect of treatment T3 compared to that of the control, due to the absence of overlap between treatment means when tested at the 5% level of significance. Treatment means of T5 and T6 were also significantly higher than the control group, however; they were moderately lower than T3. Treatment T2 registered some improvement, but was still less than combinations of treatments with T3 and T4.

In the *Kharif* season of 2023, treatment T3 had the maximum plant height and again was taller than control treatment T₀, with height increases of 31.62% and 28.44% at 25 DAS and 50 DAS, respectively. Treatment T3 was followed by T5 and T6, which also showed increased heights with values far exceeding those of T2. Treatment T2 had the least growth performance, with only 8.45% and 2.57% increases in height gains, while T1 had modest gains of 14.37% and 6.03%. The differentiation observed was greater than the CD values, showing the general level of significance was consistent across the data collected. Treatments T5 and T6 were statistically equivalent to T3 in the early stages of development but were statistically less than T3 at 50 DAS. Treatment T2 had the lowest average plant height compared to the other combined treatments; therefore, it too was statistically less than the other treatments.

The combined application of putrescine and cadaverine positively increased the plant height during both seasons, *Summer* and *Kharif* (2022 and 2023), by promoting cell elongation as they increase the mitotic activity and loosening of the cell wall, causing shoot elongation. Polyamines bind with phospholipids, enhancing membrane stability, particularly during stress conditions, and act as antioxidants to reduce oxidative stress, thereby allowing continued development (Kusano *et al.*, 2008; Alcázaret *al.*, 2010). The greater efficacy of combined treatments is probably the result of the synergistic activation of several growth-permitting pathways, indicating a non-redundant and complementary role of putrescine and cadaverine in morphogenesis (Takahashi and Kakehi, 2010).

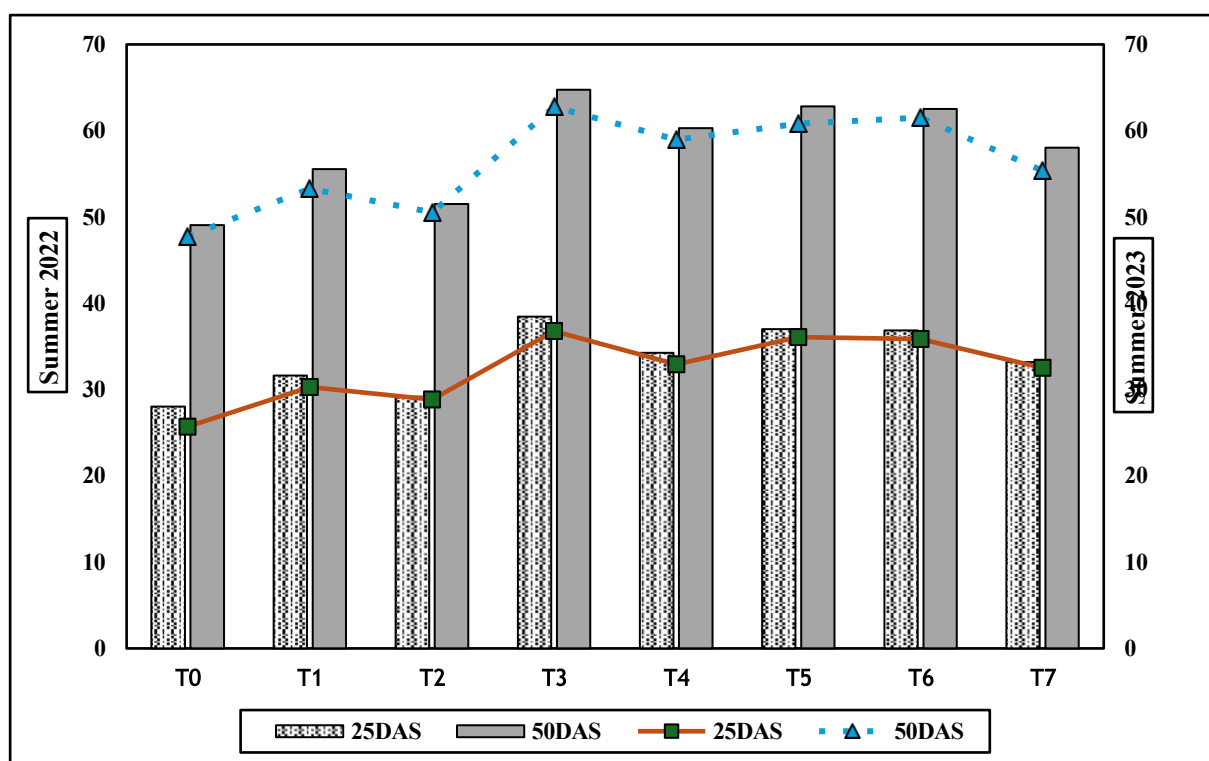
TABLE 4.1.1.a: Effect of the treatment on the plant height (cm) of black gram in *Summer* season

TREATMENTS	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	28.03 ± 0.95	49.08 ± 0.05	25.70 ± 0.49	47.74 ± 1.38
T ₁	31.62 ± 0.98 (11.34)	55.56 ± 0.54 (11.36)	29.62 ± 0.22 (15.14)	53.27 ^b ± 1.36 (10.38)
T ₂	29.19 ± 0.99 (3.96)	51.53 ± 0.84 (4.75)	27.86 ± 0.90 (10.94)	50.53 ± 1.41 (5.51)
T ₃	38.44 ± 0.89 (27.07)	64.76 ± 0.90 (24.21)	36.77 ± 0.57 (30.11)	62.76 ± 1.35 (23.92)
T ₄	34.26 ± 1.30 (18.17)	60.30 ± 0.87 (18.61)	32.93 ± 0.84 (21.95)	58.96 ± 0.84 (19.03)
T ₅	37.02 ± 0.16 (24.28)	62.82 ± 1.10 (21.88)	36.02 ± 0.47 (28.79)	60.82 ± 0.16 (21.50)
T ₆	36.87 ± 1.56 (23.97)	62.53 ± 0.75 (21.51)	35.87 ± 0.01 (28.36)	61.53 ± 1.73 (22.40)
T ₇	33.18 ± 0.52 (15.50)	58.02 ± 1.43 (15.41)	31.84 ± 0.39 (20.95)	55.35 ± 0.56 (13.75)
CD at p=0.05	2.221	2.888	1.749	3.464
Sem±	0.725	0.943	0.571	1.131

Note:

1. Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
2. Values given in parentheses indicate the percent change over the control.

Fig. 4.1.1.a: Effect of the treatment on the plant height (cm) of black gram in *Summer* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

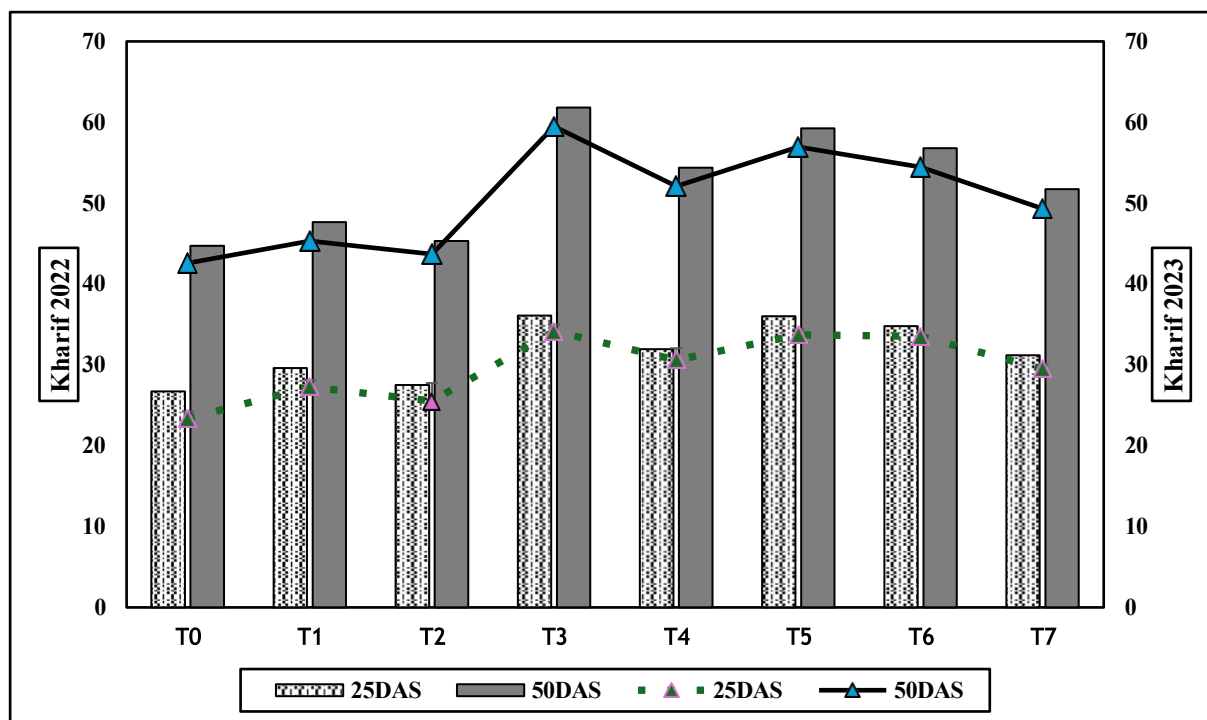
TABLE 4.1.1.b: Effect of the treatment on the plant height (cm) of black gram in *Kharif* season

TREATMENTS	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	26.70 ± 0.62	44.74 ± 2.35	23.36 ± 0.49	42.58 ± 1.11
T ₁	29.62 ± 0.49 (9.86)	47.66 ± 1.95 (6.11)	27.28 ± 0.49 (14.37)	45.31 ± 1.20 (6.03)
T ₂	27.52 ± 0.41 (2.99)	45.33 ± 1.96 (1.30)	25.52 ± 1.28 (8.45)	43.70 ± 1.42 (2.57)
T ₃	36.10 ± 0.33 (26.05)	61.82 ± 0.82 (27.62)	34.16 ± 0.46 (31.62)	59.50 ± 0.80 (28.44)
T ₄	31.93 ± 0.57 (16.37)	54.40 ± 1.72 (17.75)	30.59 ± 0.84 (23.63)	52.10 ± 1.73 (18.27)
T ₅	36.02 ± 0.65 (25.88)	59.24 ± 0.66 (24.47)	33.72 ± 0.38 (30.72)	56.95 ± 0.64 (25.24)
T ₆	34.77 ± 0.57 (23.22)	56.81 ± 1.80 (21.24)	33.51 ± 0.00 (30.28)	54.47 ± 1.81 (21.83)
T ₇	31.18 ± 0.62 (14.36)	51.71 ± 1.85 (13.48)	29.48 ± 0.26 (20.76)	49.33 ± 1.90 (13.69)
CD at p=0.05	1.701	4.979	1.836	4.419
Sem±	0.555	1.626	0.599	1.475

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig. 4.1.1.b: Effect of the treatment on the plant height (cm) of black gram in *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.1.2. Total number of leaves per plant

The effects of polyamines on black gram growth, yield, and quality were assessed during the two seasons (*Summer* and *Kharif*) of 2022 and 2023. The data on the Total Number of leaves were taken at 25 and 50 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.

The summer season of 2022, T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M) had the greatest number of leaves, and significant increases of 33.84% and 38.73% at 25 and 50 DAS, respectively. Also, T4 (Putrescine 500 μ M + Cadaverine 1000 μ M) with increases of 24.86%, T5 (Putrescine 500 μ M + Cadaverine 500 μ M) with increases of 34.46%. The least increase in the number of leaves was from T2 (Cadaverine 1000 μ M), with increases of 9.69% and 13.83% over the control. Treatments T4 and T5 also recorded significantly higher leaf numbers than control, as their mean differences exceeded the CD values. In contrast, T2 (Cadaverine 1000 μ M) showed the least increase (9.69% and 13.83%), and although higher than control, its magnitude of improvement was statistically inferior to combined treatments.

In the summer of 2023, T3 again noted the highest number of leaves, with 40.21% and 37.31% increases at 25 DAS and 50 DAS, respectively. Treatments T4 and T5 had increases of >31.6% and 30.7%. While T2, again, had the least increases at 15.01% and 9.11%, T1 (Putrescine 1500 μ M) had moderate increases of 19.42% and 18.32%. Treatments T4, T5, and T6 were also significantly superior to control and were statistically comparable among themselves at 25 DAS, but remained slightly lower than T3 at 50 DAS. Treatment T2 again recorded the lowest increase (15.01% and 9.11%) and was statistically inferior to the combined treatments.

In the *Kharif* season of 2022, Treatment T3 had the most significant number of leaves and increases of 39.16% and 36.01% at 25 and 50 DAS, respectively. T4, T5, and T6 treatments also showed considerable increases from the control, with responses ranging from 31.42% to 36.25%. Treatment T2 (Cadaverine 1000 μ M) had only the lowest levels of improvement, with increases of 11.01% and 10.41% from the control. The observed differences were greater than the CD values (2.779 and 2.928), confirming statistical significance. Treatments T4, T5, and T6 also showed significant improvement over control, as their differences exceeded CD values, though they remained slightly lower than T3.

In the *Kharif* season of 2023, T3 had the most significant number of leaves consistently from

the previous season, with increases of 39.56% and 41.45% at 25 and 50 DAS. Similarly, T4, T5, and T6 continued to show responses, with all treatments exceeding improvements of 30%. Again, the T2 treatment had the lowest levels of enhancement with increases of 7.55% and 18.63%, while T1 had lower improvements of 14.22% and 19.41%. The statistical differences between treatments are statistically significant because they exceed the critical difference values of (1.609 & 2.749). Additionally, the treatments T4, T5, and T6 resulted in a difference greater than 30% from the control. T2 had a significantly lower improvement than treatments T3 and the other combinations after 25 days of growth, only increasing by 7.55% over that period.

The increase in leaf numbers could reflect the synergistic effects of putrescine and cadaverine, two heterocyclic diamines that induce plant growth and morphogenesis. When combined, these compounds could have a more pronounced role in mitotic cell division and elongation. Polyamines are nucleic acid-binding compounds that regulate gene expression and promote cell division at the meristematic zones (Kusano *et al*, 2008). Furthermore, polyamines bind to phospholipids to stabilize membranes, which is essential for physiological cell function during active vegetative growth (Alcázaret *al.*, 2010). Polyamines also enhance the scavenging properties of reactive oxygen species (ROS) by upregulation of antioxidant enzyme rates to alleviate oxidative stress, a potential impact especially during the high-temperature summer months (Gill and Tuteja, 2010). In addition to their ability to provide cellular protection, polyamines improve root system architecture and ion transport, allowing plants to correspondingly increase and assimilate nutrient uptake in each increased leaf, resulting in higher leaf growth. Polyamine treatments have provided, but are not limited to, ameliorating abiotic stress experienced during the summer; thus, creating conditions that allow for no interruption of leaf initiation and expansion (Takahashi and Kakehi, 2010).

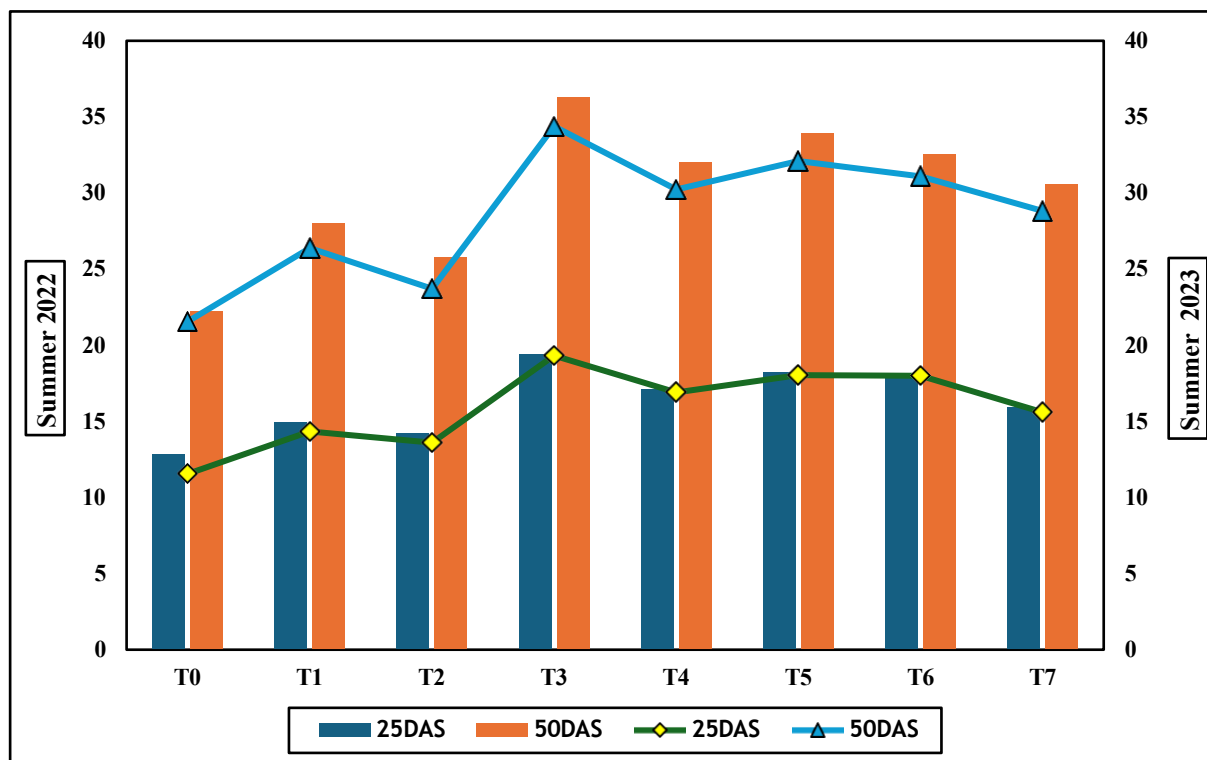
TABLE 4.1.2.a: Effect of the treatment on the number of leaves plant⁻¹ of black gram in the *Summer* season

TREATMENTS	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	12.84 ± 0.29	22.24 ± 1.55	11.55 ± 0.57	21.55 ± 0.96
T ₁	14.95 ± 0.23 (14.12)	28.05 ± 0.59 (20.69)	14.33 ± 0.33 (19.42)	26.39 ± 0.29 (18.32)
T ₂	14.21 ± 0.66 (9.69)	25.81 ± 1.44 (13.83)	13.59 ± 0.36 (15.01)	23.71 ± 0.95 (9.11)
T ₃	19.40 ± 0.77 (33.84)	36.30 ± 0.58 (38.73)	19.32 ± 0.88 (40.21)	34.38 ± 0.69 (37.31)
T ₄	17.08 ± 0.93 (24.86)	31.99 ± 1.20 (30.46)	16.89 ± 0.29 (31.63)	30.22 ± 1.05 (28.68)
T ₅	18.25 ± 1.08 (29.66)	33.94 ± 1.17 (34.46)	18.04 ± 0.42 (35.99)	32.11 ± 1.06 (32.87)
T ₆	18.05 ± 1.58 (28.88)	32.55 ± 2.51 (31.66)	18.00 ± 0.00 (35.82)	31.10 ± 2.13 (30.70)
T ₇	15.94 ± 0.47 (19.45)	30.58 ± 0.81 (27.27)	15.59 ± 0.30 (25.91)	28.82 ± 0.65 (25.21)
CD at p=0.05	2.713	4.412	1.351	3.431
Sem±	0.886	1.441	0.441	1.120

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig. 4.1.2.a: Effect of the treatment on the number of leaves plant⁻¹ of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

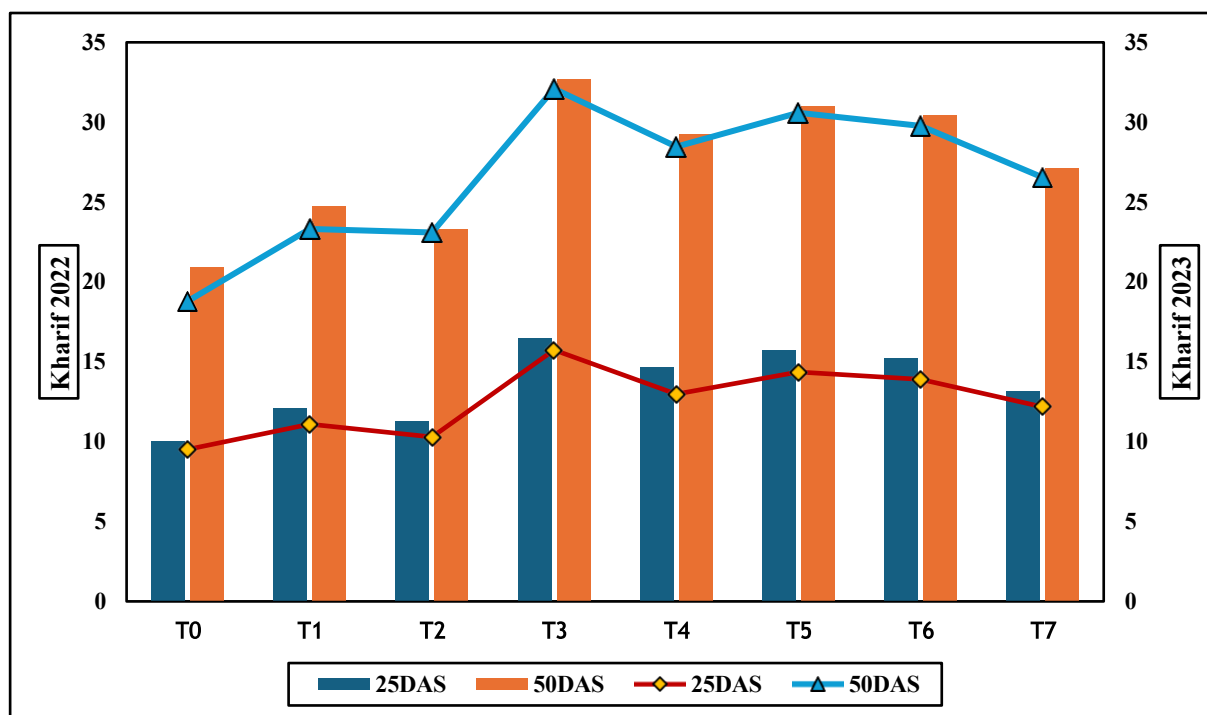
TABLE 4.1.2.b: Effect of the treatment on the number of leaves plant⁻¹ of black gram in the *Kharif* season

TREATMENTS	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	10.05 ± 0.38	20.91 ± 0.78	9.51 ± 0.03	18.79 ± 0.98
T ₁	12.09 ± 0.26 (16.90)	24.77 ± 0.81 (15.57)	11.09 ± 0.75 (14.22)	23.32 ± 0.14 (19.41)
T ₂	11.29 ± 0.65 (11.01)	23.34 ± 1.20 (10.41)	10.29 ± 0.30 (7.55)	23.10 ± 1.12 (18.63)
T ₃	16.51 ± 0.78 (39.16)	32.68 ± 0.41 (36.01)	15.74 ± 0.49 (39.56)	32.10 ± 0.50 (41.45)
T ₄	14.65 ± 0.86 (31.42)	29.25 ± 0.69 (28.50)	12.98 ± 0.82 (26.73)	28.48 ± 0.66 (34.00)
T ₅	15.76 ± 1.26 (36.25)	31.03 ± 0.69 (32.62)	14.36 ± 0.14 (33.74)	30.59 ± 0.45 (38.56)
T ₆	15.23 ± 1.65 (34.01)	30.45 ± 1.42 (31.34)	13.89 ± 0.73 (31.52)	29.76 ± 1.35 (36.85)
T ₇	13.17 ± 0.41 (23.72)	27.11 ± 0.94 (22.88)	12.17 ± 0.27 (21.83)	26.55 ± 0.82 (29.22)
CD at p=0.05	2.779	2.928	1.609	2.749
Sem	0.907	0.956	0.525	0.898

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig. 4.1.2.b: Effect of the treatment on the number of leaves plant⁻¹ of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.1.3 Total number of branches

The effects of polyamines on black gram growth, yield, and quality were assessed during the *Summer* and *Kharif* seasons of 2022 and 2023. The data on the Total Number of branches were taken at 25 and 50 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.

The summer season of 2022 found an extraordinary increase in branches over treatments. treatment T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M) recorded the maximum number of branches (5.07 and 9.17 at 25 and 50 DAS, respectively), showing increases of 49.90% and 24.95% over control. The absolute differences from control (2.53 and 2.29 branches) were much higher than the CD values (0.251 and 0.718), confirming statistical significance at both stages. Treatments T5 and T6 also showed significant improvement over control, as their mean differences exceeded the CD values, though they remained slightly lower than T3. Treatment T2 recorded the least increase (13.80% and 2.69%), and at 50 DAS the increase was marginal and statistically inferior to combined treatments. In the Summer season of 2023, T3 again produced the maximum number of branches (4.53 and 8.89 at 25 and 50 DAS), with increases of 46.43% and 27.32%, respectively, over control. The differences (2.10 and 2.43 branches) were greater than the CD values (0.613 and 0.724), indicating clear statistical superiority. Treatments T5 and T6 also recorded significant increases and were statistically superior to control at both stages, whereas T2 showed only slight improvement (12.61% and 0.72%) and remained statistically inferior to the combined applications, particularly at 50 DAS where the difference over control was negligible.

During Kharif 2022, T3 had the most branches at 25 and 50 DAS (4.30 and 8.39, respectively) with 44.03% and 27.27% higher than control, and both increases were beyond CD values (0.590 and 0.718), showing they were statistically significant. T5 and T6 were the next best-performing treatments after T3 and were both significantly different from the control at both periods. T2 was the lowest-performing treatment (6.72% and 3.02%) and was statistically different than T3 and other treatments. In Kharif 2023, T3 again had the most branches (4.21 and 8.09, respectively) at 25 and 50 DAS with increases over control of 46.16% and 24.72% and exceeded CD values (0.560 and 0.758); thus, the differences between T3 and each of the control groups have been statistically reliable across Kharif 2022 and Kharif 2023. Like Kharif 2022, T5 and T6 had significantly larger numbers of branches than control in Kharif 2023, but instead T2 only had minimal increases (4.23% and 0.87%) and was statistically similar to control at 50 DAS.

The increase in the total number of branches is attributed to the application of polyamines, such as putrescine and cadaverine, which are essential regulators in the growth and development of plants, particularly during the initial vegetative growth phases. Their promoting effects on branching resulted from stimulating cell division and expansion, especially in apical meristems and axillary bud primordia, which was conducive to increased lateral branches. At the cellular level, they stabilize DNA, fine-tune chromatin structure, and activate protein synthesis in cell cycle progression. In addition, polyamines increase chloroplast development and function, indirectly promoting branching by enhancing the availability of photosynthates. Their interactions with hormones mediate this, particularly auxins and cytokinins, because they modulate the hormonal gradient that controls axillary meristem activation and apical dominance release. There is evidence of these mechanisms in the literature; for instance, polyamines are known to upregulate CDK genes and ARFs as described during organogenesis.

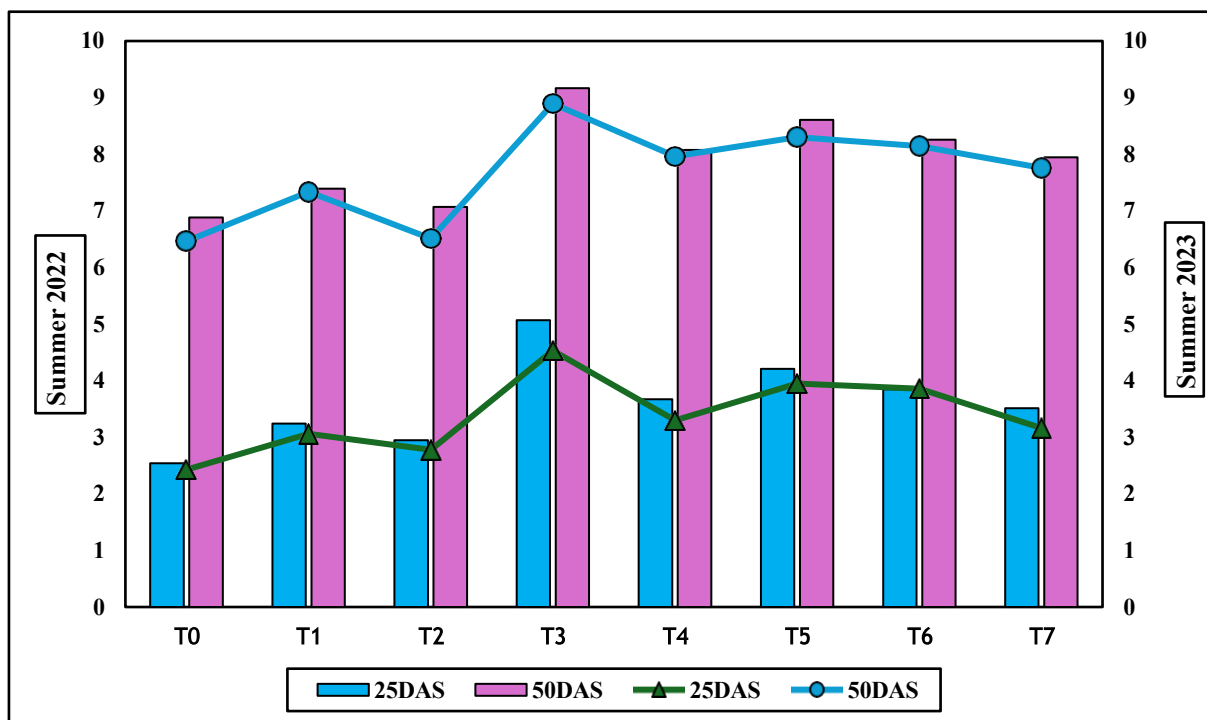
TABLE 4.1.3.a: Effect of the treatment on the total number of branches plant⁻¹ of leaves of black gram in the *Summer* season

TREATMENTS	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	2.54 ± 0.03	6.88 ± 0.27	2.43 ± 0.34	6.46 ± 0.05
T ₁	3.24 ± 0.06 (21.60)	7.39 ± 0.36 (6.90)	3.06 ± 0.26 (20.70)	7.33 ± 0.26 (11.86)
T ₂	2.95 ± 0.06 (13.80)	7.07 ± 0.08 (2.69)	2.78 ± 0.06 (12.61)	6.51 ± 0.14 (0.72)
T ₃	5.07 ± 0.17 (49.90)	9.17 ± 0.08 (24.95)	4.53 ± 0.12 (46.43)	8.89 ± 0.08 (27.32)
T ₄	3.67 ± 0.02 (30.79)	8.07 ± 0.41 (14.78)	3.30 ± 0.17 (26.46)	7.96 ± 0.08 (18.80)
T ₅	4.21 ± 0.01 (39.62)	8.61 ± 0.13 (20.06)	3.95 ± 0.29 (38.57)	8.30 ± 0.34 (22.13)
T ₆	3.85 ± 0.08 (33.97)	8.26 ± 0.06 (16.67)	3.86 ± 0.10 (37.13)	8.14 ± 0.38 (20.63)
T ₇	3.51 ± 0.09 (27.64)	7.95 ± 0.12 (13.42)	3.16 ± 0.10 (23.29)	7.76 ± 0.17 (16.71)
CD at p=0.05	0.251	0.718	0.613	0.724
Sem±	0.082	0.234	0.200	0.236

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.3.a: Effect of the treatment on the total number of branches plant⁻¹ of leaves of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

TABLE 4.1.3.b: Effect of the treatment on the Total number of branches of black gram in the *Kharif* season

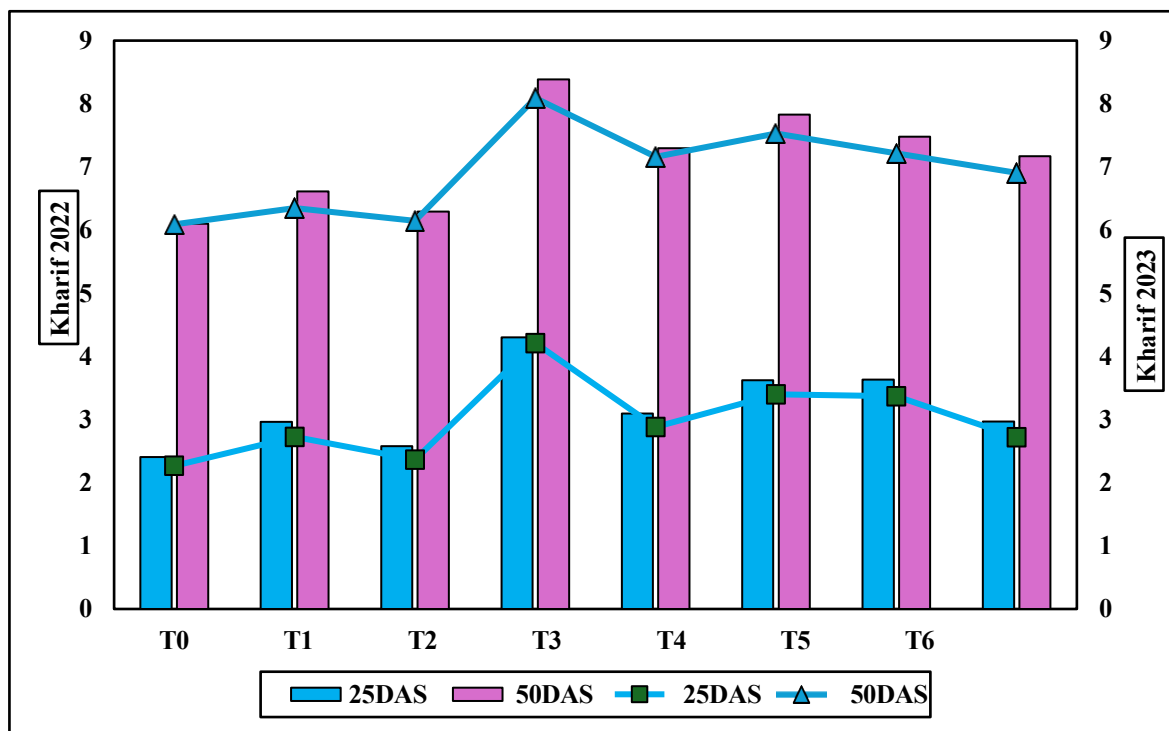
TREATMENTS	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	2.41 ± 0.08	6.10 ± 0.27	2.27 ± 0.11	6.09 ± 0.37
T ₁	2.96 ± 0.37 (18.79)	6.61 ± 0.36 (7.72)	2.72 ± 0.30 (16.77)	6.35 ± 0.33 (4.04)
T ₂	2.58 ± 0.04 (6.72)	6.29 ± 0.08 (3.02)	2.37 ± 0.10 (4.23)	6.06 ± 0.08 (0.87)
T ₃	4.30 ± 0.12 (44.03)	8.39 ± 0.08 (27.27)	4.21 ± 0.16 (46.16)	8.09 ± 0.05 (24.72)
T ₄	3.09 ± 0.17 (22.20)	7.29 ± 0.41 (16.36)	2.88 ± 0.13 (21.20)	7.16 ± 0.36 (14.94)
T ₅	3.62 ± 0.24 (33.52)	7.83 ± 0.13 (22.06)	3.40 ± 0.27 (33.33)	7.53 ± 0.10 (19.12)
T ₆	3.63 ± 0.10 (33.70)	7.48 ± 0.06 (18.41)	3.37 ± 0.02 (32.74)	7.21 ± 0.09 (15.57)
T ₇	2.97 ± 0.12 (18.88)	7.17 ± 0.12 (14.88)	2.72 ± 0.11 (16.67)	6.90 ± 0.14 (11.78)
CD at p=0.05	0.590	0.718	0.560	0.758
Sem±	0.193	0.234	0.183	0.247

Note:

1-Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM

2-Values given in parentheses indicate the percent change over the control.

Fig 4.1.3.b: Effect of the treatment on the Total Number of Branches of leaves of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.1.4. LEAF AREA

The effects of foliar application of polyamines on black gram growth, yield, and quality were assessed during the two seasons (*Summer* and *Kharif*) of 2022 and 2023. Leaf area was measured 25 and 50 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.

During both summer years, all treatments exhibited an increase in leaf area compared to the control treatment at both 25 DAS and 50 DAS. The application method with the most increase was T3 (combined application of Putrescine (1500 μ M) + Cadaverine (1000 μ M)) with the highest leaf area measurements of 414.97 cm² and 715.69 cm² at 25 DAS and 50 DAS, respectively in 2022, and percentage improvement over the control of 27.30% and 25.55%

concerning leaf area at 25 DAS and 50 DAS, respectively, 2022. The absolute difference between the control group of 113.27 cm² and 182.83 cm² was statistically significant due to its magnitude relative to either CD value (14.510 or 37.829), with greater frequency and rates of increase noted by treatments T5 and T6 versus T4 and T7 (both of which had less than the CD threshold rate/value). Treatment T2 (1000 µM cadaverine) did exhibit marginal improvement in both conditions (1.18% to 6.49%), but had a smaller increase (statistically significant) at 25 days post-application when compared to other treatments using the CD; thus, while the gains from T2 were relatively low, they were nevertheless valid as statistical measures of effectiveness.

T3 had a similar trend in 2023 Leaf Area for Treatment T3 again was the largest, yielding 405.41 cm² at 25 DAS and 703.22 cm² at 50 DAS (improvement value = 27.90% at 25 DAS and 26.02% at 50 DAS). The differences of 113.10 cm² at 25 DAS and 182.98 cm² at 50 DAS are also far greater than the respective Critical Difference (CD) values of 14.636 and 37.897, resulting in statistical superiority. T5 and T6 followed, but were still greater than Control for both dates. T2 was by far the least effective treatment and did not significantly differ from control.

During the *Kharif* season of 2022, Treatment T3 recorded 401.88 cm² at 25 DAS and 689.06 cm² at 50 DAS, showing increases of 28.18% and 25.90% over control. The differences were far above the CD values (14.514 and 28.251), confirming statistical significance. Treatments T5 and T6 also recorded significant enhancement over control, while T2 exhibited the least improvement (1.23%–3.21%) and was statistically inferior to the combined treatments.

During Kharif 2023, T3 exhibited its superiority with leaf area of 387.71 cm² (25 DAS) and 661.96 cm² (50 DAS), which are increases of 29.18% and 27% over the control. Their increase was well above the expected CD values (11.267 cm² and 32.608 cm²), indicating that the treatment effect was consistent across both seasons. The treatments T5 and T6 had a significant increase as well; however, T2 had a small (marginal) increase and therefore will be at a disadvantage.

The enhanced leaf area in effective treatments could be determined by the role of polyamines in cell division and expansion, which is mainly mediated by the activation of DNA replication, elevated expression of cell cycle-associated genes (i.e., Cyclin D, CDKA), and the stabilization of cell membranes. Alkaline agents induce the activity of ribosomes and stimulate protein synthesis, which is required for rapid leaf tissue development. Moreover, putrescine and cadaverine have been found to postpone leaf senescence, presumably through reprogramming

reactive oxygen species (ROS) sequestration and enhancing chloroplast integrity. They could also facilitate the integration of nitrogen, which is used for centric leaf tissue elongation. These assertions are consistent with the literature demonstrating that polyamines could contribute to leaf biomass, chlorophyll content, and activities of photosynthetic enzymes, eventually resulting in better leaf area and longevity.

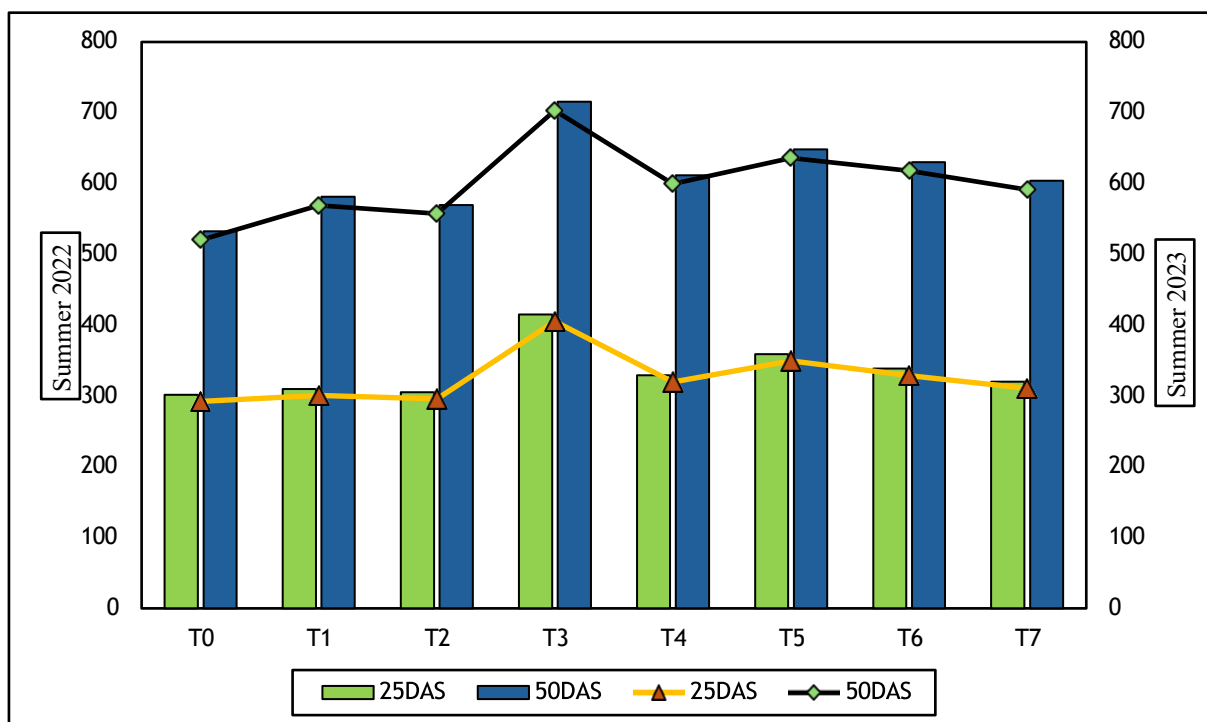
TABLE 4.1.4.a: Effect of the treatment on the leaf area (cm² plant⁻¹) of black gram in the *Summer* season

TREATMENTS	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	301.70 ± 5.91	532.86 ± 7.29	292.31 ± 5.97	520.24 ± 7.60
T ₁	310.03 ± 4.24 (2.69)	581.69 ± 7.98 (8.39)	300.57 ± 4.24 (2.75)	569.36 ± 7.98 (8.63)
T ₂	305.30 ± 3.30 (1.18)	569.87 ± 10.0 (6.49)	295.84 ± 3.30 (1.19)	557.54 ± 10.0 (6.69)
T ₃	414.97 ± 8.56 (27.30)	715.69 ± 10.0 (25.55)	405.41 ± 8.55 (27.90)	703.22 ± 9.99 (26.02)
T ₄	329.20 ± 2.27 (8.35)	611.67 ± 17.5 (12.89)	319.74 ± 2.27 (8.58)	599.34 ± 17.5 (13.20)
T ₅	359.00 ± 5.10 (15.96)	648.52 ± 9.77 (17.84)	349.44 ± 5.19 (16.35)	636.28 ± (18.24)
T ₆	338.57 ± 4.83 (10.89)	630.28 ± 22.1 (15.46)	328.91 ± 4.95 (11.13)	617.85 ± (15.80)
T ₇	320.40 ± 4.05 (5.84)	603.89 ± 9.54 (11.76)	310.91 ± 4.02 (5.98)	591.56 ± (12.06)
CD at p=0.05	14.510	37.829	14.636	37.897
Sem±	4.738	12.352	4.779	12.374

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.4.a: Effect of the treatment on the leaf area ($\text{cm}^2 \text{ plant}^{-1}$) of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine (1500 μmol), T2= Cadaverine (1000 μmol), T3= Putrescine (1500 μmol) + Cadaverine (1000 μmol), T4= Putrescine (3000 μmol) + Cadaverine (500 μmol), T5= Putrescine (750 μmol) + Cadaverine (2000 μmol), T6= Putrescine (750 μmol) + Cadaverine (500 μmol) and T7= Putrescine (3000 μmol) + Cadaverine (2000 μmol)

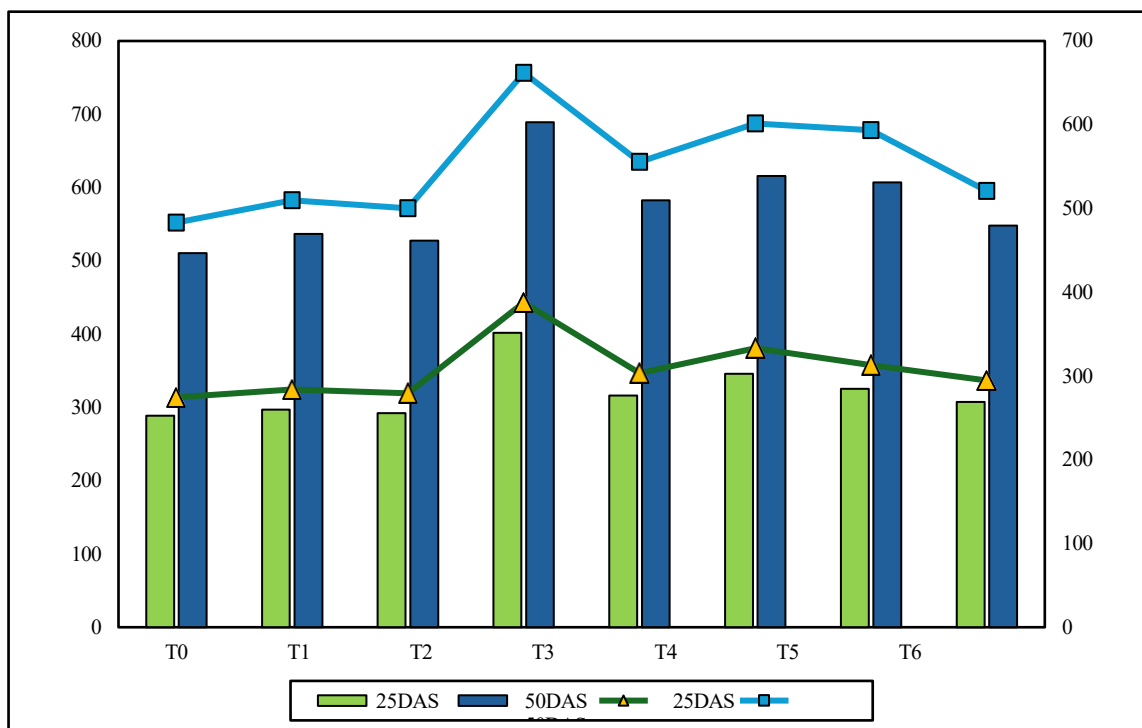
TABLE 4.1.4.b: Effect of the treatment on leaf area (cm² plant⁻¹) of black gram in the *Kharif* season

TREATMENTS	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	288.61 ± 5.91	510.57 ± 6.72	274.57 ± 1.47	483.22 ± 6.96
T ₁	296.94 ± 4.24 (2.81)	536.81 ± 11.4 (4.89)	284.11 ± 4.40 (3.36)	509.70 ± 11.4 (5.19)
T ₂	292.21 ± 3.30 (1.23)	527.50 ± 3.33 (3.21)	279.37 ± 3.13 (1.72)	500.39 ± 3.33 (3.43)
T ₃	401.88 ± 8.56 (28.18)	689.06 ± 16.7 (25.90)	387.71 ± 4.05 (29.18)	661.96 ± 16.7 (27.00)
T ₄	316.11 ± 2.27 (8.70)	582.73 ± 9.50 (12.38)	303.31 ± 2.16 (9.47)	555.7 ± 9.36 (13.05)
T ₅	345.91 ± 5.10 (16.57)	615.65 ± 8.37 (17.07)	333.34 ± 5.15 (17.63)	601.57 ± 4.85 (19.67)
T ₆	325.48 ± 4.83 (11.33)	607.04 ± 9.43 (15.89)	312.84 ± 4.82 (12.23)	593.43 ± 18.2 (18.57)
T ₇	307.31 ± 4.05 (6.09)	548.18 ± 9.74 (6.86)	294.67 ± 4.05 (6.82)	520.89 ± 9.91 (7.23)
CD at p=0.05	14.514	28.251	11.267	32.608
Sem±	4.739	9.225	3.679	10.647

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.4.b: Effect of the treatment on leaf area ($\text{cm}^2 \text{ plant}^{-1}$) of black gram in the *Kharif* season



Note: T0= Control, T1= Putrescine (1500 μmol), T2= Cadaverine (1000 μmol), T3= Putrescine (1500 μmol) + Cadaverine (1000 μmol), T4= Putrescine (3000 μmol) + Cadaverine (500 μmol), T5= Putrescine (750 μmol) + Cadaverine (2000 μmol), T6= Putrescine (750 μmol) + Cadaverine (500 μmol) and T7= Putrescine (3000 μmol) + Cadaverine (2000 μmol)

4.1.5. LEAF AREA INDEX

The effects of foliar application of polyamines on black gram growth, yield, and quality were assessed during the two seasons (summer and *Kharif*) of 2022 and 2023. Leaf area index was measured 25 and 50 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 T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M), LAI (leaf area index) at 25 DAS (days after sowing) had the highest mean of 1.38 and was significantly different from the control at 1.01 (a difference of 0.37, > CD of 0.048). After 50 DAS, LAI for T3 increased to 2.39 and was again significantly different (a difference of 0.61, > CD of 0.126) from the control (1.78). T5 (LAI of 1.20 and 2.16 at 25 and 50 DAS, respectively) and T6 (LAI of 1.13 and 2.10 at 25 and 50 DAS, respectively) were also significantly different from the control, as their differences exceeded the respective CDs. In contrast, T1 and T2 showed little to no improvement after 25 DAS and were statistically similar to the control. This trend continued throughout Summer 2023. During 25 DAS, LAI for T3 was 1.35 and was significantly different from the control (0.97; a difference of 0.38, > CD of 0.049). By 50 DAS, T3 had continued to exhibit significant superiority over the control (LAI 2.34 and 1.73, respectively) (a difference of 0.61, > CD of 0.126). Both T5 and T6 demonstrated improvements over the control at 25 and 50 DAS, while T1 and T2 either showed nominal increases over their controls or had statistically similar LAIs to those of their controls.

In the *Kharif* season of 2022, LAI exhibited a similar trend as the previous year. LAI (1.34) was significantly superior to the control (0.96) at 25 DAS, with a difference (0.38) greater than the critical difference (0.048). At 50 DAS, T3 (2.30) was also significantly greater than control (1.70), with a difference (0.60) greater than the critical difference (0.094). T5 and T6 were both significantly greater than the control, and T1 and T2 had little improvement over the control and were statistically similar.

In *Kharif* 2023, T3 again had the maximum LAI at both 25 DAS (1.29) and 50 DAS (2.21) with significant differences from the control (0.92 at 25 DAS and 1.61 at 50 DAS) of 0.37 and 0.60, respectively, which were both significantly higher than their respective critical differences (0.036 and 0.110). T5 and T6 were also significantly greater than the control, while T2 was least improved and only marginally greater than the control.

The noticeable increase in LAI from the polyamine treatments, especially with the combined

treatments of Putrescine and Cadaverine, may be due to polyamines regulating cell division, expansion, and development of tissue. Polyamines bring about DNA replication and upregulate essential genes (like Cyclin D and CDKA) involved in cell cycle regulation, which promotes a fast-tracked cell cycle and leaf development. Polyamines also help maintain the integrity of membranes while also activating nitrogen assimilation and delaying senescence by scavenging reactive oxygen species (ROS), which allows the chloroplasts to be functional for a more extended period and to photosynthesize. As a result, the rate of protein synthesis and cell proliferation increased, according to the literature, which indicates that the application of polyamines enhances leaf biomass and chlorophyll content.

TABLE 4.1.5.a: Effect of the treatment on the leaf area index of black gram in the *Summer* season

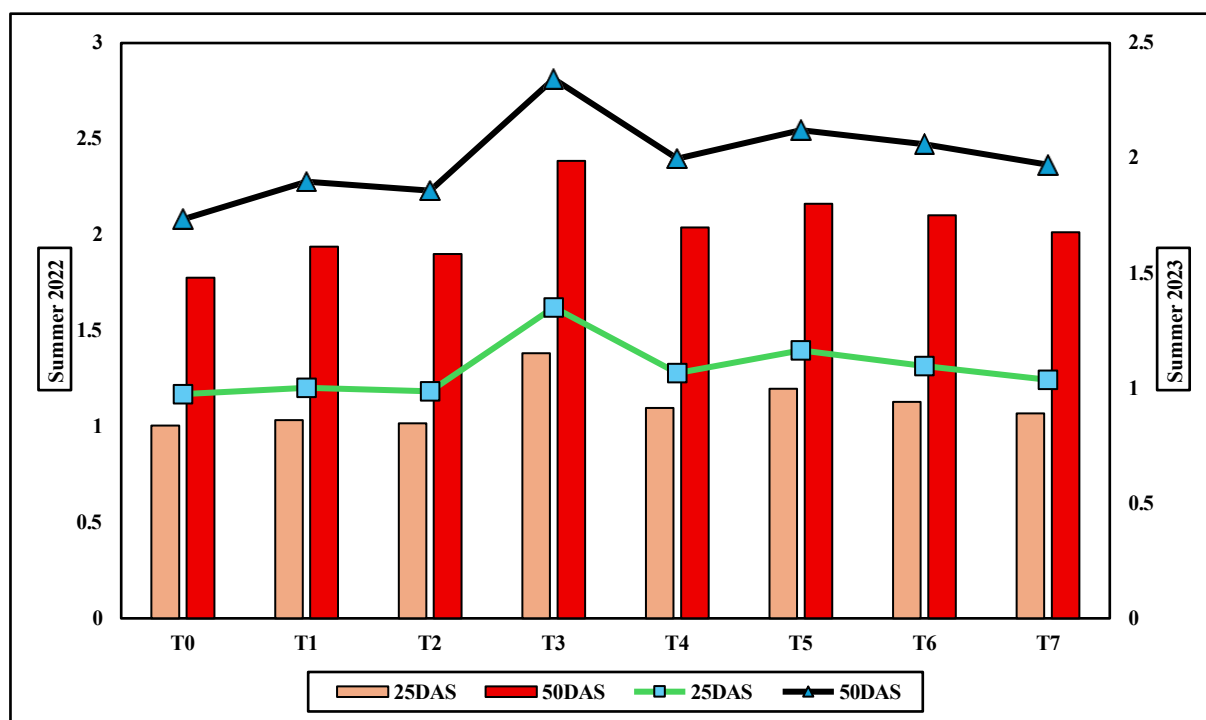
TREATMENTS	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	1.01 ± 0.02	1.78 ± 0.02	0.97 ± 0.02	1.73 ± 0.02
T ₁	1.03 ± 0.01 (2.69)	1.94 ± 0.02 (8.39)	1.00 ± 0.01 (2.75)	1.90 ± 0.02 (8.63)
T ₂	1.02 ± 0.01 (1.18)	1.90 ± 0.03 (6.49)	0.99 ± 0.01 (1.19)	1.86 ± 0.03 (6.69)
T ₃	1.38 ± 0.02 (27.30)	2.39 ± 0.03 (25.55)	1.35 ± 0.02 (27.90)	2.34 ± 0.03 (26.02)
T ₄	1.10 ± 0.00 (8.35)	2.04 ± 0.05 (12.89)	1.07 ± 0.00 (8.58)	2.00 ± 0.05 (13.20)
T ₅	1.20 ± 0.01 (15.96)	2.16 ± 0.03 (17.84)	1.16 ± 0.01 (16.35)	2.12 ± 0.03 (18.24)
T ₆	1.13 ± 0.01 (10.89)	2.10 ± 0.07 (15.46)	1.10 ± 0.01 (11.13)	2.06 ± 0.07 (15.80)
T ₇	1.07 ± 0.01 (5.84)	2.01 ± 0.03 (11.76)	1.04 ± 0.01 (5.98)	1.97 ± 0.03 (12.06)
CD at p=0.05	0.048	0.126	0.049	0.126
Sem±	0.016	0.041	0.016	0.041

Note:

Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM

Values given in parentheses indicate the percent change over the control.

Fig. 4.1.5.a: Effect of the treatment on the leaf area index of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

TABLE 4.1.5.b: Effect of the treatment on the leaf area index of black gram in the *Kharif* season

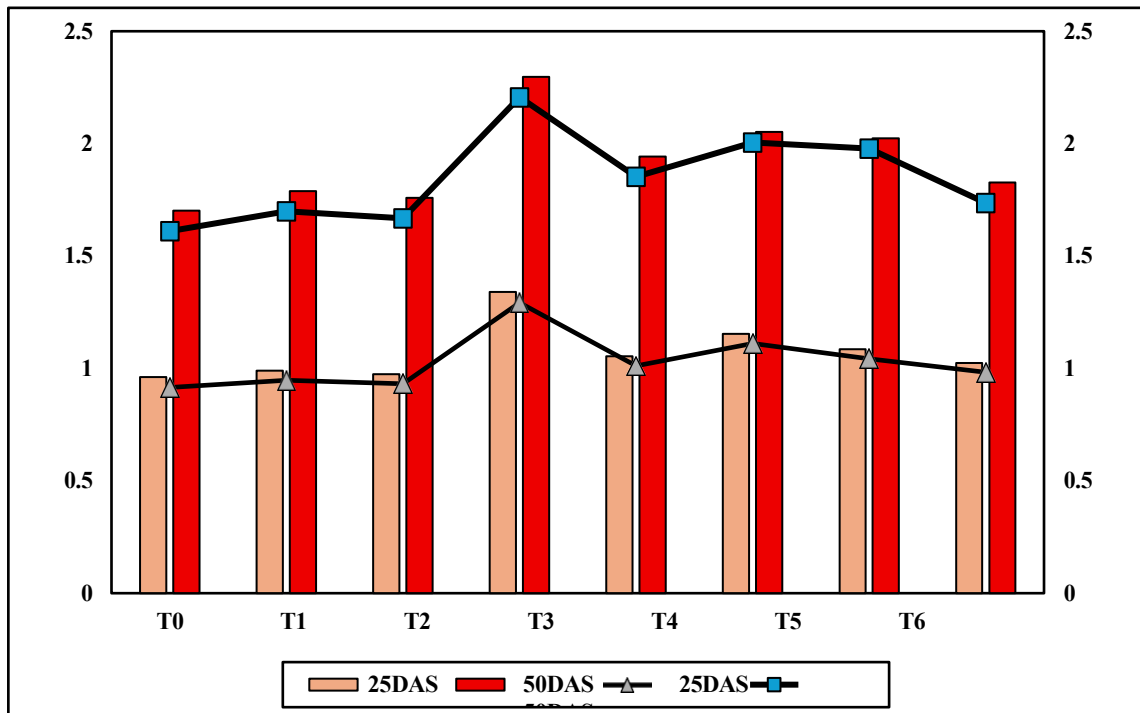
TREATMENTS	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	0.96 ± 0.02	1.70 ± 0.02	0.92 ± 0.00	1.61 ± 0.02
T ₁	0.99 ± 0.01 (2.81)	1.79 ± 0.03 (4.89)	0.95 ± 0.01 (3.36)	1.70 ± 0.03 (5.19)
T ₂	0.97 ± 0.01 (1.23)	1.76 ± 0.01 (3.21)	0.93 ± 0.01 (1.72)	1.67 ± 0.01 (3.43)
T ₃	1.34 ± 0.02 (28.18)	2.30 ± 0.05 (25.90)	1.29 ± 0.01 (29.18)	2.21 ± 0.05 (27.00)
T ₄	1.05 ± 0.00 (8.70)	1.94 ± 0.03 (12.38)	1.01 ± 0.00 (9.47)	1.85 ± 0.03 (13.05)
T ₅	1.15 ± 0.01 (16.57)	2.05 ± 0.02 (17.07)	1.11 ± 0.01 (17.63)	2.01 ± 0.01 (19.67)
T ₆	1.08 ± 0.01 (11.33)	2.02 ± 0.03 (15.89)	1.04 ± 0.01 (12.23)	1.98 ± 0.06 (18.57)
T ₇	1.02 ± 0.01 (6.09)	1.83 ± 0.03 (6.86)	0.98 ± 0.01 (6.82)	1.74 ± 0.03 (7.23)
CD at p=0.05	0.048	0.094	0.036	0.110
Sem±	0.016	0.031	0.012	0.051

Note:

Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM

Values given in parentheses indicate the percent change over the control.

Fig 4.1.5.b: Effect of the treatment on the leaf area index of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.1.6. LEAF AREA DURATION

The effects of foliar application of polyamines on black gram growth, yield, and quality were assessed during the two seasons (summer and *Kharif*) of 2022 and 2023. Leaf area duration was measured 25 and 50 days after sowing (DAS), with detailed results in Table 4.1.6.a and 4.1.6.b and illustrated in Figures 4.1.6.a and 4.1.6.b.

During the Summer season of 2022, significant differences among treatments were observed on the basis of CD at 5% level (CD = 2.822). The highest LAD (42.4 days) was recorded under T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M), which was significantly superior to control (31.8 days), as the difference (10.6 days) exceeded the CD value. Treatments T5 (39.1 days), T6 (38.4 days), T4 (37.3 days), and T7 (37.0 days) were also significantly superior to control since their differences (7.3, 6.6, 5.5, and 5.2 days, respectively) were greater than CD. T1 (35.6 days) and T2 (34.8 days) showed moderate improvement and were significantly higher than control but remained inferior to T3. A similar trend was observed during Summer 2023 (CD = 2.827). T3 recorded the maximum LAD (41.7 days) and was significantly superior to control (31.2 days), as the difference (10.5 days) exceeded CD. Treatments T5 (38.5 days), T6 (37.8 days), T4 (36.6 days), and T7 (36.3 days) were also significantly higher than control. T1 (34.9 days) and T2 (34.1 days) showed comparatively lower increases but remained significantly superior to control.

During the Kharif season of 2022, significant variation among treatments was observed (CD = 2.254). T3 recorded the highest LAD (40.7 days), which was significantly superior to control (30.5 days), as the difference (10.2 days) exceeded CD. Treatments T5 (36.9 days) and T6 (37.0 days) were also significantly superior to control. T4 (35.4 days) showed significant improvement, whereas T1 (32.4 days) and T2 (31.8 days) exhibited smaller increases; T2 remained statistically closer to control due to lower difference. In Kharif 2023 (CD = 2.602), T3 again recorded the maximum LAD (39.0 days) and was significantly superior to control (28.8 days), as the difference (10.2 days) exceeded CD. Treatments T5 (36.2 days) and T6 (36.4 days) also showed significant superiority over control. T4 (33.7 days) was significantly higher than control, whereas T1 (30.6 days) and T2 (30.1 days) showed marginal increases; T2 was statistically at par or close to control due to smaller difference relative to CD. T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M) had the highest level of LAD in each year and across all seasons. The other treatments were not statistically as significant as T3, with T5 and T6 being the next most significant statistically on a CD basis. The treatment with the least

amount of improvement on a CD basis compared to the control group was T2 (Cadaverine 1000 μ M only).

The observed increase in LAD with polyamine application can be attributed to their effect on delaying senescence, maintaining membrane stability, enhancing chlorophyll specificity, and rate of photosynthesis. Particularly with the use of a combination of polyamines, the level of protein synthesis and nitrogen metabolism improved while being able to scavenge reactive oxygen species (ROS), thereby prolonging functional leaf area. The physiological and morphological implications of improved LAD promote prolonged canopy duration, prolonged light interception, and subsequently enhanced assimilatory production during critical time-critical growth stages, resulting in increased LAD. These physiological enhancements are further supported by previous literature highlighting the effectiveness of polyamines in maintaining leaf area and leaf function at essential growth stages.

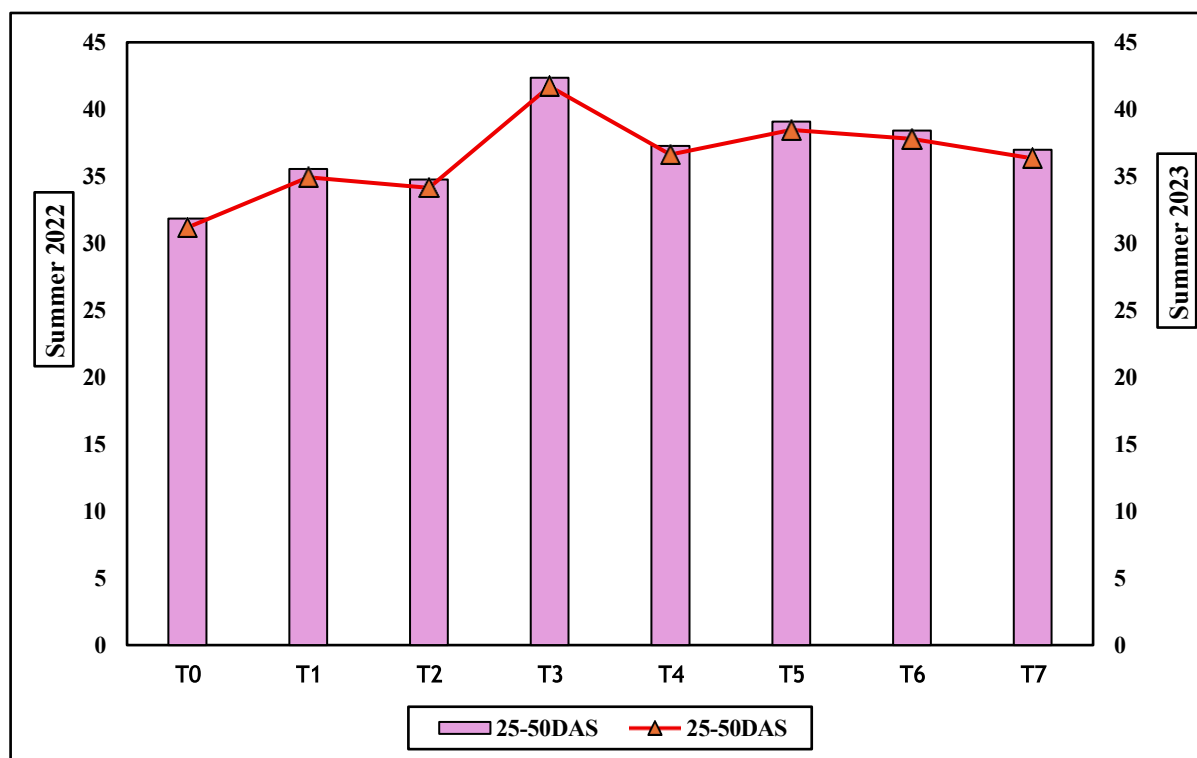
TABLE 4.1.6.a: Effect of the treatment on the leaf area duration (days) of black gram in the Summer season

TREATMENTS	Summer (2022)	Summer (2023)
	25-50DAS	25-50DAS
T ₀	31.8 ± 0.6	31.2 ± 0.6
T ₁	35.6 ± 0.5 (10.47)	34.9 ± 0.5 (10.73)
T ₂	34.8 ± 0.8 (8.44)	34.1 ± 0.8 (8.68)
T ₃	42.4 ± 0.6 (24.83)	41.7 ± 0.6 (25.26)
T ₄	37.3 ± 1.5 (14.55)	36.6 ± 1.5 (14.88)
T ₅	39.1 ± 0.8 (18.55)	38.5 ± 0.8 (18.95)
T ₆	38.4 ± 1.6 (17.13)	37.8 ± 1.6 (17.49)
T ₇	37.0 ± 0.6 (13.90)	36.3 ± 0.6 (14.22)
CD at p=0.05	2.822	2.827
Sem±	0.921	0.923

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.6.a: Effect of the treatment on the leaf area duration of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

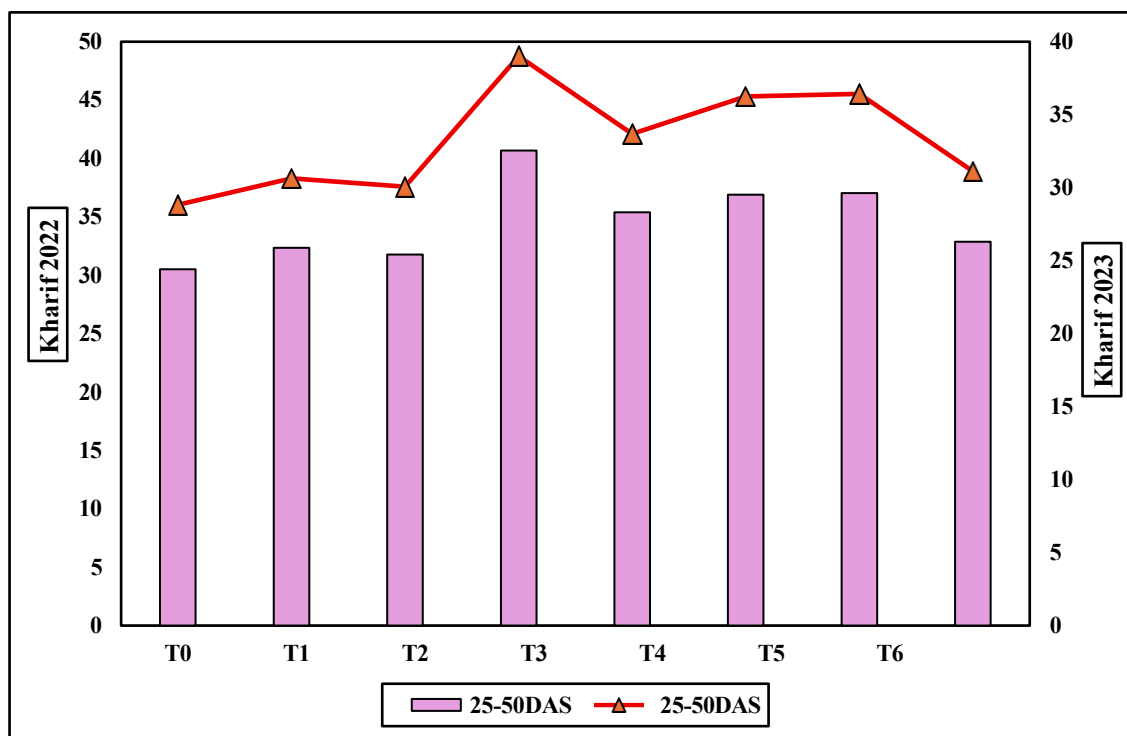
TABLE 4.1.6.b: Effect of the treatment on the leaf area duration of black gram in the *Kharif* season

TREATMENTS	<i>Kharif</i> (2022)	<i>Kharif</i> (2023)
	25-50DAS	25-50DAS
T ₀	30.5 ± 0.6	28.8 ± 0.6
T ₁	32.4 ± 0.7 (5.68)	30.6 ± 0.7 (5.91)
T ₂	31.8 ± 0.1 (3.97)	30.1 ± 0.1 (4.09)
T ₃	40.7 ± 1.0 (24.96)	39.0 ± 1.2 (26.10)
T ₄	35.4 ± 0.8 (13.75)	33.7 ± 0.8 (14.40)
T ₅	36.9 ± 0.7 (17.26)	36.2 ± 0.6 (20.46)
T ₆	37.0 ± 0.7 (17.56)	36.4 ± 1.3 (20.84)
T ₇	32.9 ± 0.8 (7.16)	31.1 ± 0.8 (7.39)
CD at p=0.05	2.254	2.602
Sem±	0.736	0.850

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.6.b: Effect of the treatment on the leaf area duration of black gram in the *Kharif* season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.1.7. DAYS TAKEN FOR 50% FLOWERING

The effects of foliar application of polyamines on black gram growth, yield, and quality were assessed during the two seasons (summer and *Kharif*) of 2022 and 2023. Days taken for 50% flowering were measured, with detailed results in Table 4.1.7.a and 4.1.7.b and illustrated in Figures 4.1.7.a and 4.1.7.b.

During Summer 2022, significant differences among treatments were observed (CD at $p=0.05 = 1.101$). The control (T0) took the maximum number of days (45.71 days) to reach 50% flowering. The minimum days were recorded under T3 (Putrescine 1500 μM + Cadaverine 1000 μM), i.e., 40.35 days, which was significantly earlier than control as the difference (5.36 days) exceeded the CD value. Treatment T5 (41.37 days) and T6 (42.37 days) were also significantly earlier than control, as their differences (4.34 and 3.34 days, respectively) were greater than CD. T4 (43.34 days), T1 (43.71 days), and T7 (43.68 days) also showed significant reduction compared to control. However, T2 (44.71 days) showed only a slight reduction (1.00 day), which was marginally lower and statistically less pronounced compared to superior treatments.

During Summer 2023 (CD = 1.835), a similar trend was observed. The control recorded the highest number of days (46.37 days), whereas T3 recorded the minimum (41.30 days), showing a significant reduction of 5.07 days over control. Treatments T5 (41.67 days) and T6 (42.71 days) were also significantly earlier than control. T4, T1, and T7 showed moderate but significant reduction, whereas T2 (45.00 days) remained closer to control and was statistically less effective compared to combination treatments.

In Kharif 2022, significant variation was observed among treatments (CD = 1.083). The control took maximum days (46.94 days) for 50% flowering. The earliest flowering was recorded under T3 (41.58 days), which was significantly earlier than control as the difference (5.36 days) exceeded CD. Treatments T5 (42.53 days) and T6 (43.60 days) were also significantly earlier than control. T4, T1, and T7 showed moderate but significant reductions, while T2 (45.94 days) showed minimal reduction and was statistically closer to control. In Kharif 2023 (CD = 1.794), the same pattern persisted. The control recorded 47.59 days, whereas T3 recorded the minimum (42.47 days), which was significantly earlier as the difference (5.12 days) exceeded CD. Treatments T5 (42.90 days) and T6 (43.88 days) also

showed significant reduction over control. T4 and T1 were moderately earlier, while T2 (46.23 days) remained comparatively closer to control.

The foliar application of polyamines affected the days to 50% flowering. For both summer seasons 2022 and 2023, the average number of days to flowering (level of flowering response) was lowest under treatment T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M). It appears T3 reached flowering sooner, which also suggests T3 is the combination of polyamines that influences flowering at the proper time. This quicker flowering response was likely also due to the interaction of putrescine/cadaverine. These two apply in unison and interact synergistically. This may also help enhance hormonal regulation, for example, when interacting with auxins and gibberellins involved in floral induction and flowering. Polyamines are also engaged with membrane stabilization; however, stabilizing membranes ultimately regulates transcription of some genes and lowers oxidative stress, which would allow for a more supportive internal environment for their early reproductive development.

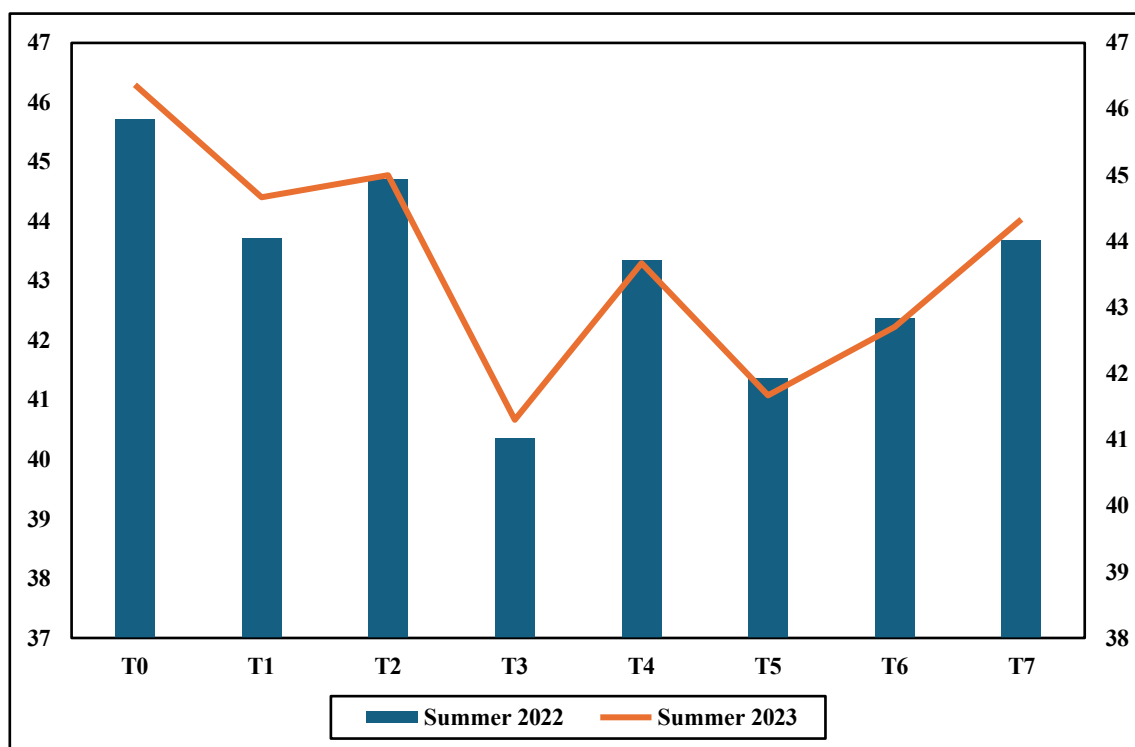
TABLE 4.1.7.a: Effect of the treatment on the days taken for 50% flowering of black gram in the *Summer* season

TREATMENTS	<i>Summer</i> (2022)	<i>Summer</i> (2023)
T ₀	45.71 ± 0.35	46.37 ± 0.08
T ₁	43.71 ± 0.35 (-4.58)	44.67 ± 0.88 (-3.81)
T ₂	44.71 ± 0.35 (-2.23)	45.00 ± 0.57 (-3.04)
T ₃	40.35 ± 0.33 (-13.28)	41.30 ± 0.35 (-12.27)
T ₄	43.34 ± 0.33 (-5.45)	43.67 ± 0.66 (-6.18)
T ₅	41.37 ± 0.31 (-10.48)	41.67 ± 0.33 (-11.27)
T ₆	42.37 ± 0.31 (-7.87)	42.71 ± 0.30 (-8.57)
T ₇	43.68 ± 0.33 (-4.65)	44.33 ± 0.33 (-4.59)
CD at p=0.05	1.101	1.835
Sem±	0.359	0.599

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.7.a: Effect of the treatment on the days taken for 50% flowering of black gram in the *Summer* season



Note: T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

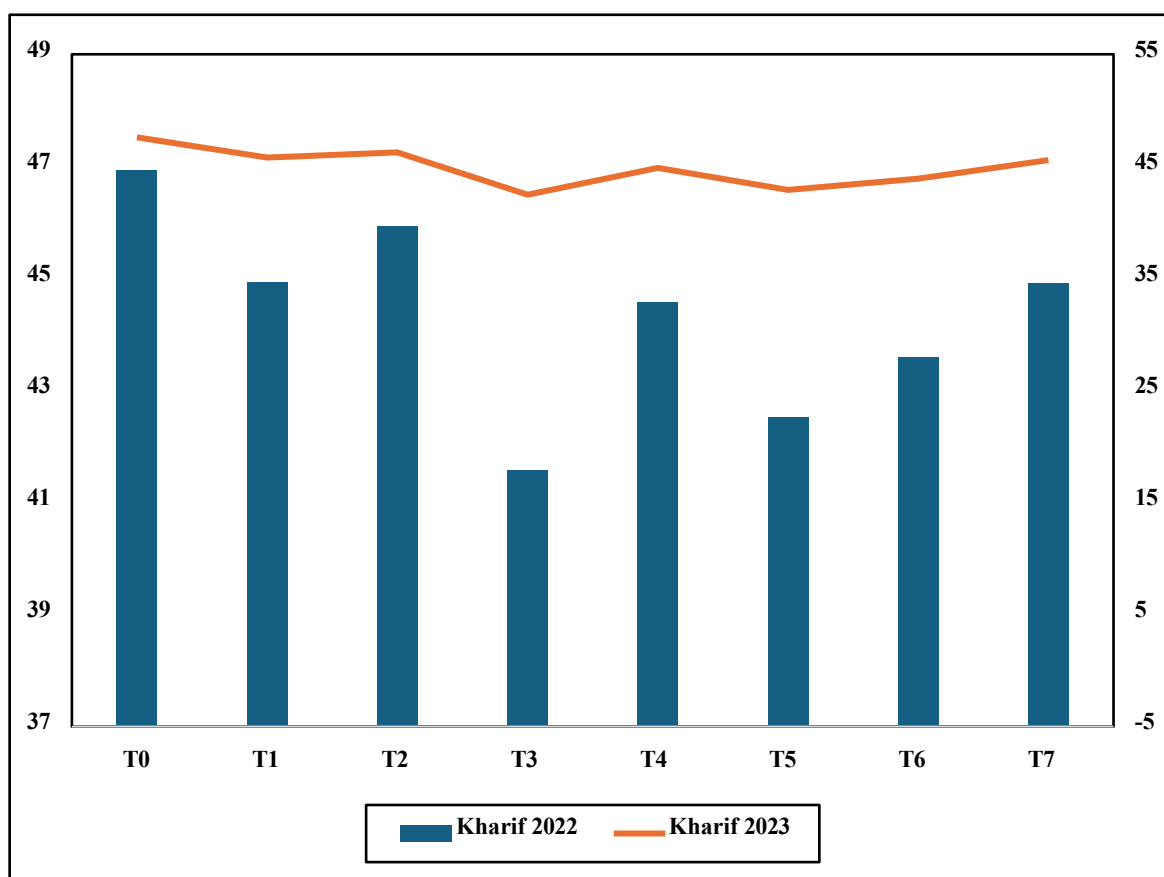
TABLE 4.1.7.b: Effect of the treatment on the days taken for 50% flowering of black gram in the *Kharif* season

TREATMENTS	<i>Kharif</i> (2022)	<i>Kharif</i> (2023)
T ₀	46.94 ± 0.34	47.59 ± 0.86
T ₁	44.94 ± 0.35 (-4.45)	45.80 ± 0.84 (-3.89)
T ₂	45.94 ± 0.35 (-2.17)	46.23 ± 0.57 (-2.93)
T ₃	41.58 ± 0.34 (-12.88)	42.47 ± 0.38 (-12.06)
T ₄	44.57 ± 0.34 (-5.30)	44.86 ± 0.63 (-6.08)
T ₅	42.53 ± 0.25 (-10.35)	42.90 ± 0.33 (-10.92)
T ₆	43.60 ± 0.31 (-7.64)	43.88 ± 0.26 (-8.44)
T ₇	44.91 ± 0.32 (-4.52)	45.55 ± 0.34 (-4.47)
CD at p=0.05	1.083	1.794
Sem±	0.353	0.586

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.7.b: Effect of the treatment on the days taken for 50% flowering of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.1.8. DAYS TAKEN FOR MATURITY

The effects of foliar application of polyamines on black gram growth, yield, and quality were assessed during the two seasons (summer and *Kharif*) of 2022 and 2023. Days taken for maturity were measured, with detailed results in Table 4.1.8.a and 4.1.8.b and illustrated in Figures 4.1.8.a and 4.1.8.b.

During Summer 2022, significant differences among treatments were observed (CD at $p=0.05 = 1.214$). The control (T0) took the maximum number of days (65.67 days) to reach maturity. The minimum days were recorded under T3 (Putrescine 1500 μM + Cadaverine 1000 μM) with 60.48 days, which was 8.58% earlier than control. The reduction of 5.19 days over control exceeded the CD value, indicating statistical significance. Treatment T5 (61.33 days) reduced maturity duration by 7.07%, and T6 (62.59 days) by 4.92% compared to control; both differences (4.34 and 3.08 days, respectively) were greater than CD and hence statistically significant. T4 (63.39 days), T1 (63.67 days), and T7 (63.70 days) also showed significant reductions (3.60%, 3.15%, and 3.10%, respectively), as their differences exceeded CD. However, T2 (64.71 days) showed only 1.49% reduction (0.96 day), which was less than CD and therefore remained statistically at par with control.

During Summer 2023 (CD = 1.944), similar results were observed. The control recorded 66.33 days, whereas T3 recorded 61.00 days, showing an 8.74% reduction over control. The difference of 5.33 days exceeded CD, confirming statistical significance. T5 (61.67 days) and T6 (62.67 days) reduced maturity by 7.57% and 5.85%, respectively, and were significantly superior to control. T4 (63.67 days) showed 4.19% reduction and was also significant. T1 (64.67 days) and T7 (64.33 days) showed smaller reductions (2.58% and 3.11%) and were marginally significant, whereas T2 (65.00 days) reduced maturity by only 2.05% and remained closer to control.

In *Kharif* 2022, significant variation was observed among treatments (CD = 1.245). The control took 66.62 days to reach maturity. T3 recorded the minimum (61.47 days), which was 8.37% earlier than control. The reduction of 5.15 days exceeded CD, indicating significant superiority. T5 (62.29 days) and T6 (63.56 days) reduced maturity by 6.94% and 4.82%, respectively, and were significantly earlier than control. T4 (64.37 days) and T1 (64.62 days) also showed significant reductions (3.49% and 3.09%). However, T2 (65.69 days) showed only 1.42% reduction (0.93 day), which was less than CD and thus statistically at par with control.

During Kharif 2023 ($CD = 2.001$), the same trend persisted. The control recorded 67.16 days, while T3 recorded 61.83 days, resulting in an 8.63% reduction over control. The difference of 5.33 days exceeded CD , confirming statistical significance. T5 (62.23 days) and T6 (63.55 days) showed reductions of 7.93% and 5.68%, respectively, and were significantly earlier than control. T4 (64.55 days) also showed significant reduction (4.05%). T1 (65.65 days) and T7 (65.01 days) showed moderate reductions (2.31% and 3.31%), whereas T2 (65.93 days) showed only 1.87% reduction and remained statistically closer to control.

The expected decrease in days taken for maturity seen under polyamine treatments appears very promising, especially under combined applications of putrescine 1500 μM and cadaverine 1000 μM , as seen here. This indicates a likely acceleration of physiological and developmental stages in black gram, allowing for an expected timely maturity. The reduction could result from polyamines acting synergistically to improve the critical source-sink relationship while enhancing nutrient allocation and managing the similarity of oxidative stress, such that the crop cycle can be completed faster. Moderate combinations also produced outcomes, while individual ones had minimal consequences, suggesting that both concentration and combinations of polyamines can provide improved cropping duration for better management and production in the field.

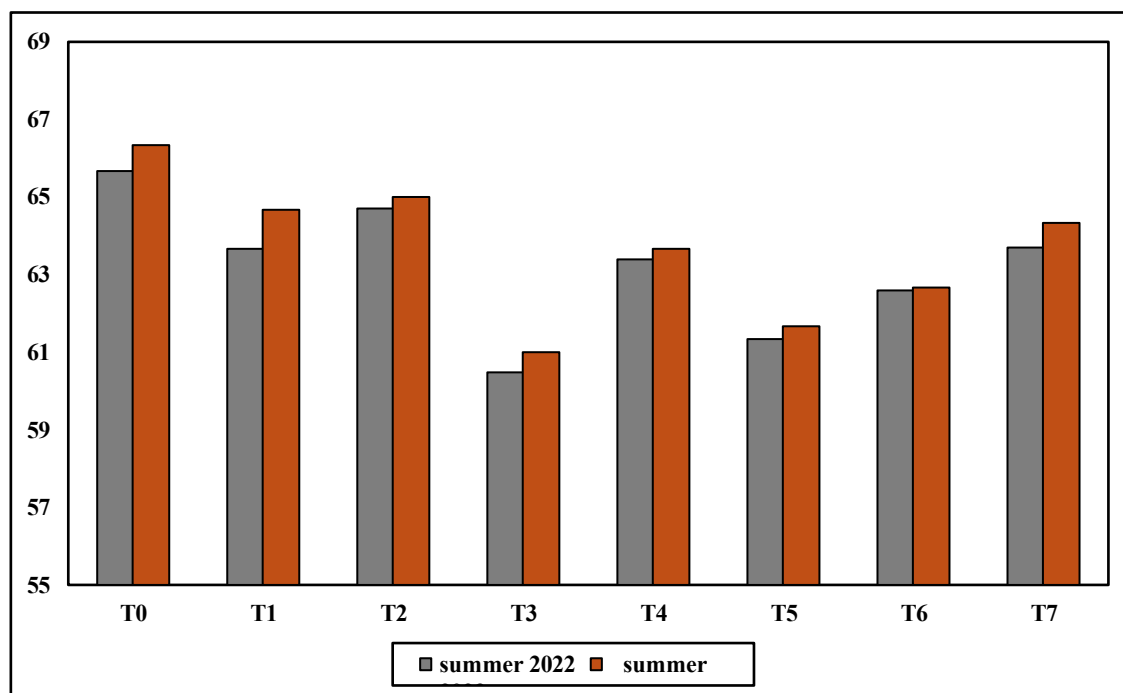
TABLE 4.1.8.a: Effect of the treatment on the days taken for the maturity of black gram in the *Summer* season

TREATMENTS	<i>Summer</i> (2022)	<i>Summer</i> (2023)
T ₀	65.67 ± 0.32	66.33 ± 0.88
T ₁	63.67 ± 0.33 (-3.15)	64.67 ± 0.88 (-2.58)
T ₂	64.71 ± 0.29 (-1.49)	65.00 ± 0.57 (-2.05)
T ₃	60.48 ± 0.35 (-8.58)	61.00 ± 0.57 (-8.74)
T ₄	63.39 ± 0.30 (-3.60)	63.67 ± 0.66 (-4.19)
T ₅	61.33 ± 0.33 (-7.07)	61.67 ± 0.33 (-7.57)
T ₆	62.59 ± 0.59 (-4.92)	62.67 ± 0.33 (-5.85)
T ₇	63.70 ± 0.34 (-3.10)	64.33 ± 0.33 (-3.11)
CD at p=0.05	1.214	1.944
Sem±	0.396	0.635

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.8.a: Effect of the treatment on the days taken for maturity of black gram in the *Summer* season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

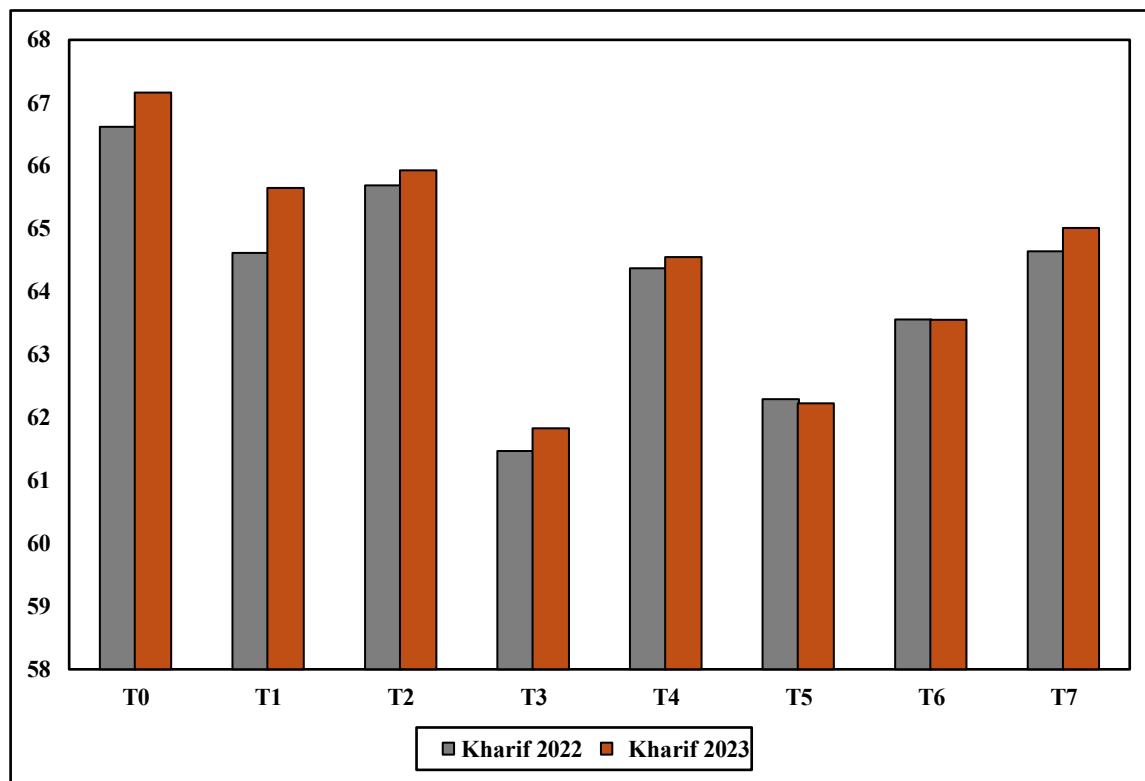
TABLE 4.1.8.b: Effect of the treatment on the days taken for maturity of black gram in the *Kharif* season

TREATMENTS	<i>Kharif</i> (2022)	<i>Kharif</i> (2023)
T ₀	66.62 ± 0.36	67.16 ± 1.07
T ₁	64.62 ± 0.31 (-3.09)	65.65 ± 0.88 (-2.31)
T ₂	65.69 ± 0.29 (-1.42)	65.93 ± 0.58 (-1.87)
T ₃	61.47 ± 0.36 (-8.37)	61.83 ± 0.51 (-8.63)
T ₄	64.37 ± 0.30 (-3.49)	64.55 ± 0.66 (-4.05)
T ₅	62.29 ± 0.35 (-6.94)	62.23 ± 0.22 (-7.93)
T ₆	63.56 ± 0.61 (-4.82)	63.55 ± 0.28 (-5.68)
T ₇	64.64 ± 0.33 (-3.05)	65.01 ± 0.55 (-3.31)
CD at p=0.05	1.245	2.001
Sem±	0.406	0.653

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.8.b: Effect of the treatment on the days taken for maturity of black gram in the *Kharif* season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.1.9. ROOT LENGTH

The effects of foliar application of polyamines on black gram growth, yield, and quality were assessed during the two seasons (summer and *Kharif*) of 2022 and 2023. Root length was measured at 25 and 50 days after sowing (DAS), with detailed results presented in Table 4.1.9.a and 4.1.9.b and illustrated in Figures 4.1.9.a and 4.1.9.b.

During Summer 2022, significant variation among treatments was observed ($CD_{0.05} = 0.242$ at 25 DAS and 0.400 at 50 DAS). At 25 DAS, T3 recorded the maximum root length (15.08 cm), which was 43.51% higher than control (8.52 cm), and the difference exceeded CD, indicating statistical significance. At 50 DAS, T3 (22.19 cm) showed 27.84% higher root length than control (16.01 cm), which was also significant on CD basis. Treatments T5 and T6 were statistically at par with T3 and significantly superior to control, showing 24–41% increase over control.

During Summer 2023, a similar trend was recorded ($CD = 0.569$ at 25 DAS and 0.351 at 50 DAS). T3 showed 40.88% and 31.06% higher root length than control at 25 and 50 DAS, respectively, and remained significantly superior as differences exceeded CD values.

In Kharif 2022 and 2023, significant differences were again observed (CD ranged from 0.400 to 0.588). T3 recorded the highest root length at both stages, showing 41–42% increase at 25 DAS and 31–32% increase at 50 DAS over control, and was statistically superior. Treatments T5 and T6 also showed significant improvement over control, whereas individual applications exhibited comparatively moderate increases. Overall, T3 (Putrescine 1500 μM + Cadaverine 1000 μM) consistently recorded significantly higher root length (27–43% increase over control) across seasons and years on CD basis, indicating the beneficial effect of combined polyamine application on root development.

The increase in root length observed in response to polyamine treatments, with a specific emphasis on T3 (putrescine 1500 μM + cadaverine 1000 μM), indicates a synergistic nature of the compounds in facilitating early root growth in black grams. Polyamines help roots grow longer by encouraging cells to divide and expand in the root tips, while also helping to keep cell membranes stable and managing growth and stress-related genes. Both the increased root lengths at both 25 and 50 DAS suggest the potential for improved water and nutrient uptake capacity and improved plant vigor. Since the foundation for overall root length is established in

early growth, the size of the roots could also indicate enhanced water and nutrient uptake capacity and improved plant vigor. T5 and T6 combinations of moderate concentrations of the polyamines also had better results than individual treatments. Overall, together the results suggest that the concentration of polyamines and the ratio of polyamines would be critical in determining how roots develop in the early developmental stage.

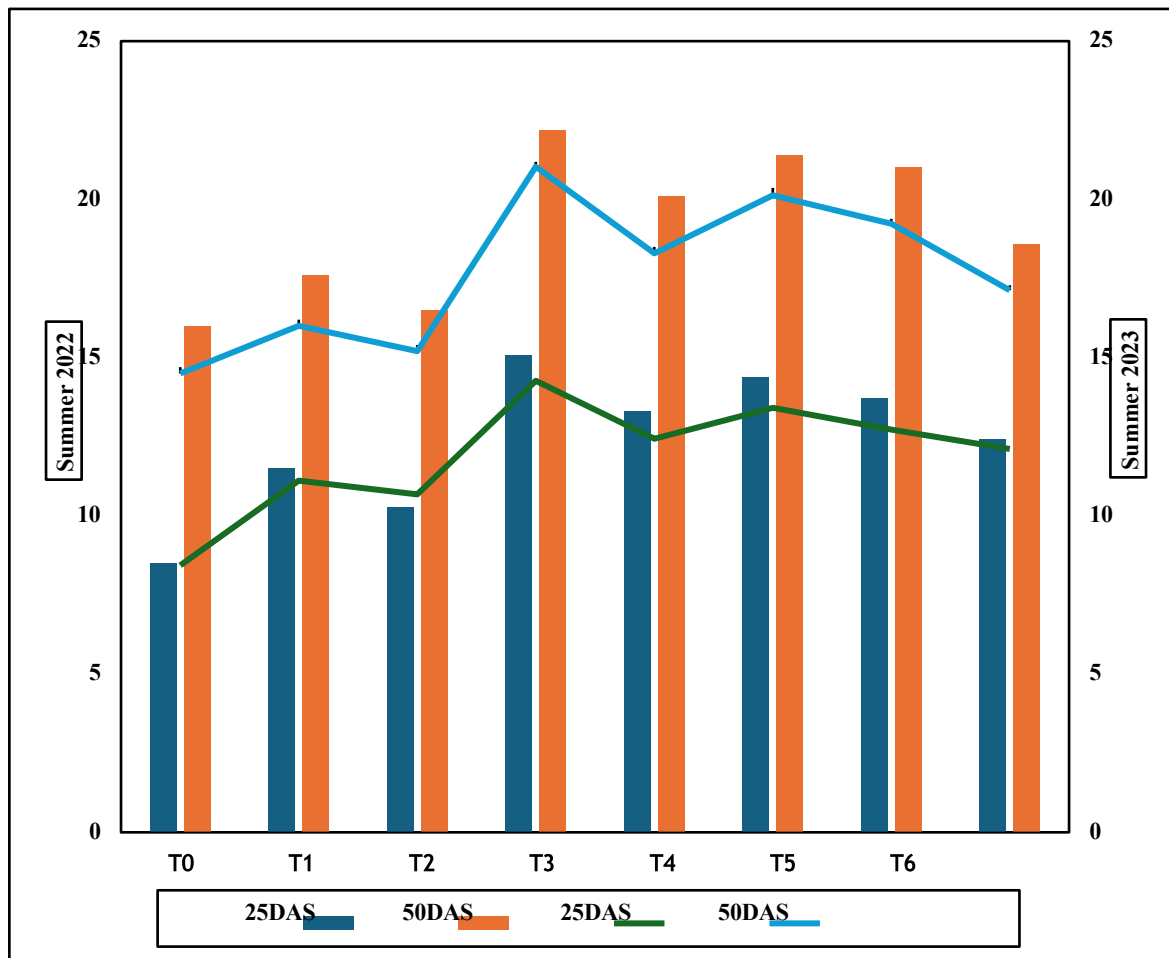
TABLE 4.1.9.a: Effect of the treatment on the root length (cm) of black gram in the *Summer* season

TREATMENTS	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	8.52 ± 0.04	16.01 ± 0.16	8.44 ± 0.28	14.50 ± 0.11
T ₁	11.50 ± 0.15 (25.94)	17.61 ± 0.06 (9.07)	11.12 ± 0.24 (24.11)	16.00 ± 0.11 (9.38)
T ₂	10.28 ± 0.10 (17.18)	16.51 ± 0.14 (3.03)	10.68 ± 0.29 (20.98)	15.20 ± 0.11 (4.61)
T ₃	15.08 ± 0.09 (43.51)	22.19 ± 0.04 (27.84)	14.27 ± 0.18 (40.88)	21.03 ± 0.08 (31.06)
T ₄	13.32 ± 0.13 (36.06)	20.09 ± 0.11 (20.32)	12.43 ± 0.13 (32.14)	18.30 ± 0.11 (20.77)
T ₅	14.38 ± 0.09 (40.76)	21.41 ± 0.10 (25.22)	13.42 ± 0.07 (37.12)	20.13 ± 0.13 (27.98)
T ₆	13.73 ± 0.08 (37.97)	21.01 ± 0.16 (23.80)	12.73 ± 0.08 (33.73)	19.23 ± 0.08 (24.61)
T ₇	12.43 ± 0.03 (31.48)	18.58 ± 0.17 (13.82)	12.11 ± 0.23 (30.33)	17.13 ± 0.08 (15.37)
CD at p=0.05	0.242	0.400	0.569	0.351
Sem±	0.079	0.130	0.186	0.114

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.9.a: Effect of the treatment on the root length (cm) of black gram in the *Summer* season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

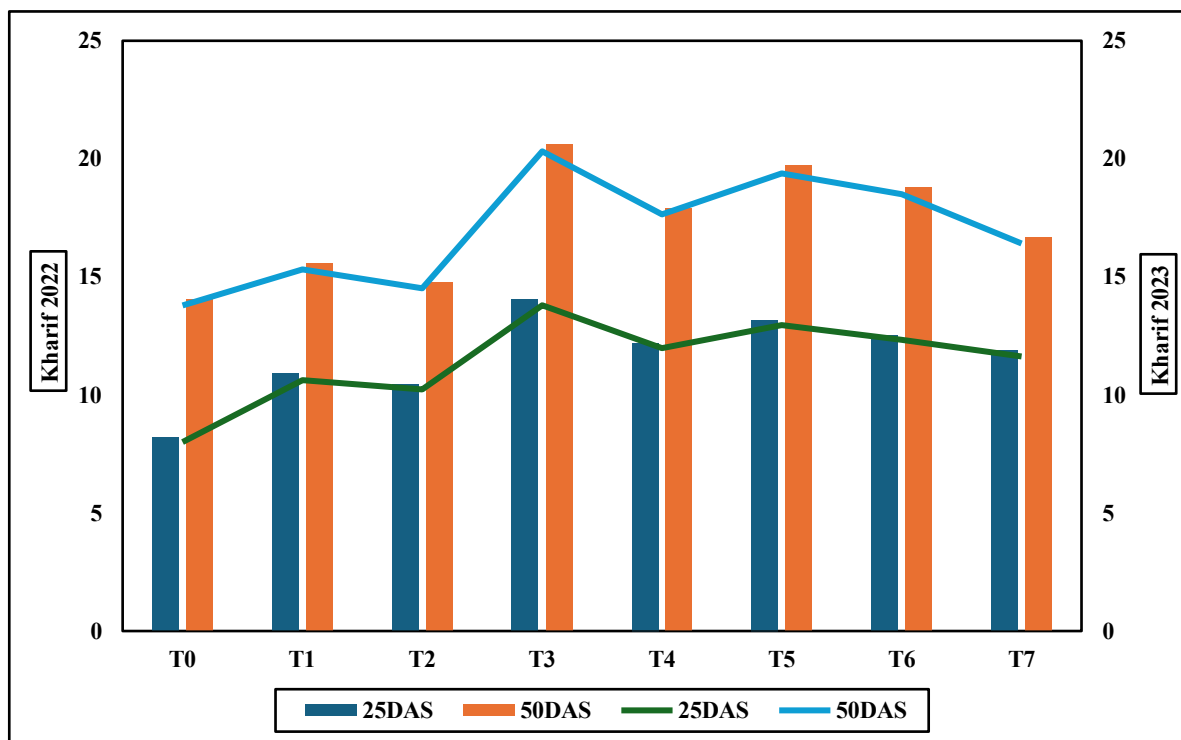
TABLE 4.1.9.b: Effect of the treatment on the root length (cm) of black gram in the *Kharif* season

TREATMENTS	KHARIF (2022)		KHARIF (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	8.20 ± 0.27	14.06 ± 0.11	8.00 ± 0.25	13.79 ± 0.11
T ₁	10.89 ± 0.26 (24.69)	15.57 ± 0.14 (9.68)	10.63 ± 0.25 (24.71)	15.32 ± 0.12 (9.97)
T ₂	10.44 ± 0.32 (21.45)	14.75 ± 0.14 (4.68)	10.23 ± 0.34 (21.74)	14.51 ± 0.13 (4.96)
T ₃	14.05 ± 0.16 (41.61)	20.62 ± 0.06 (31.80)	13.80 ± 0.19 (41.99)	20.32 ± 0.05 (32.12)
T ₄	12.20 ± 0.14 (32.76)	17.88 ± 0.13 (21.38)	11.98 ± 0.14 (33.21)	17.65 ± 0.14 (21.88)
T ₅	13.17 ± 0.05 (37.73)	19.72 ± 0.14 (28.69)	12.95 ± 0.04 (38.21)	19.38 ± 0.15 (28.84)
T ₆	12.51 ± 0.06 (34.41)	18.80 ± 0.11 (25.23)	12.34 ± 0.08 (35.14)	18.51 ± 0.10 (25.49)
T ₇	11.90 ± 0.26 (31.05)	16.69 ± 0.10 (15.76)	11.62 ± 0.25 (31.14)	16.41 ± 0.11 (15.98)
CD at p=0.05	0.588	0.403	0.576	0.400
Sem±	0.192	0.131	0.188	0.131

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.9.b: Effect of the treatment on the root length (cm) of black gram in the *Kharif* season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.1.10. ROOT NODULES

The effects of foliar application of polyamines on black gram growth, yield, and quality were assessed during the two seasons (summer and *Kharif*) of 2022 and 2023. Root nodules were measured at 25 and 50 days after sowing (DAS), with detailed results presented 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, the treatments for T3 produced significantly different results than the other treatments that measured either 2.879 or 4.076 (CD for 25DAS and 50DAS, respectively). The treatment that had the most nodules on July 25th was T3 (63.20) and provided a 50.72 percent improvement over the Control (31.15). The difference between T3 and the Control (32.05) exceeded the CD for 25DAS, meaning T3 was statistically better than the Control. Then at 50DAS, T3 (72.90) produced 38.42 percent more nodules than the Control (44.89). This was also statistically different from the Control using the CD as a comparison. Treatments T5 (46.44 and 33.47) and T6 (42.49 and 32.26) were statistically similar to T3 and significantly better than the Control.

In summer 2023, T3 and the other treatments produced similar results (CD 3.135 and 3.010 for 25DAS and 50DAS, respectively). T3 produced 61.72 and 69.96 nodules at 25DAS and 50DAS, respectively, for a 51.09 percent and 37.91 percent increase over the Control. T3 continued to produce a statistically greater quantity of nodules at both 25DAS and 50DAS than the Control treatment because the difference values between T3 and the Control were greater than the CD. While treatments T5 and T6 continued to produce a statistically significant number of nodules more than the Control treatment, they were still less than T3; thus, T1 and T2 had much lower percentage increases in nodules than T3.

During Kharif 2022, significant variation was observed (CD = 1.159 at 25 DAS and 2.700 at 50 DAS). T3 recorded maximum nodules (60.86 and 68.65 at 25 and 50 DAS), showing 51.31% and 37.91% increase over control (29.63 and 42.62), and differences exceeded CD values. Treatments T5 and T6 also showed significant improvement ranging from 33–48% over control.

Similarly, in Kharif 2023 (CD = 1.687 at 25 DAS and 2.502 at 50 DAS), T3 recorded 60.00 and 67.60 nodules, reflecting 52.30% and 38.16% increase over control, and was statistically superior. Other combination treatments remained significantly better than control, whereas T1 and T2 showed moderate enhancement.

The significant increase in root nodules as a result of the polyamine treatments, most particularly T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M), explained the contribution of these compounds to improving symbiotic nitrogen fixation in black gram. Polyamines affect the root–rhizobia processes by promoting root hair formation, membrane stabilization, and modulation signalling pathways of nodulation. Furthermore, polyamines likely influence the regulation of oxidative stress and gene expression that support the initial nodule inception and the development of additional nodules. Individual applications of plant growth products, including other combinations/assays, indicated that combinatorial effects were improved in the treatment compared with their applications, demonstrating the impact of concentration synergy. Improved nodulation leads not only to greater nitrogen assimilation but also can enhance plant vigor and productivity in both summer crops and *Kharif* ones.

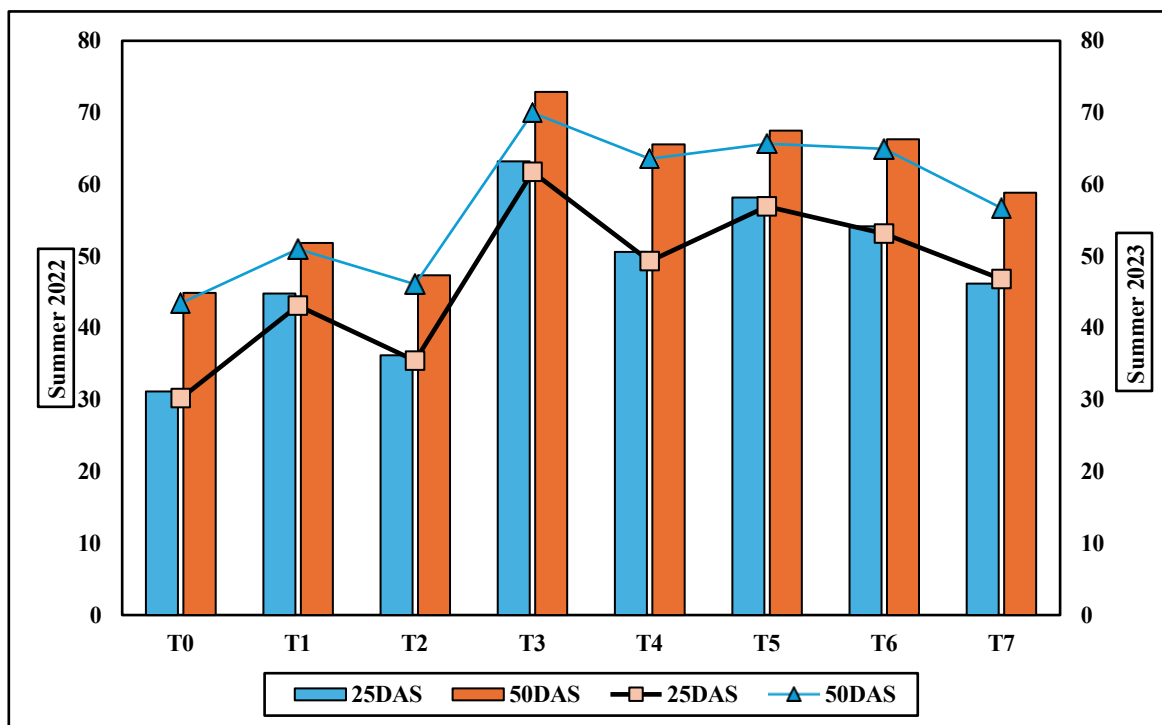
TABLE 4.1.10.a: Effect of the treatment on the number of root nodules plant⁻¹ of black gram in the *Summer* season

TREATMENTS	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	31.15 ± 0.58	44.89 ± 0.96	30.19 ± 0.50	43.44 ± 0.82
T ₁	44.78 ± 2.11 (30.44)	51.86 ± 0.95 (13.43)	43.10 ± 2.82 (29.96)	50.99 ± 0.73 (14.80)
T ₂	36.19 ± 0.48 (13.94)	47.33 ± 0.79 (5.16)	35.41 ± 0.52 (14.73)	46.07 ± 0.64 (5.70)
T ₃	63.20 ± 0.55 (50.72)	72.90 ± 1.39 (38.42)	61.72 ± 0.45 (51.09)	69.96 ± 0.96 (37.91)
T ₄	50.60 ± 0.85 (38.44)	65.56 ± 2.17 (31.53)	49.31 ± 0.81 (38.78)	63.55 ± 1.59 (31.64)
T ₅	58.15 ± 0.51 (46.44)	67.48 ± 1.23 (33.47)	56.93 ± 0.47 (46.97)	65.64 ± 0.96 (33.82)
T ₆	54.16 ± 0.61 (42.49)	66.26 ± 0.58 (32.26)	53.14 ± 0.64 (43.19)	64.96 ± 0.46 (33.13)
T ₇	46.17 ± 0.57 (32.54)	58.82 ± 1.69 (23.68)	46.78 ± 1.73 (35.46)	56.70 ± 1.35 (23.38)
CD at p=0.05	2.879	4.076	3.135	3.010
Sem±	0.940	1.331	1.024	0.983

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Figure 4.1.10.a: Effect of the treatment on the number of root nodules plant⁻¹ of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

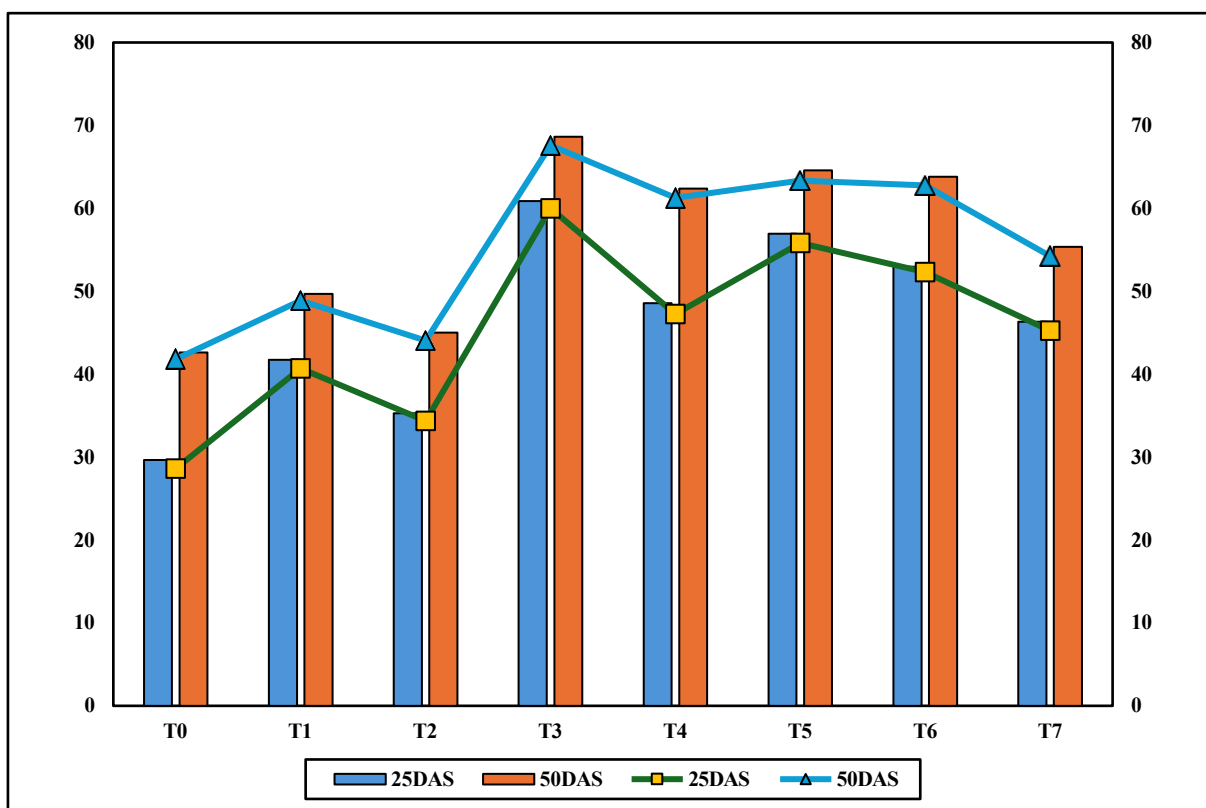
TABLE 4.1.10.b: Effect of the treatment on the number of root nodules plant⁻¹ of black gram in the *Kharif* season

TREATMENTS	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	29.63 ± 0.37	42.62 ± 0.74	28.62 ± 0.40	41.81 ± 0.64
T ₁	41.72 ± 0.61 (28.97)	49.69 ± 0.74 (14.22)	40.67 ± 0.56 (29.63)	48.86 ± 0.74 (14.44)
T ₂	35.27 ± 0.42 (15.97)	45.02 ± 0.59 (5.32)	34.36 ± 0.39 (16.71)	44.04 ± 0.67 (5.06)
T ₃	60.86 ± 0.99 (51.31)	68.65 ± 0.89 (37.91)	60.00 ± 0.98 (52.30)	67.60 ± 0.88 (38.16)
T ₄	48.58 ± 0.68 (39.00)	62.37 ± 1.31 (31.66)	47.23 ± 0.62 (39.41)	61.25 ± 1.26 (31.74)
T ₅	56.92 ± 0.55 (47.94)	64.59 ± 1.00 (34.01)	55.83 ± 0.52 (48.74)	63.34 ± 1.01 (34.00)
T ₆	52.94 ± 0.56 (44.03)	63.81 ± 0.40 (33.20)	52.32 ± 0.61 (45.30)	62.75 ± 0.43 (33.38)
T ₇	46.34 ± 0.45 (36.05)	55.37 ± 1.29 (23.03)	45.26 ± 0.49 (36.76)	54.23 ± 1.16 (22.91)
CD at p=0.05	1.159	2.700	1.687	2.502
Sem±	0.379	0.882	0.551	0.817

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Figure 4.1.10.b: Effect of the treatment on the root nodules plant⁻¹ of black gram in the *Kharif* season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.2. BIOCHEMICAL PARAMETER

4.2.1 Chlorophyll index (SPAD)

The effects of foliar application of polyamines on chlorophyll index in black gram were evaluated at 25 and 50 DAS during the two seasons of (summer and *Kharif*) of 2022 and 2023. The detailed results are presented in Table 4.2.1. a and Table 4.2.1.b and illustrated in Figures 4.2.1.a and 4.2.1.b

In the summer of 2022 considerable comparative results are evident (CD 0.05 =23 at 25 and 50 DAS). T3 had the highest Chlorophyll Index (40.55) which is significantly higher (20.72%) than control (32.15) and above CD =0.05. At 50 DAS T3 (60.97) was also significantly higher than Control (50.57) by 17.06%. T5 and T6 were both statistically superior to control. In the summer of 2023 similar results were observed (CD =2.569 at 25 DAS and 0.302 at 50 DAS). T3 39.40 and 58.67 were also significantly higher than control (21.79% and 18.35%) at 25 and 50 DAS respectively. Treatments T5 and T6 were significantly higher than T1, T2 etc.

During Kharif 2022, significant variation was observed (CD = 2.569 at 25 DAS and 0.290 at 50 DAS). T3 recorded the highest chlorophyll index (38.17 and 57.27), showing 22.49% and 18.39% increase over control (29.59 and 46.73), and differences exceeded CD values. Treatments T5 and T6 were next superior.

In the Kharif 2023 season (25 DAS CD=1.995, 50 DAS CD=0.271), T3 obtained 37.08 for chlorophyll content at 25 DAS and 56.10 at 50 DAS, which is an increase of 24.41% and 19.19% respectively compared to control (28.03 and 45.33), and was statistically more significant. T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M) consistently showed a significant (17–24% above the control) improvement in chlorophyll index throughout all seasons and years (based on CD). The increase in chlorophyll index when using combined polyamine application is assumed to be due to an increase in chlorophyll biosynthesis, stabilization of membranes, and enhanced antioxidant activity; therefore, maintaining photosynthesizing efficiency.

The higher chlorophyll readings resulting from combined applications of cadaverine and putrescine, particularly T3, further indicate a regulatory role played by polyamines in both chloroplast function and pigment metabolism. As previously mentioned, polyamines bind to negatively charged phospholipids and proteins located within thylakoid membranes, resulting in stabilization of chloroplast ultrastructure and maintenance of structural integrity in photosynthesis. Through this structural stabilization process, the greater chlorophyll

concentrations developed by these treatments likely resulted in decreased degradation of chlorophyll and elevated specifications for pigment concentration relative to previous growing stages. Enhanced chlorophyll levels may also have been influenced by increased assimilation of nitrogen due to nitrogen being a component part of the chlorophyll molecule. It is plausible that the combined application of polyamines to root zones improved nodular development and the processes of nitrogen fixation and therefore increased nitrogen availability for chlorophyll biosynthesis through the creation of greater chlorophyll through the accumulation of greater amounts of internal developed root systems. In addition, as stated previously, polyamines are also capable of acting as very strong antioxidants and eliminating reactive oxygen species (ROS), therefore reducing the oxidative damage produced on chlorophyll and minimizing the oxidative degradation of lipids that occur within the thylakoid membrane. These biological mechanisms may have contributed to prolonged periods of greater chlorophyll production, which was particularly evident at 50 days after sowing (DAS).

The higher efficacy of applying treatments in tandem versus applying individual treatments provides evidence of the synergistic interaction between putrescine and cadaverine in the regulation of photosynthetic metabolism. Putrescine is mainly used to stabilize membranes and modulate the activity of enzymes while cadaverine appears to aid in the synthesis of pigments and providing cellular protection. The combined effects of these compounds may provide an optimal redox balance, increased anatomical support for photosynthesis, and improved levels of chlorophyll-related gene expression.

The increased retention of chlorophyll at later growth stages suggests a greater source strength of the plant, which may have facilitated the uptake and movement of carbohydrates to developing reproductive organs as such potentially contribute to improved yield attributes. The findings of this study validate previously reported work demonstrating that polyamines enhance the stability and physiological efficiency of chlorophyll in legumes both under normal and stress conditions (Singh *et al.*, 2022; Malik *et al.*, 2022).

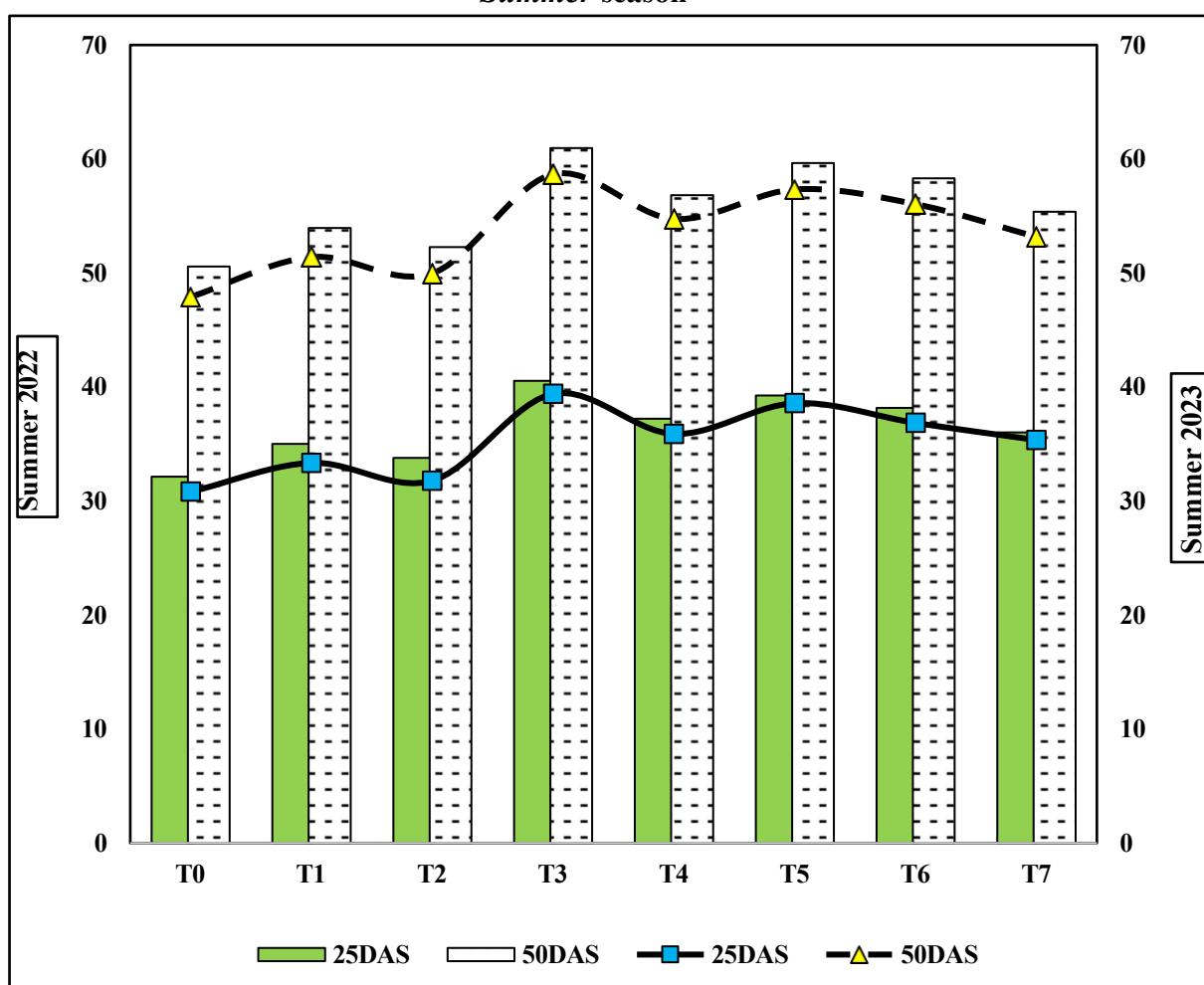
TABLE 4.2.1.a: Effect of the treatment on the chlorophyll index of black gram in the *Summer* season

Treatments	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	32.15 ± 0.05	50.57 ± 0.05	30.82 ± 1.28	47.90 ± 0.08
T ₁	35.02 ± 0.08 (8.19)	53.94 ± 0.08 (6.24)	33.35 ± 0.92 (7.60)	51.40 ± 0.08 (6.81)
T ₂	33.78 ± 0.08 (4.83)	52.27 ± 0.05 (3.25)	31.7 ± 1.24 (3.04)	49.97 ± 0.05 (4.14)
T ₃	40.55 ± 0.11 (20.72)	60.97 ± 0.11 (17.06)	39.40 ± 0.31 (21.79)	58.67 ± 0.11 (18.35)
T ₄	37.22 ± 0.08 (13.61)	56.84 ± 0.08 (11.03)	35.88 ± 0.68 (14.12)	54.74 ± 0.08 (12.48)
T ₅	39.25 ± 0.11 (18.06)	59.64 ± 0.08 (15.20)	38.58 ± 0.57 (20.13)	57.34 ± 0.08 (16.45)
T ₆	38.18 ± 0.08 (15.80)	58.30 ± 0.08 (13.26)	36.85 ± 0.60 (16.37)	56.04 ± 0.12 (14.51)
T ₇	36.02 ± 0.08 10.740	55.37 ± 0.11 (8.67)	35.35 ± 0.65 (12.82)	53.17 ± 0.11 (9.91)
CD at p=0.05	0.293	0.286	2.569	0.302
Sem±	0.096	0.093	0.839	0.099

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.1.a: Effect of the treatment on the chlorophyll index of black gram in the Summer season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

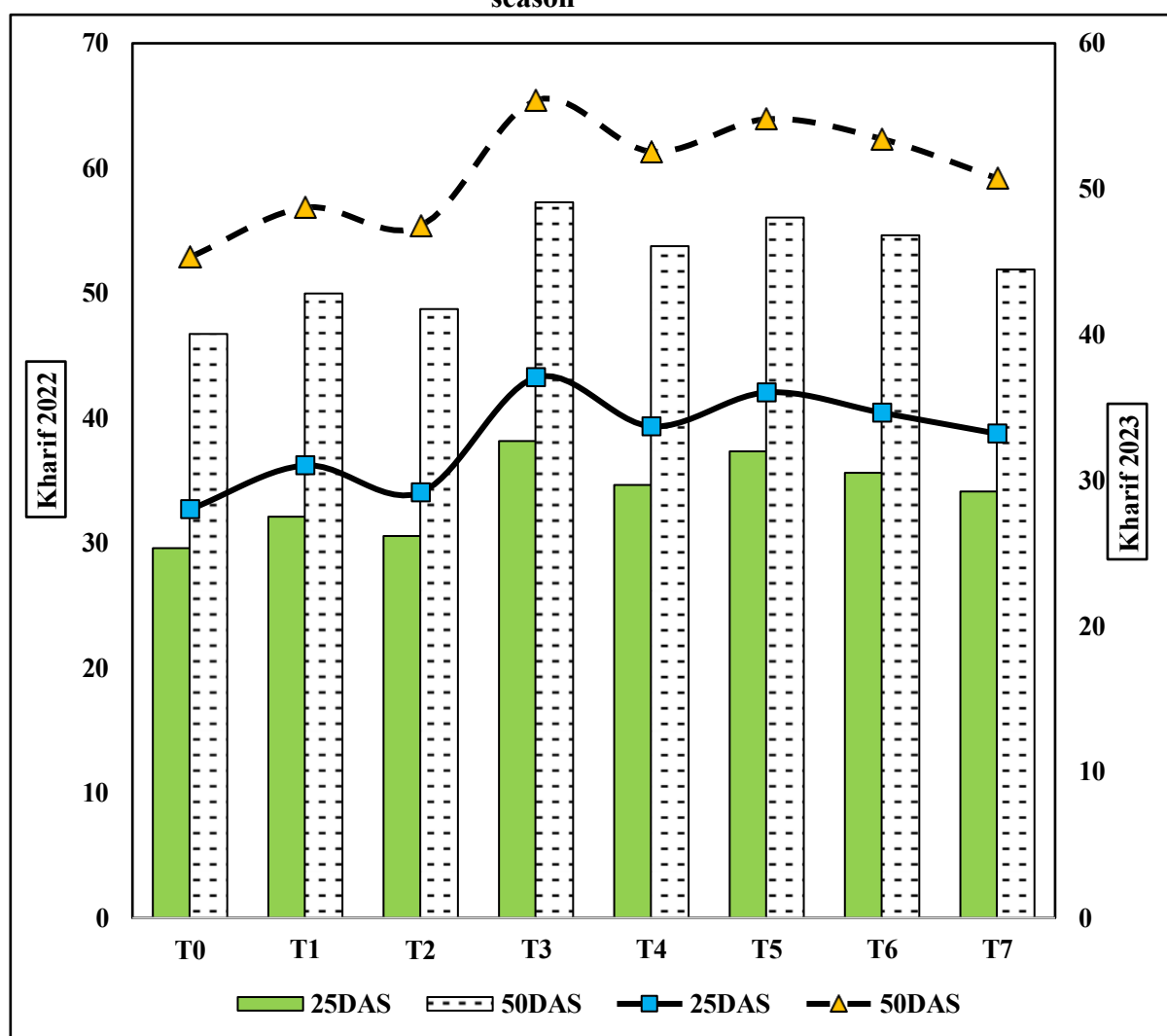
TABLE 4.2.1.b: Effect of the treatment on the chlorophyll index of black gram in the *Kharif* season

Treatments	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	29.59 ± 1.28	46.73 ± 0.08	28.03 ± 0.79	45.33 ± 0.08
T ₁	32.12 ± 0.92 (7.89)	49.97 ± 0.08 (6.47)	31.04 ± 0.72 (9.70)	48.73 ± 0.08 (6.98)
T ₂	30.55 ± 1.24 (3.16)	48.73 ± 0.08 (4.10)	29.18 ± 1.06 (3.95)	47.47 ± 0.12 (4.49)
T ₃	38.17 ± 0.31 (22.49)	57.27 ± 0.08 (18.39)	37.08 ± 0.23 (24.41)	56.10 ± 0.06 (19.19)
T ₄	34.65 ± 0.68 (14.62)	53.77 ± 0.08 (13.08)	33.72 ± 0.47 (16.87)	52.57 ± 0.08 (13.76)
T ₅	37.35 ± 0.57 (20.79)	56.07 ± 0.08 (16.65)	36.05 ± 0.50 (22.25)	54.80 ± 0.05 (17.27)
T ₆	35.62 ± 0.60 (16.94)	54.63 ± 0.08 (14.46)	34.65 ± 0.62 (19.11)	53.43 ± 0.08 (15.16)
T ₇	34.12 ± 0.65 (13.29)	51.90 ± 0.11 (9.96)	33.22 ± 0.43 (15.61)	50.73 ± 0.08 (10.64)
CD at p=0.05	2.569	0.290	1.995	0.271
Sem±	0.839	0.095	0.652	0.089

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.1.b: Effect of the treatment on the chlorophyll index of black gram in the *Kharif* season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.2.2 Chlorophyll content

The effects of foliar application of polyamines on chlorophyll index in black gram were evaluated at 25 and 50 DAS during the two seasons (summer and *Kharif*) of 2022 and 2023. The detailed results are presented in Table 4.2.2. and Table 4.2.2.b and illustrated in Figures 4.2.2.a and 4.2.2.b

During Summer 2022, significant differences were observed among treatments ($CD_{0.05} = 0.777$ at 25 DAS and 0.178 at 50 DAS). At 25 DAS, T3 recorded the maximum chlorophyll content (2.10 mg g^{-1}), showing 40.78% increase over control (1.24 mg g^{-1}). The difference exceeded CD, indicating statistical significance. At 50 DAS, T3 (2.40 mg g^{-1}) recorded 35.66% higher chlorophyll content than control (1.54 mg g^{-1}), which was also significant on CD basis. Treatments T5 (33.03% and 28.41%) and T6 (28.09% and 23.92%) were statistically superior to control. In Summer 2023, similar trends were observed ($CD = 0.177$ at both stages). T3 recorded 1.89 and 2.15 mg g^{-1} at 25 and 50 DAS, reflecting 45.41% and 39.83% increase over control (1.03 and 1.29 mg g^{-1}), respectively. The differences exceeded CD values, confirming statistical superiority. T5 and T6 also showed significant increases over control.

During Kharif 2022, significant variation was recorded ($CD = 0.146$ at 25 DAS and 0.172 at 50 DAS). T3 recorded the highest chlorophyll content (1.69 and 1.97 mg g^{-1}), showing 47.40% and 43.39% increase over control (0.89 and 1.11 mg g^{-1}), respectively. These differences were greater than CD values. T5 and T6 were the next superior treatments. Similarly, in Kharif 2023 ($CD = 0.125$ at 25 DAS and 0.151 at 50 DAS), T3 recorded 1.61 and 1.94 mg g^{-1} , representing 47.59% and 43.77% increase over control (0.84 and 1.09 mg g^{-1}), and remained statistically superior. Other combination treatments also recorded significant improvement over control.

Overall, T3 (Putrescine $1500 \text{ } \mu\text{M}$ + Cadaverine $1000 \text{ } \mu\text{M}$) consistently recorded significantly higher chlorophyll content (35–48% increase over control) across seasons and years.

The increasing level of chlorophyll content and the chlorophyll synthesis highlighted in all polyamine treatments, primarily T3, indicate a synergistic effect of putrescine and cadaverine on chlorophyll biosynthesis, stability, and protection. The polyamines stimulate chlorophyll synthesis and operate against the degradation of chlorophyll through enzyme activity, thereby initiating a delay in degradation when plants are normal or stressed. Putrescine plays a crucial role in stabilizing chloroplast membranes, mitigating oxidative damage, and promoting nitrogen assimilation, all of which are essential for chlorophyll synthesis (Hussain *et al.*, 2021). Cadaverine also plays a role in the stabilization of pigments and photosynthetic efficiency

through the improvement of cellular homeostasis and tolerance to stress (Yari *et al.*, 2022). greater effect observed in combined treatments is likely due to the additive impacts on antioxidant capacity, assimilation of nutrients, and enzyme activity. The decline in chlorophyll content in the control and low concentration treatments suggests that there was inadequate physiological assistance to form optimal pigment development. These results are consistent with past studies that show greater polyamines help maintain chlorophyll contents under abiotic stress and low nutrient conditions (Malik *et al.*, 2022; Singh *et al.*, 2022). Increases in chlorophyll content may act to support better biomass accumulation and yields over time. The synergistic effect of using a combination of putrescine and cadaverine appears to be greater than if either chemical is applied separately. For example, both chemicals are able to maintain or improve redox balances; increase enzyme activity; and/or enhance pigment metabolism. Furthermore, if chlorophyll levels were well-maintained, photosynthesis would be more efficient in terms of producing carbohydrates, and ultimately result in more biomass, which could lead to improved yield potential.

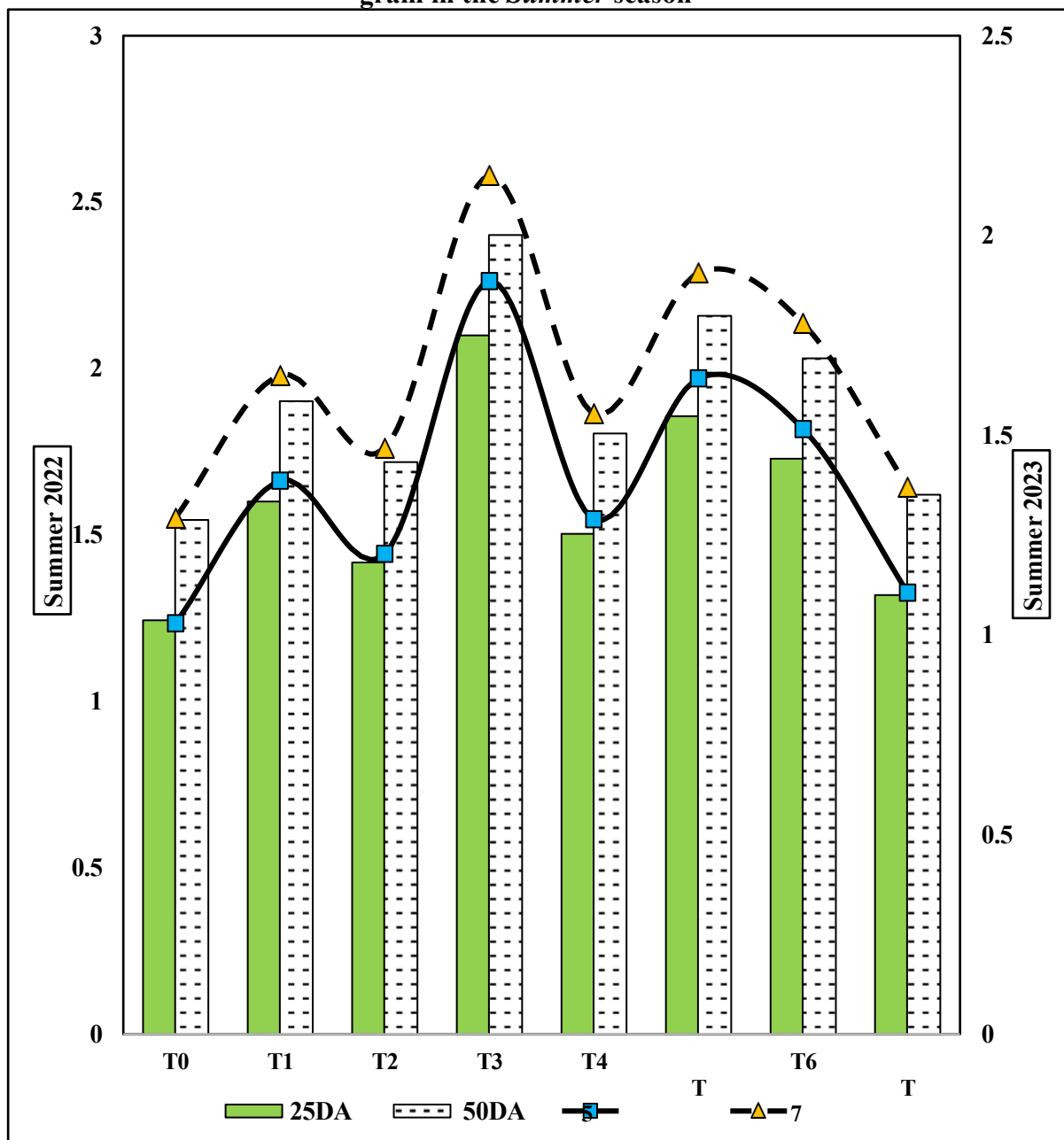
TABLE 4.2.2.a: Effect of the treatment on the chlorophyll content (mg g⁻¹ FW) of black gram in the summer season

Treatments	<i>Summer (2022)</i>		<i>Summer (2023)</i>	
	25DAS	50DAS	25DAS	50DAS
T ₀	1.24 ± 0.00	1.54 ± 0.01	1.03 ± 0.01	1.29 ± 0.00
T ₁	1.60 ± 0.04 (22.30)	1.90 ± 0.04 (18.76)	1.39 ± 0.03 (25.74)	1.65 ± 0.04 (21.62)
T ₂	1.42 ± 0.05 (12.25)	1.72 ± 0.05 (10.10)	1.20 ± 0.05 (14.43)	1.47 ± 0.05 (11.83)
T ₃	2.10 ± 0.08 (40.78)	2.40 ± 0.08 (35.66)	1.89 ± 0.08 (45.41)	2.15 ± 0.08 (39.83)
T ₄	1.50 ± 0.04 (17.31)	1.81 ± 0.04 (14.41)	1.29 ± 0.04 (20.18)	1.55 ± 0.04 (16.75)
T ₅	1.86 ± 0.04 (33.03)	2.16 ± 0.05 (28.41)	1.64 ± 0.04 (37.33)	1.91 ± 0.04 (32.16)
T ₆	1.73 ± 0.06 (28.09)	2.03 ± 0.06 (23.92)	1.52 ± 0.06 (32.06)	1.78 ± 0.06 (27.30)
T ₇	1.32 ± 0.06 (5.77)	1.62 ± 0.06 (4.70)	1.11 ± 0.06 (6.89)	1.37 ± 0.06 (5.56)
CD at p=0.05	0.777	0.178	0.177	0.177
Sem±	0.058	0.058	0.058	0.058

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.2.a: Effect of the treatment on the chlorophyll content (mg g^{-1} FW) of black gram in the *Summer* season



T0= Control, T1= Putrescine ($1500\mu\text{mol}$), T2= Cadaverine ($1000\mu\text{mol}$), T3= Putrescine ($1500\mu\text{mol}$) + Cadaverine ($1000\mu\text{mol}$), T4= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$), T5= Putrescine ($750\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$), T6= Putrescine ($750\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$) and T7= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$)

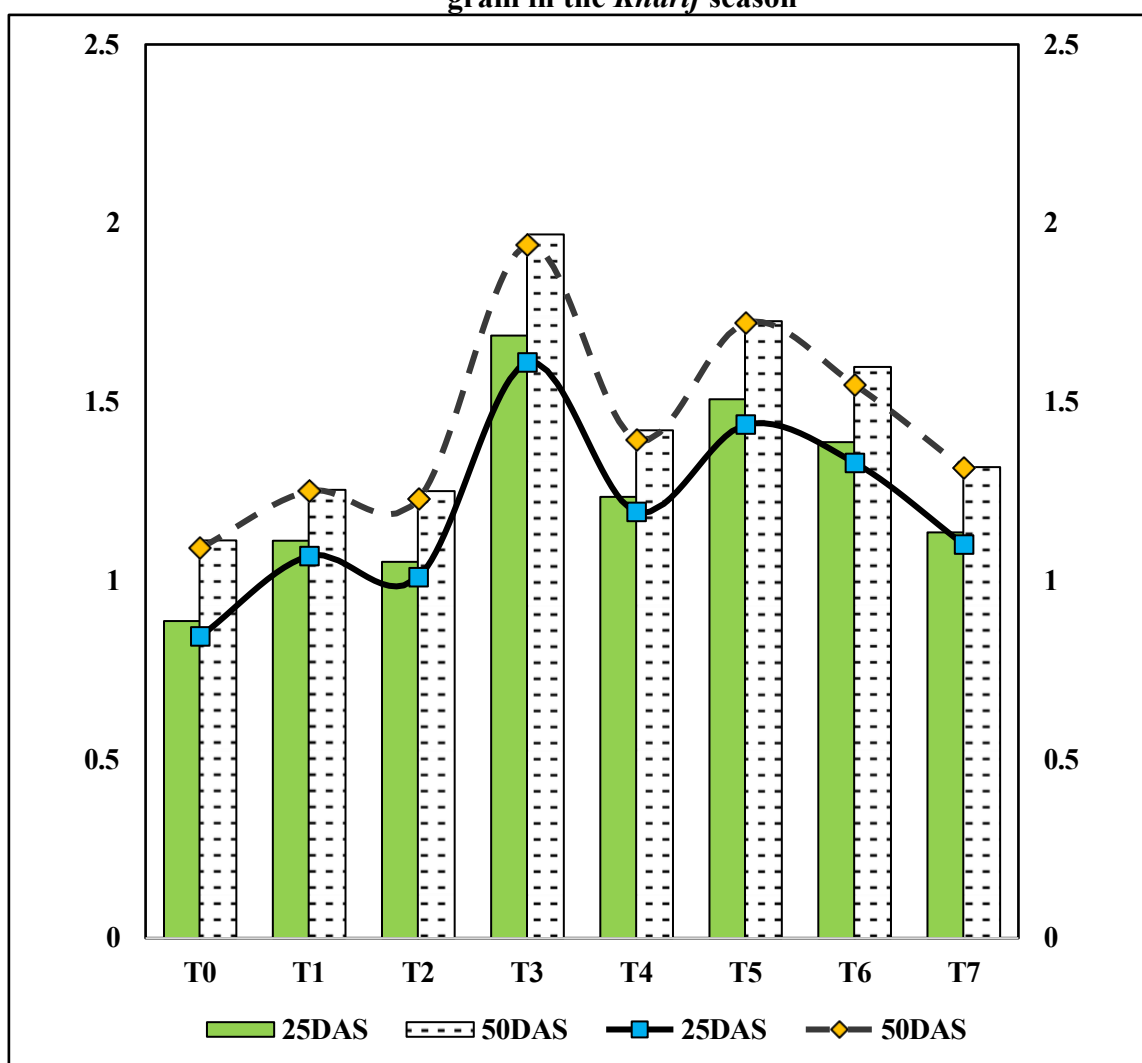
TABLE 4.2.2.b: Effect of the treatment on the chlorophyll index (mg g⁻¹ FW) of black gram in the *Kharif* season

Treatments	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	0.89 ± 0.01	1.11 ± 0.01	0.84 ± 0.00	1.09 ± 0.02
T ₁	1.11 ± 0.03 (20.26)	1.25 ± 0.06 (11.28)	1.07 ± 0.02 (21.07)	1.25 ± 0.06 (12.80)
T ₂	1.05 ± 0.02 (15.76)	1.25 ± 0.03 (11.06)	1.01 ± 0.01 (16.43)	1.23 ± 0.04 (11.14)
T ₃	1.69 ± 0.07 (47.40)	1.97 ± 0.08 (43.39)	1.61 ± 0.06 (47.59)	1.94 ± 0.05 (43.77)
T ₄	1.23 ± 0.05 (28.15)	1.42 ± 0.03 (21.70)	1.19 ± 0.05 (29.21)	1.39 ± 0.02 (21.72)
T ₅	1.51 ± 0.05 (41.16)	1.73 ± 0.04 (35.53)	1.44 ± 0.03 (41.25)	1.72 ± 0.06 (36.65)
T ₆	1.39 ± 0.04 (36.10)	1.60 ± 0.06 (30.39)	1.33 ± 0.03 (36.53)	1.55 ± 0.05 (29.53)
T ₇	1.14 ± 0.01 (21.87)	1.32 ± 0.06 (15.57)	1.10 ± 0.00 (23.36)	1.31 ± 0.00 (16.99)
CD at p=0.05	0.146	0.172	0.125	0.151
Sem±	0.048	0.056	0.041	0.049

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.2.b: Effect of the treatment on the chlorophyll content (mg g⁻¹ FW) of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500μmol), T2= Cadaverine (1000μmol), T3= Putrescine (1500μmol) + Cadaverine (1000μmol), T4= Putrescine (3000μmol) + Cadaverine (500μmol), T5= Putrescine (750μmol) + Cadaverine (2000μmol), T6= Putrescine (750μmol) + Cadaverine (500μmol) and T7= Putrescine (3000μmol) + Cadaverine (2000μmol)

4.2.3 Total protein content in leaves

The effects of foliar application of polyamines on total protein content in black gram were evaluated at 25 and 50 DAS during the two seasons (summer and *Kharif*) of 2022 and 2023.

The detailed results are presented in Table 4.2.3. and Table 4.2.3.b and illustrated in Figures 4.2.3.a and 4.2.3.b

In the summer of 2022, there were significant differences in treatment ($CD_{0.05} = 1.476$ at 25 DAS and 2.176 at 50 DAS). The highest level of protein was obtained for T3 with significant increases over control (17.86 and 19.56 mg g⁻¹) of 19.13% and 18.98% (22.09 and 24.14 mg g⁻¹) at 25 DAS and 50 DAS, respectively. The CD showed a statistically significant difference. In the summer of 2022, T3 also had the highest level of protein (20.63 mg g⁻¹ and 22.44 mg g⁻¹) compared to the control (16.46 mg g⁻¹ and 18.18 mg g⁻¹). Both improvements were significant compared to the control, as well as to other treatments. T5 and T6 recorded appreciable increases in protein.

According to studies done during Kharif 2022, various trials created significant variances across all treatment types used (Control versus T2 through T6) and the period over which they occurred (Days After Sowing). For example, at 25 days after sowing, the coefficient of determination for the protein content was 1.129, while at 50 days, it was 1.301. At both points in time, T3 yielded the highest amount of protein (19.13 mg g⁻¹ at 25 DAS and 20.94 mg g⁻¹ at 50 DAS). Additionally, T3 provided the greatest increase from the control (15.10 mg g⁻¹ at 25 DAS and 16.60 mg g⁻¹ at 50 DAS) with a 21.07% and 20.76% higher protein content than the control for T3, respectively. Furthermore, T5 and T6 also yielded significantly higher increases (163.05-117.18% greater than the control). Similarly, during the Kharif 2023 growing season, T3 yielded similar results with respect to protein yield (18.32 mg g⁻¹ at 25 DAS and 19.99 mg g⁻¹ at 50 DAS), producing a 25.71% and 25.82% increase when compared to the control at both times for T3 respectively (13.61 mg g⁻¹ at 25 DAS and 14.80 mg g⁻¹ at 50 DAS). Overall, the combined application of putrescine and cadaverine (T3) did produce the highest amount of protein on average across years and growing seasons.

Enhanced levels of total soluble protein due to the application of polyamines (T3) indicate how polyamines can influence nitrogen metabolism and protein synthesis in plants because they are closely associated with metabolism in cells, regulating the expression of genes, and regulating the activities of enzymes that help to synthesize amino acids and proteins. The increased levels of total soluble protein observed with the use of the combined treatment seem

to suggest an elevation in protein levels was caused by a combination of enhanced nitrogen assimilation and also an increased activity of the enzymes involved in the synthesis of proteins. Polyamines also help stabilize the cell membranes and the ribosomal structures within the cells, enabling efficient translation and synthesis of proteins. Further, their contribution to maintaining homeostasis in the cell and buffering oxidative stress on biochemical processes further leads to the accumulation of total soluble proteins.

The enhanced levels of total soluble proteins obtained from the combined treatments also indicate a potential synergistic interaction between putrescine and cadaverine that may result in increased metabolic efficiency and/or nitrogen utilization. The increased levels of total soluble protein at later stages of growth suggest that the plants were undergoing more metabolic activity and physiological efficiency at later stages, which ultimately will lead to greater growth and yield performance.

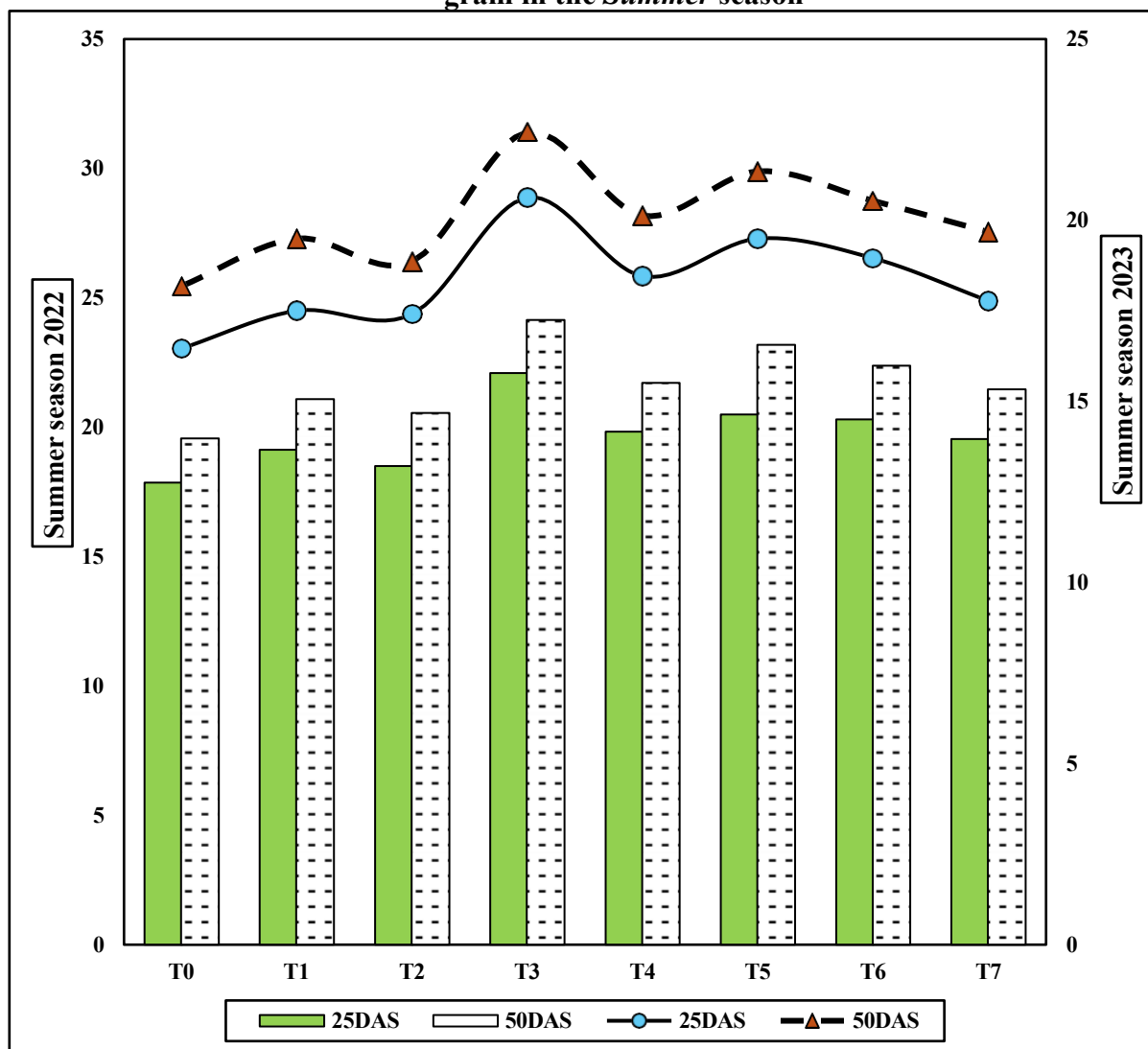
TABLE 4.2.3.a: Effect of the treatment on Total soluble protein (mg g⁻¹ FW) of black gram in the *Summer* season

Treatments	Summer (2022)		Summer (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	17.86 ± 0.09	19.56 ± 0.39	16.46 ± 0.12	18.18 ± 0.37
T ₁	19.12 ± 0.48 (6.58)	21.09 ± 0.44 (7.24)	17.50 ± 0.42 (5.95)	19.49 ± 0.42 (6.74)
T ₂	18.50 ± 0.45 (3.43)	20.55 ± 0.26 (4.80)	17.41 ± 0.22 (5.46)	18.86 ± 0.27 (3.60)
T ₃	22.09 ± 0.87 (19.13)	24.14 ± 1.49 (18.98)	20.63 ± 0.15 (20.20)	22.44 ± 0.40 (18.97)
T ₄	19.82 ± 0.25 (9.89)	21.71 ± 0.62 (9.91)	18.45 ± 0.86 (10.80)	20.13 ± 0.39 (9.67)
T ₅	20.49 ± 0.15 (12.84)	23.18 ± 0.39 (15.61)	19.49 ± 0.78 (15.56)	21.35 ± 0.52 (14.85)
T ₆	20.30 ± 0.55 (12.00)	22.38 ± 0.34 (12.60)	18.95 ± 0.28 (13.14)	20.54 ± 1.41 (11.47)
T ₇	19.54 ± 0.23 (8.58)	21.47 ± 0.60 (8.88)	17.77 ± 0.15 (7.39)	19.68 ± 0.39 (7.60)
CD at p=0.05	1.476	2.176	1.494	1.430
Sem±	0.482	0.711	0.488	0.660

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.3.a: Effect of the treatment on Total protein content (mg g⁻¹ FW) of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine (1500μmol), T2= Cadaverine (1000μmol), T3= Putrescine (1500μmol) + Cadaverine (1000μmol), T4= Putrescine (3000μmol) + Cadaverine (500μmol), T5= Putrescine (750μmol) + Cadaverine (2000μmol), T6= Putrescine (750μmol) + Cadaverine (500μmol) and T7= Putrescine (3000μmol) + Cadaverine (2000μmol)

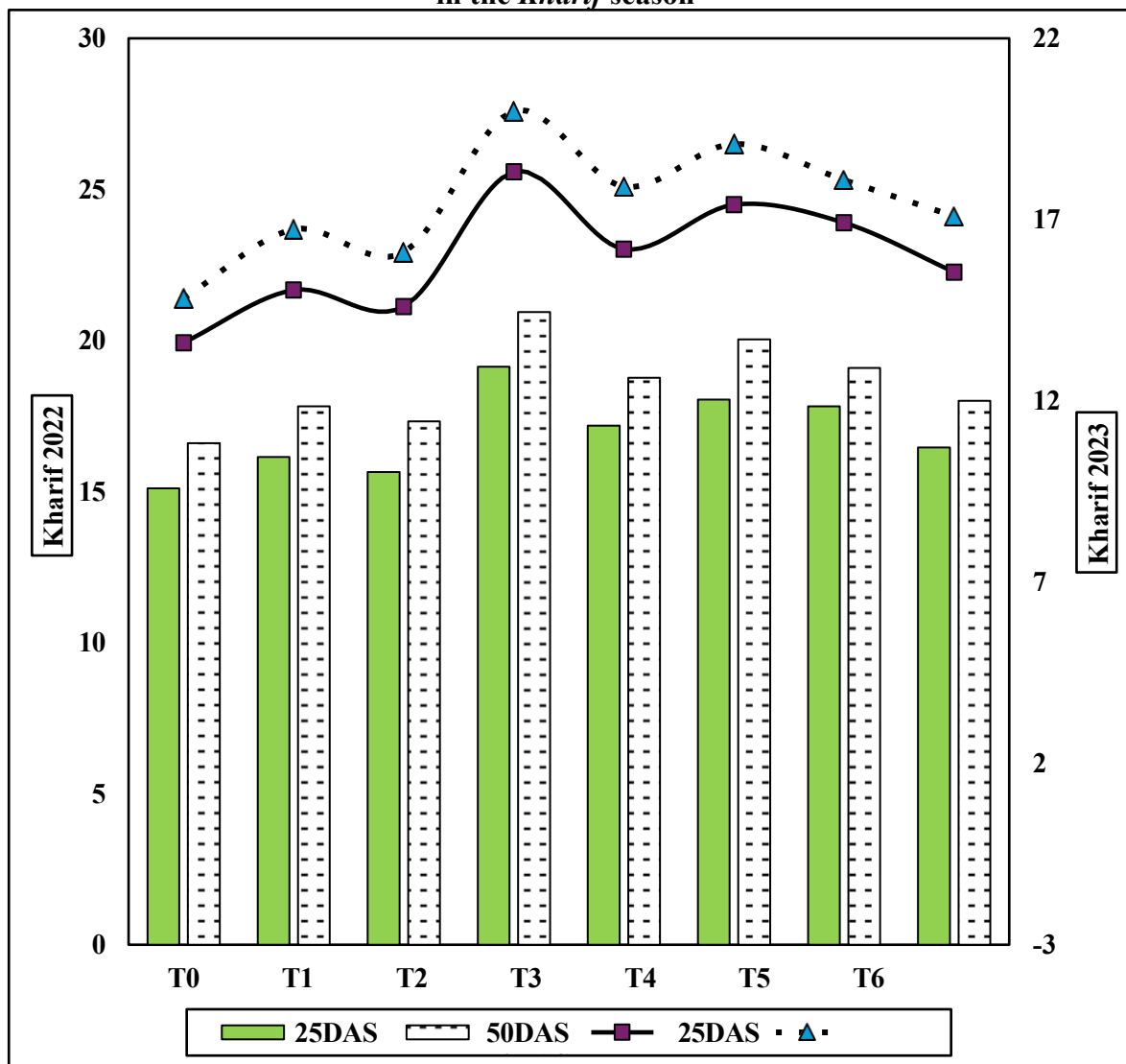
TABLE 4.2.3.b: Effect of the treatment on total protein content (mg g⁻¹ FW) of black gram in the *Kharif* season

Treatments	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	15.10 ± 0.19	16.60 ± 0.19	13.61 ± 0.35	14.8 ± 0.27
T ₁	16.14 ± 0.15 (6.45)	17.82 ± 0.25 (6.86)	15.06 ± 0.36 (9.62)	16.73 ± 0.40 (11.37)
T ₂	15.65 ± 0.24 (3.47)	17.32 ± 0.16 (4.18)	14.60 ± 0.35 (6.82)	16.10 ± 0.19 (7.88)
T ₃	19.13 ± 0.86 (21.07)	20.94 ± 0.02 (20.76)	18.32 ± 0.98 (25.71)	19.99 ± 1.02 (25.82)
T ₄	17.18 ± 0.25 (12.12)	18.77 ± 0.35 (11.58)	16.19 ± 0.23 (15.94)	17.91 ± 0.39 (17.19)
T ₅	18.05 ± 0.15 (16.31)	20.04 ± 0.19 (17.18)	17.41 ± 0.27 (21.85)	19.09 ± 0.19 (22.30)
T ₆	17.82 ± 0.32 (15.25)	19.09 ± 0.24 (13.05)	16.91 ± 0.31 (19.55)	18.09 ± 0.27 (18.02)
T ₇	16.46 ± 0.19 (8.25)	18.00 ± 0.12 (7.80)	15.55 ± 0.16 (12.52)	17.09 ± 0.15 (13.25)
CD at p=0.05	1.129	1.301	1.353	1.355
Sem±	0.369	0.425	0.442	0.443

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.3.b: Effect of the treatment on total protein content (mg^{-1} FW) of black gram in the *Kharif* season



T0= Control, T1= Putrescine ($1500\mu\text{mol}$), T2= Cadaverine ($1000\mu\text{mol}$), T3= Putrescine ($1500\mu\text{mol}$) + Cadaverine ($1000\mu\text{mol}$), T4= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$), T5= Putrescine ($750\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$), T6= Putrescine ($750\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$) and T7= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$)

4.2.4. Total carbohydrate content

The effect of foliar application of putrescine and cadaverine on total carbohydrate content in black gram leaves was recorded at 25 and 50 DAS during the two seasons (*Summer* and *Kharif*) of 2022 and 2023. Data are tabulated in Table 4.2.4.a and 4.2.4.b and illustrated in Figure 4.2.4.a. and 4.2.3.b.

There were large treatment differences throughout the study period of Summer 2022 ($CD_{0.05}$ - 0.292 at 25 DAS and 0.596 at 50 DAS). At 25 DAS, T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M) had the greatest carbohydrate content (8.41 mg g⁻¹); this represents an increase of 9.25% compared to control (7.63 mg g⁻¹) and was substantially different than CD value. At 50 DAS, T3 had again the greatest carbohydrate content (9.61 mg g⁻¹) with a percentage increase of 10.69% over control (8.58 mg g⁻¹) with a significant difference from CD. Treatments T5 and T6 also had higher carbohydrate content; these ranged 5.89–8.96% higher than control. Data from Summer 2023 exhibited similar trends to Summer 2022 with treatment differences being statistically significant ($CD = 0.368$ at 25 DAS and 0.248 at 50 DAS). At 25 and 50 DAS, T3 exhibited the highest carbohydrate content (8.13 and 9.05 mg g⁻¹), resulting in increases of 9.00% and 7.79% above control (7.41 and 8.35 mg g⁻¹), which were statistically significant on a CD basis. Treatments T5 and T6 also exhibited moderate increases of 5.29–7.87% over control.

During Kharif 2022, significant variation was recorded among treatments ($CD = 0.401$ at 25 DAS and 0.359 at 50 DAS). Treatment T3 recorded the highest carbohydrate content (7.94 and 8.84 mg g⁻¹) with 9.24% and 7.60% increase over control (7.21 and 8.17 mg g⁻¹), which exceeded CD values. Other treatments such as T5 and T6 also showed improvement ranging from 5.16–7.36% over control. Similarly, during Kharif 2023, treatment differences were significant ($CD = 0.404$ at 25 DAS and 0.245 at 50 DAS). T3 recorded the maximum carbohydrate content (7.84 and 8.76 mg g⁻¹), showing 9.28% and 8.06% increase over control (7.11 and 8.05 mg g⁻¹). Treatments T5 and T6 also recorded higher carbohydrate content with increases between 5.48–7.45% over control.

Overall, the combined application of putrescine and cadaverine (T3) consistently recorded the highest carbohydrate content (7–11% increase over control) across seasons and years.

The carbohydrate content of plants treated with polyamines, especially T3, has increased significantly. This can be attributed to the enhanced photosynthetic capacity and increased metabolic potential of black gram plants. Polyamines function in the regulation of carbon metabolism through the stimulation of enzymes involved in photosynthesis and the synthesis of

carbohydrates. The increased chlorophyll content found through combined treatment may have led to improved light interception, consequently leading to improved carbon fixation, with a resultant increase in the accumulation of photosynthates in plant tissue. Furthermore, polyamines have a role in maintaining the structural integrity of the chloroplasts and protecting the photosynthetic apparatus from oxidative stress, thereby allowing for a sustained rate of photosynthesis throughout the growth cycle of the plant.

Polyamines also affect the activities of key enzymes related to carbohydrate production and translocation, such as Rubisco and sucrose phosphate synthase, which are important components of the process. It is likely that through the combined application of putrescine and cadaverine, there has been increased metabolic coordination between carbon assimilation and energy metabolism, resulting in a greater amount of carbohydrate accumulation.

Maintaining an improved carbohydrate status through polyamine treatment will likely increase growth, biomass accumulation, and reproductive growth within the black gram. This study demonstrates the importance of polyamines in regulating the metabolism of carbohydrates and improving the physiological efficiency of legume crops.

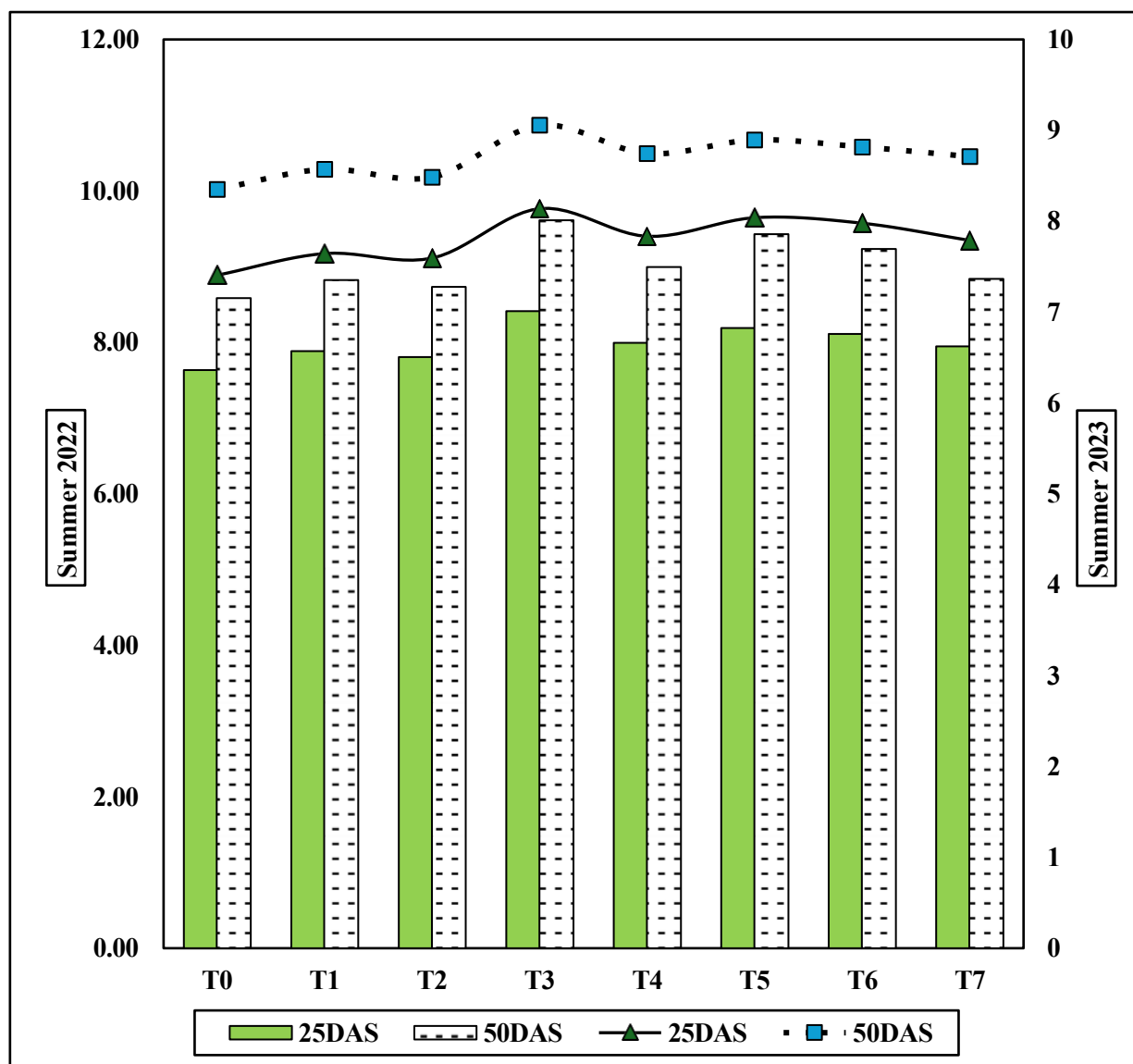
TABLE 4.2.4.a: Effect of the treatment on total carbohydrate content (mg g⁻¹ FW) of black gram in the *Summer* season

Treatments	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	7.63 ± 0.10	8.58 ± 0.10	7.41 ± 0.10	8.35 ± 0.06
T ₁	7.88 ± 0.05 (3.17)	8.82 ± 0.24 (2.71)	7.64 ± 0.06 (3.12)	8.57 ± 0.09 (2.59)
T ₂	7.81 ± 0.11 (2.21)	8.73 ± 0.15 (1.72)	7.59 ± 0.11 (2.48)	8.48 ± 0.06 (1.57)
T ₃	8.41 ± 0.09 (9.25)	9.61 ± 0.07 (10.69)	8.13 ± 0.11 (9.00)	9.05 ± 0.11 (7.79)
T ₄	8.00 ± 0.09 (4.52)	8.99 ± 0.29 (4.57)	7.83 ± 0.16 (5.45)	8.74 ± 0.04 (4.51)
T ₅	8.19 ± 0.09 (6.78)	9.43 ± 0.17 (8.96)	8.04 ± 0.16 (7.87)	8.89 ± 0.14 (6.12)
T ₆	8.11 ± 0.07 (5.89)	9.23 ± 0.32 (7.04)	7.98 ± 0.12 (7.17)	8.82 ± 0.04 (5.29)
T ₇	7.95 ± 0.09 (3.92)	8.84 ± 0.19 (2.89)	7.79 ± 0.11 (4.91)	8.71 ± 0.04 (4.15)
CD at p=0.05	0.292	0.596	0.368	0.248
Sem±	0.095	0.195	0.120	0.081

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.4.a: Effect of the treatment on total carbohydrate content (mg g⁻¹ FW) of black gram in the *Summer* season



T0= Control, T1= Putrescine (1500μmol), T2= Cadaverine (1000μmol), T3= Putrescine (1500μmol) + Cadaverine (1000μmol), T4= Putrescine (3000μmol) + Cadaverine (500μmol), T5= Putrescine (750μmol) + Cadaverine (2000μmol), T6= Putrescine (750μmol) + Cadaverine (500μmol) and T7= Putrescine (3000μmol) + Cadaverine (2000μmol)

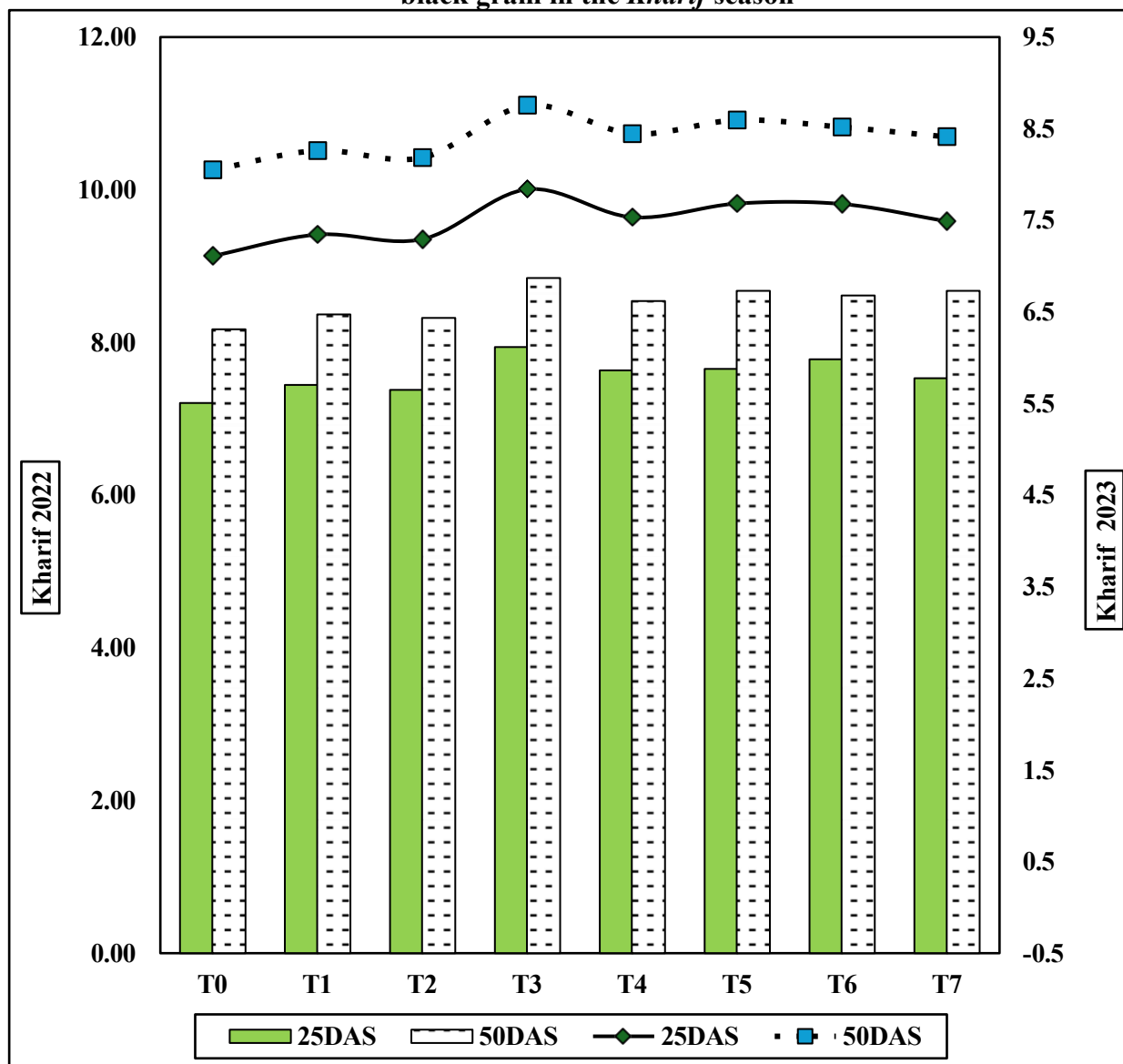
TABLE 4.2.4.b: Effect of the treatment on total carbohydrate content (mgg⁻¹ FW) of black gram in the *Kharif* season

Treatments	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	7.21 ± 0.10	8.17 ± 0.06	7.11 ± 0.10	8.05 ± 0.06
T ₁	7.44 ± 0.06 (3.21)	8.37 ± 0.09 (2.32)	7.34 ± 0.06 (3.18)	8.26 ± 0.09 (2.56)
T ₂	7.38 ± 0.11 (2.33)	8.32 ± 0.04 (1.80)	7.29 ± 0.11 (2.51)	8.18 ± 0.06 (1.63)
T ₃	7.94 ± 0.11 (9.24)	8.84 ± 0.11 (7.60)	7.84 ± 0.11 (9.28)	8.76 ± 0.11 (8.06)
T ₄	7.63 ± 0.16 (5.60)	8.54 ± 0.04 (4.36)	7.53 ± 0.16 (5.60)	8.44 ± 0.04 (4.67)
T ₅	7.66 ± 0.25 (5.88)	8.68 ± 0.14 (5.83)	7.68 ± 0.21 (7.45)	8.59 ± 0.14 (6.33)
T ₆	7.78 ± 0.12 (7.36)	8.62 ± 0.04 (5.16)	7.68 ± 0.12 (7.38)	8.52 ± 0.04 (5.48)
T ₇	7.53 ± 0.12 (4.35)	8.68 ± 0.21 (5.83)	7.49 ± 0.11 (5.04)	8.41 ± 0.04 (4.29)
CD at p=0.05	0.401	0.359	0.404	0.245
Sem±	0.131	0.117	0.132	0.080

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.4.b: Effect of the treatment on total carbohydrate content (mg g^{-1} FW) of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine ($1500\mu\text{mol}$), T2= Cadaverine ($1000\mu\text{mol}$), T3= Putrescine ($1500\mu\text{mol}$) + Cadaverine ($1000\mu\text{mol}$), T4= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$), T5= Putrescine ($750\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$), T6= Putrescine ($750\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$) and T7= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$)

4.2.5 Total phenol content

The impact of foliar application of polyamines on total phenol content in black gram leaves was evaluated at 25 and 50 DAS during both summer and *Kharif* seasons of 2022 and 2023. The findings are briefly summarized in Table 4.2.5.a and 4.2.5.b and illustrated in Figure 4.2.5.a. and 4.2.5.b.

There were significant differences in the types of treatments applied throughout the experiments conducted during Summer 2022 within 25 days (DAS). However, those differences diminished significantly by 50 DAS, no longer providing evidence of treatment differences. During 25 DAS, T3 (Putrescine 1500 μM and Cadaverine 1000 μM) had the greatest amount of phenols recorded (0.287 mg g^{-1}), exceeding that of T1 (control) by 26.19%, which exceeded the critical difference (CD). T5 and T6 also exhibited similar increases (22.85% and 21.91%, respectively) from that of T1. T3 had the highest amount of phenols (0.329 mg g^{-1} ; 14.79% greater than T1; 0.280 mg g^{-1}) at 50 DAS and provided no statistical significance to show differences among treatments.

Similarly, during Summer 2023, treatments had statistically significant differences at 25 DAS (CD = 0.016) compared to no significant differences at 50 DAS. The phenols' highest concentration (0.278 mg g^{-1}) had occurred within T3 at 25 DAS, with an increase of 26.41% over the control (0.205 mg g^{-1}). While T5 and T6 also increased in phenol content (22.96% and 21.99%, respectively) when compared to the control (T1), their statistical significance was found at 50 DAS with a phenol value of 0.320 mg g^{-1} for T3 compared to 0.268 mg g^{-1} .

The differences between treatments were statistically significant at 25 DAS (CD = 0.017), while they were statistically non-significant at 50 DAS during the *Kharif* 2022 growing season. The treatment with the highest phenol content was T3, which had a phenol content of 0.274 mg g^{-1} at 25 DAS and was 26.68% higher than the control, which had a phenol content of 0.201 mg g^{-1} . At 25 DAS, T5 and T6 also had statistically significant differences when compared to the control, with phenol content increases of 18.49% and 17.97%, respectively. At 50 DAS, the phenol content of T3 was equal to 0.318 mg g^{-1} , which was a 15.29% increase over the control (0.269 mg g^{-1}), but there were no significant differences in these treatments over the control.

In the *Kharif* 2023 growing season, differences between treatments were again statistically significant at 25 DAS (CD = 0.017) but not at 50 DAS. The maximum phenol content during

this season was recorded for T3 (0.264 mg g⁻¹) and represented a 26.25% increase over the control (0.195 mg g⁻¹). At 50 DAS, T3 had a phenol content of 0.307 mg g⁻¹, which was a 15.44% increase compared to the control (0.259 mg g⁻¹); however, there were no significant differences in treatment group phenol content in relation to the control.

When combining both growing seasons and treatment years, the average increase in phenol concentration over the control was 15–27% for T3 due to the combined use of putrescine and cadaverine.

The enhanced phenol levels observed in treatments with polyamines provide evidence of their contribution to secondary metabolite synthesis. The antioxidant properties of phenols protect the cellular components of plants from oxidative stress (by scavenging reactive oxygen species, ROS). Polyamines in plants are known to function in the regulation of the phenylpropanoid metabolic pathway, which is the site for the biosynthesis of phenolic compounds. The fact that phenol content was enhanced in the combined treatment groups is likely due to an increase in the activity of phenylalanine ammonia-lyase (PAL), the key enzyme involved in the biosynthesis of phenolic compounds. The increase in phenolic compounds in these treated plants may improve their antioxidant defense mechanisms and enhance their tolerance to environmental stress.

The phenol contents measured at 25 DAS were greater compared to the phenol contents of the other treatments, which suggests that the application of polyamines stimulates early metabolic responses in relation to defense against and protection from environmental stress. Although it can be seen from the data collected at 50 DAS that phenol content had continued to rise in all of the treatment groups, the relationships observed in the phenol levels were statistically non-significant, which indicates that the catabolism of phenolic products may stabilize by the later growth stages. As a result of this research, there is further evidence supporting the conclusion that combined applications of putrescine and cadaverine have been shown to increase the rates of phenolic metabolism, thus increasing the antioxidant defense system and effectively improving the physiological performance of black gram plants.

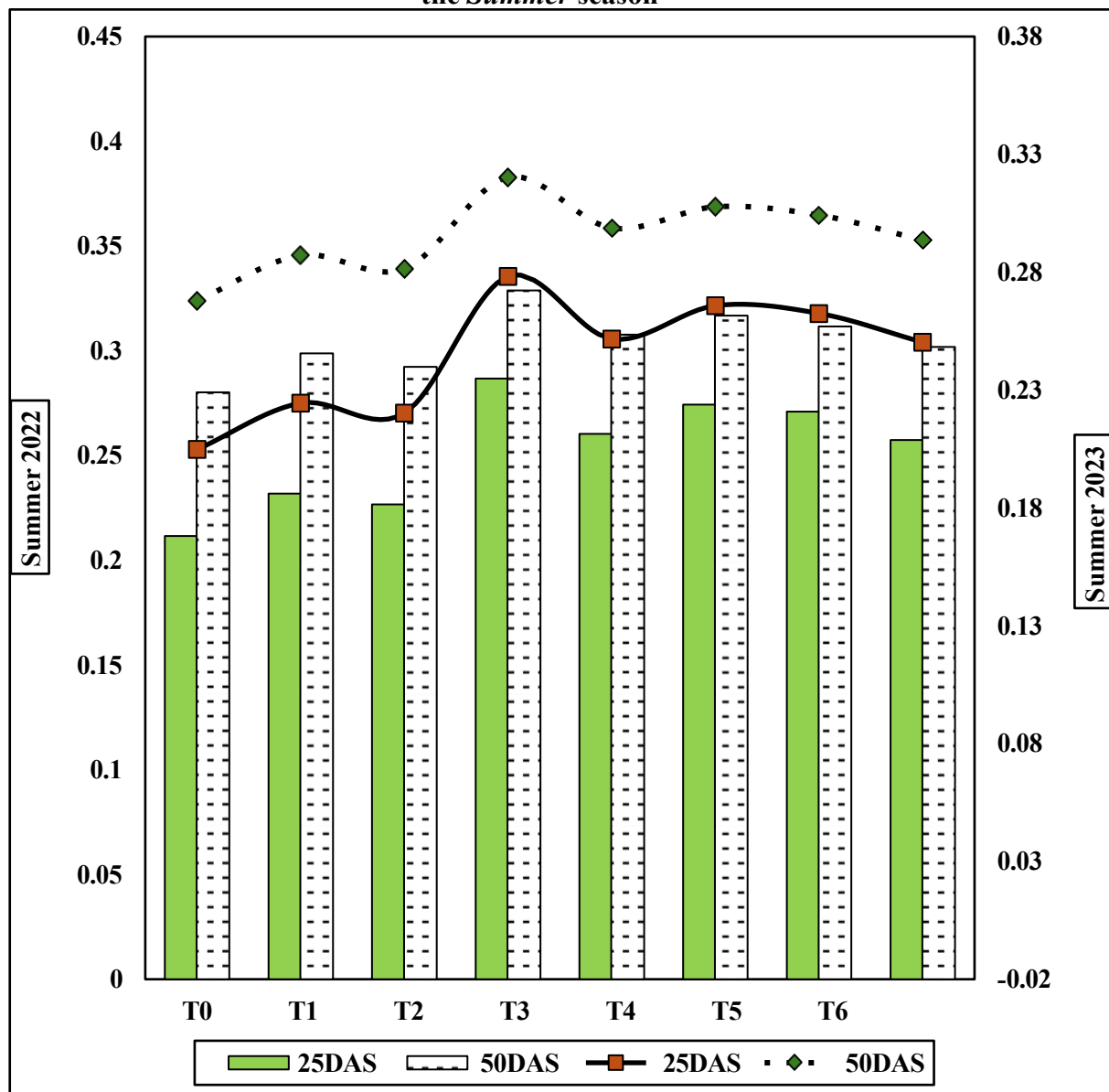
TABLE 4.2.5.a: Effect of the treatment on total phenol content (mg g⁻¹ FW) of black gram in the *Summer* season (2022 and 2023)

Treatments	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	0.212 ± 0.00	0.280 ± 0.02	0.205 ± 0.00	0.268 ± 0.01
T ₁	0.232 ± 0.00 (8.73)	0.299 ± 0.01 (6.25)	0.224 ± 0.00 (8.77)	0.287 ± 0.01 (6.72)
T ₂	0.227 ± 0.00 (6.64)	0.292 ± 0.02 (4.18)	0.220 ± 0.00 (7.02)	0.281 ± 0.01 (4.79)
T ₃	0.287 ± 0.01 (26.19)	0.329 ± 0.02 (14.79)	0.278 ± 0.01 (26.41)	0.320 ± 0.02 (16.36)
T ₄	0.260 ± 0.00 (18.71)	0.308 ± 0.00 (8.95)	0.252 ± 0.00 (18.64)	0.299 ± 0.00 (10.32)
T ₅	0.274 ± 0.00 (22.85)	0.317 ± 0.01 (11.58)	0.266 ± 0.00 (22.96)	0.308 ± 0.01 (12.95)
T ₆	0.271 ± 0.00 (21.91)	0.312 ± 0.00 (10.12)	0.263 ± 0.00 (21.99)	0.304 ± 0.00 (11.94)
T ₇	0.257 ± 0.00 (17.78)	0.302 ± 0.00 (7.20)	0.250 ± 0.00 (18.18)	0.294 ± 0.11 (8.74)
CD at p=0.05	0.017	NS	0.016	NS
Sem±	0.005	0.016	0.005	0.015

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.5.a: Effect of the treatment on total phenol content (mg g⁻¹ FW) of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

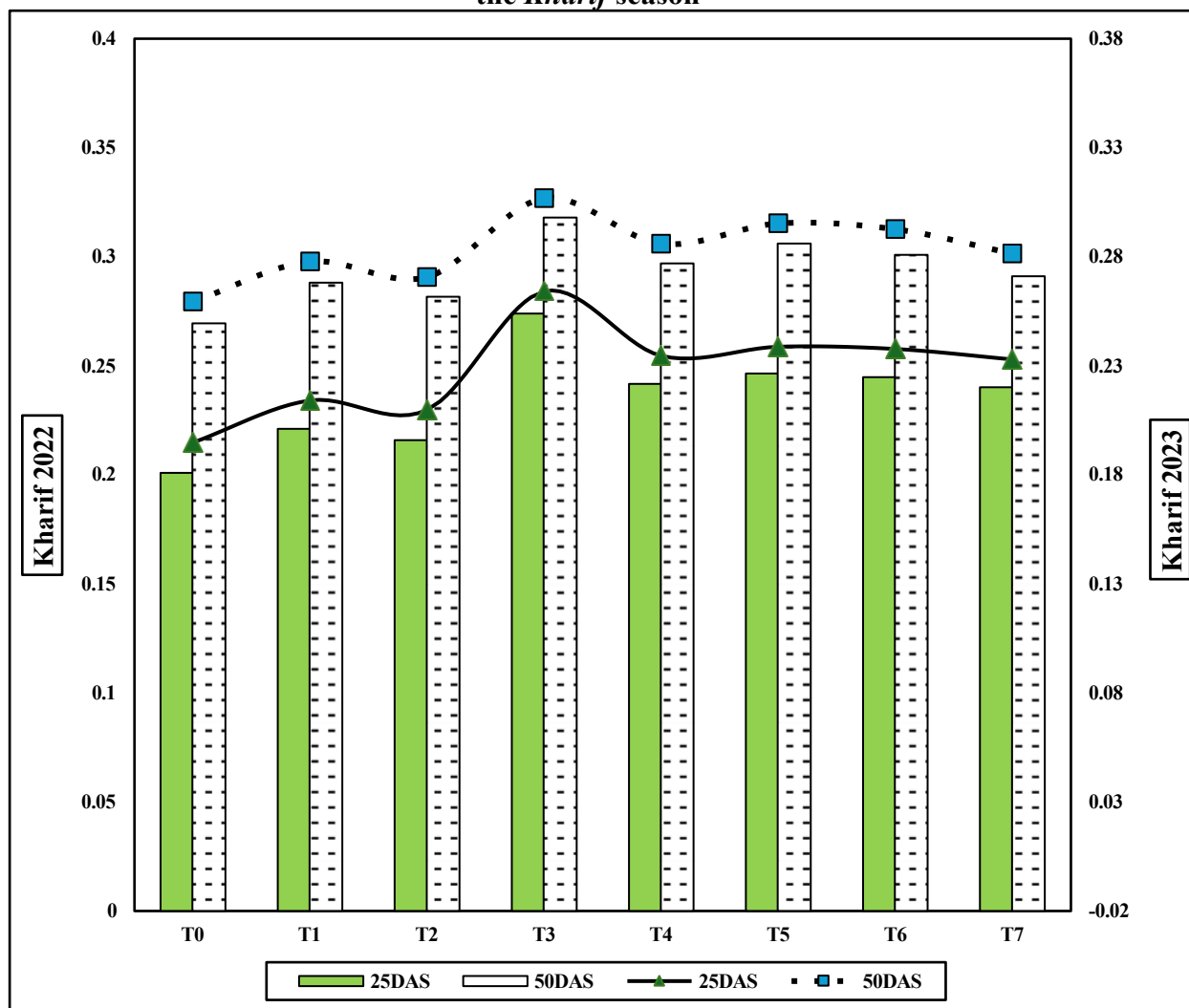
TABLE 4.2.5.b: Effect of the treatment on total phenol content (mg⁻¹ FW) of black gram in the *Kharif* season

Treatments	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	0.201 ± 0.00	0.269 ± 0.02	0.195 ± 0.00	0.259 ± 0.01
T ₁	0.221 ± 0.00 (9.15)	0.288 ± 0.01 (6.48)	0.214 ± 0.00 (9.01)	0.278 ± 0.01 (6.61)
T ₂	0.216 ± 0.00 (6.97)	0.282 ± 0.02 (4.34)	0.210 ± 0.00 (7.14)	0.271 ± 0.02 (4.14)
T ₃	0.274 ± 0.01 (26.68)	0.318 ± 0.02 (15.29)	0.264 ± 0.01 (26.25)	0.307 ± 0.02 (15.44)
T ₄	0.242 ± 0.00 (16.90)	0.297 ± 0.00 (9.27)	0.235 ± 0.00 (16.95)	0.286 ± 0.00 (9.27)
T ₅	0.246 ± 0.01 (18.49)	0.306 ± 0.01 (11.99)	0.239 ± 0.01 (18.40)	0.295 ± 0.01 (12.14)
T ₆	0.245 ± 0.00 (17.97)	0.301 ± 0.00 (10.48)	0.238 ± 0.00 (18.05)	0.293 ± 0.00 (11.34)
T ₇	0.240 ± 0.00 (16.39)	0.291 ± 0.00 (7.46)	0.233 ± 0.00 (16.36)	0.281 ± 0.00 (7.83)
CD at p=0.05	0.017	NS	0.017	NS
Sem±	0.005	0.016	0.006	0.015

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.5.b: Effect of the treatment on total phenol content (mg^{-1} FW) of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine ($1500\mu\text{mol}$), T2= Cadaverine ($1000\mu\text{mol}$), T3= Putrescine ($1500\mu\text{mol}$) + Cadaverine ($1000\mu\text{mol}$), T4= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$), T5= Putrescine ($750\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$), T6= Putrescine ($750\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$) and T7= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$)

4.2.6. TOTAL FLAVANOL CONTENT

The total flavanol content of black gram was evaluated at 25 and 50 DAS across the summer and *Kharif* seasons of 2022 and 2023 to assess the effect of different foliar applications of polyamines. The results are presented in Table 4.2.6.a and 4.2.6.b and depicted in Figures 4.2.6.a and 4.2.6.b.

During Summer 2022, significant differences among treatments were observed. Treatment 3 (Putrescine 1500 μM + Cadaverine 1000 μM) produced the highest concentration of flavanols (0.567 mg/g) at 25 days after sowing, which was significantly higher than the control (0.479 mg/g), exceeding the CD value. Also, at 50 days after sowing, treatment 3 had the highest flavanol concentration (0.645 mg/g), a significant increase relative to the control (0.525 mg/g). Treatments 5 and 4 produced relatively high concentrations of flavanols and had increases ranging from 11.70 to 16.84% compared to the control. Like the previous summer, significant differences in treatments were observed during summer 2023 (CD = 0.027 at 25 days after sowing and 0.042 at 50 days after sowing). Treatment 3 had the highest concentration of flavanols at 25 days after sowing (0.537 mg/g), a 16.62% increase over the control (0.447 mg/g). At 50 days after sowing, treatment 3 had a concentration of 0.591 mg/g and a 19.97% increase over the control (0.473 mg/g). Treatments 5 and 4 also had significant increases of 13.35 to 18.01% over the control.

There were differences in treated conditions during *Kharif* 2022 between treatments (C.D. = 0.025 at 25 DAS and 0.040 at 50 DAS). At 25 and 50 DAS, T3 had the highest amount of flavanol (0.512 mg g⁻¹ and 0.568 mg g⁻¹ versus 0.427 mg g⁻¹ and 0.453 mg g⁻¹, respectively) and displayed the most significant comparative increase (i.e., 16.58% and 20.25%) of any treatment versus control. T4 and T5 were also higher than the control by 14.29–18.66%.

For *Kharif* 2023, differences between treatments were observed to be significant (C.D. = 0.026 at 25 D.A.S. and 0.040 at 50 D.A.S.). At 25 and 50 D.A.S., T3 had maximum amounts of flavanol (0.495 mg g⁻¹ and 0.548 mg g⁻¹, respectively) and greater than control (0.408 mg g⁻¹ and 0.433 mg g⁻¹, respectively) by 17.57% and 20.98% over control, respectively. Other treatments such as T5 and T4 produced significant increases in amounts of flavanol compared to control (14.40–19.34%).

In summary, T3 consistently produced greater amounts of flavanol compared to control throughout all growing seasons and years ($\approx$ 16–21% greater).

The higher levels of flavanols in the polyamine treated plants suggest that polyamines play a significant role in regulating the metabolism of secondary metabolites in plants. The flavanols are

a significant group of phenolic compounds, and an important function of these compounds in plants is that they provide strong antioxidant properties, which help protect the plant, as part of its defenses.

The activity of several enzymes responsible for synthesizing flavonoid and flavanol compounds is stimulated by polyamines in the phenylpropanoid pathway; therefore by increasing honed synthesis of these compounds, overall levels of flavanols in the combined treatment were much higher than those observed when only one polyamine was used. Furthermore, polyamines help to preserve cellular redox balance in plants, as well as providing protection to plant tissues from oxidative stress by scavenging reactive oxygen species (ROS). The increased levels of flavanols in the combined treatment may, therefore, help to enhance the antioxidant defense system and provide physiological stability to plants under stress.

The better performance of the combined treatment as compared to the individual treatments supports the idea that there is an additive effect between putrescine and cadaverine in terms of enhancing metabolic regulation and stimulating the synthesis of protective secondary metabolites, which may ultimately lead to improved plant growth and productivity in black gram.

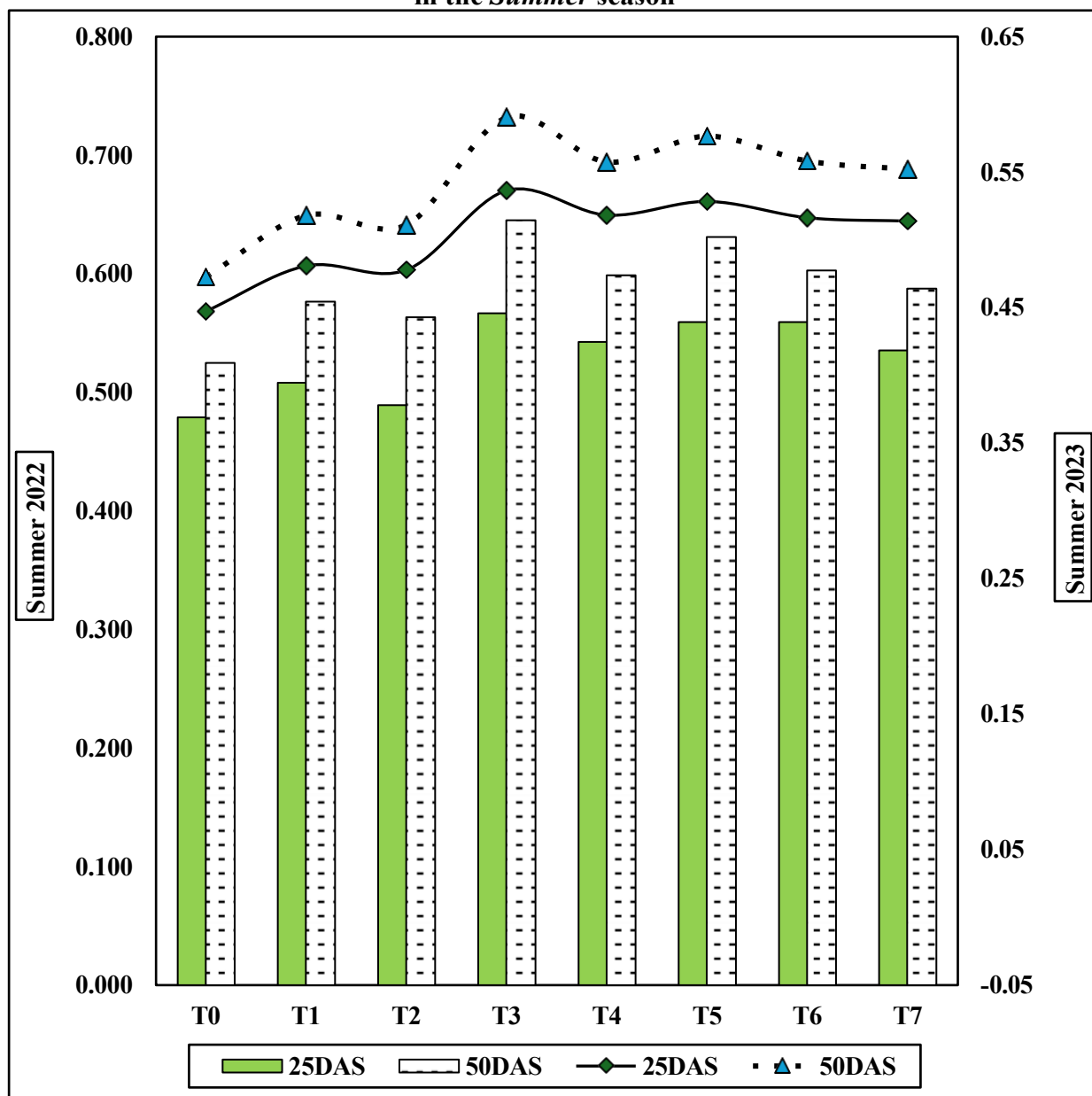
TABLE 4.2.6.a: Effect of the treatment on total flavanol content (mg g⁻¹ FW) of black gram in the *Summer* season

Treatments	<i>Summer</i> (2022)		<i>Summer</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	0.479 ± 0.00	0.525 ± 0.02	0.447 ± 0.01	0.473 ± 0.01
T ₁	0.508 ± 0.03 (5.72)	0.576 ± 0.00 (8.98)	0.481 ± 0.01 (6.96)	0.518 ± 0.00 (8.77)
T ₂	0.489 ± 0.02 (2.10)	0.563 ± 0.01 (6.87)	0.478 ± 0.00 (6.37)	0.511 ± 0.00 (7.52)
T ₃	0.567 ± 0.01 (15.48)	0.645 ± 0.02 (18.66)	0.537 ± 0.00 (16.62)	0.591 ± 0.01 (19.97)
T ₄	0.542 ± 0.00 (11.70)	0.599 ± 0.02 (12.38)	0.518 ± 0.00 (13.67)	0.557 ± 0.02 (15.21)
T ₅	0.559 ± 0.00 (14.36)	0.631 ± 0.02 (16.84)	0.528 ± 0.00 (15.33)	0.577 ± 0.01 (18.01)
T ₆	0.559 ± 0.01 (14.37)	0.603 ± 0.00 (12.96)	0.516 ± 0.00 (13.35)	0.558 ± 0.00 (15.32)
T ₇	0.535 ± 0.00 (10.53)	0.588 ± 0.01 (10.70)	0.514 ± 0.01 (12.94)	0.552 ± 0.01 (14.39)
CD at p=0.05	0.057	0.057	0.027	0.042
Sem±	0.019	0.019	0.009	0.014

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.6.a: Effect of the treatment on Total flavanol content (mg g^{-1} FW) of black gram in the *Summer* season



Note:

T0= Control, T1= Putrescine ($1500\mu\text{mol}$), T2= Cadaverine ($1000\mu\text{mol}$), T3= Putrescine ($1500\mu\text{mol}$) + Cadaverine ($1000\mu\text{mol}$), T4= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$), T5= Putrescine ($750\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$), T6= Putrescine ($750\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$) and T7= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$)

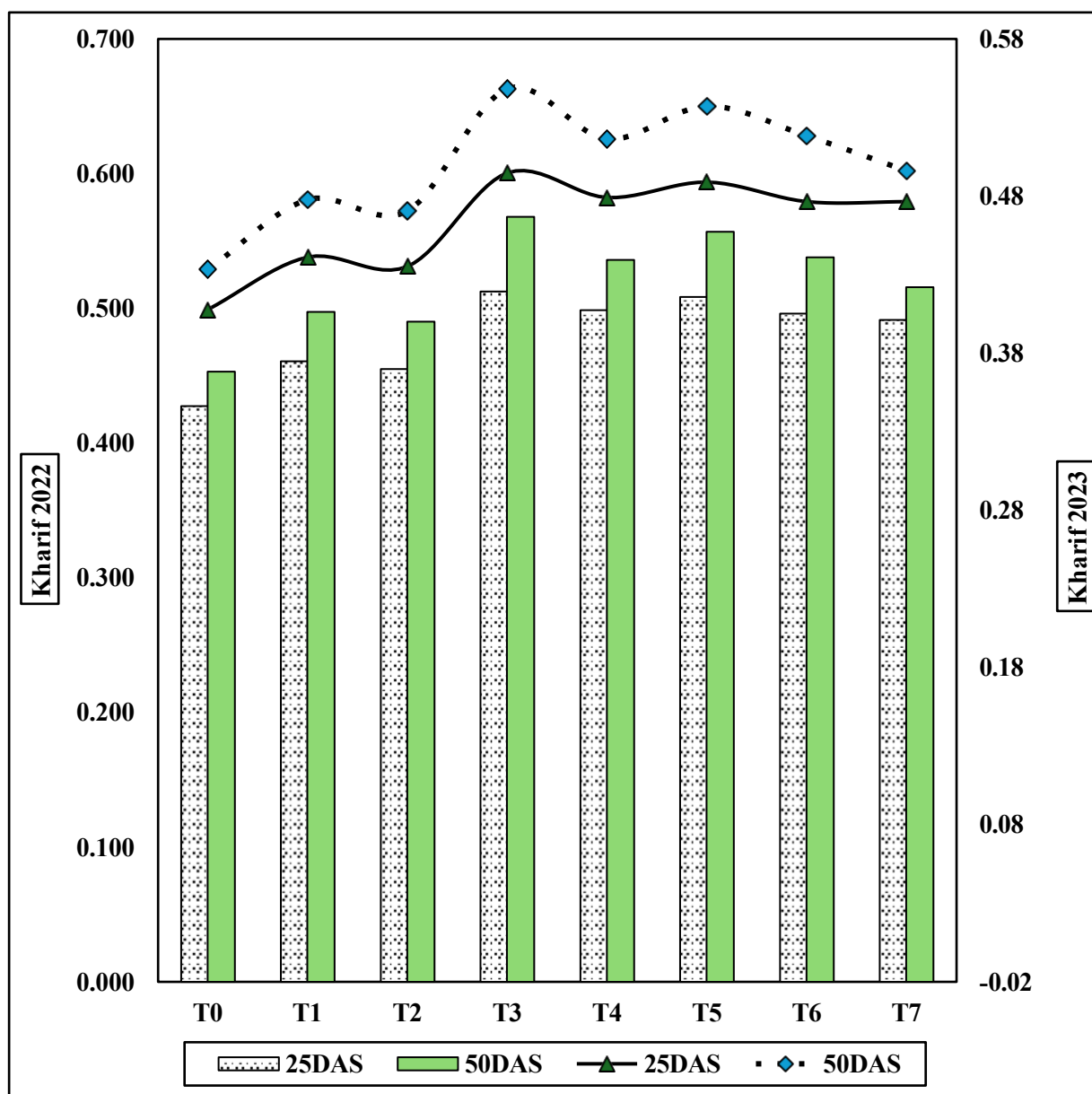
TABLE 4.2.6.b: Effect of the treatment on total flavanol content (mg⁻¹ FW) of black gram in the *Kharif* season

Treatments	<i>Kharif</i> (2022)		<i>Kharif</i> (2023)	
	25DAS	50DAS	25DAS	50DAS
T ₀	0.427 ± 0.01	0.453 ± 0.01	0.408 ± 0.01	0.433 ± 0.01
T ₁	0.461 ± 0.01 (7.23)	0.497 ± 0.00 (8.94)	0.441 ± 0.01 (7.56)	0.478 ± 0.00 (9.30)
T ₂	0.455 ± 0.00 (6.03)	0.490 ± 0.00 (7.60)	0.435 ± 0.00 (6.31)	0.471 ± 0.00 (7.92)
T ₃	0.512 ± 0.00 (16.58)	0.568 ± 0.01 (20.25)	0.495 ± 0.00 (17.57)	0.548 ± 0.01 (20.98)
T ₄	0.499 ± 0.00 (14.29)	0.536 ± 0.02 (15.49)	0.479 ± 0.00 (14.87)	0.516 ± 0.02 (16.08)
T ₅	0.508 ± 0.00 (15.94)	0.557 ± 0.01 (18.66)	0.489 ± 0.00 (16.58)	0.537 ± 0.01 (19.34)
T ₆	0.496 ± 0.00 (13.83)	0.538 ± 0.00 (15.80)	0.476 ± 0.00 (14.40)	0.518 ± 0.00 (16.39)
T ₇	0.492 ± 0.01 (13.03)	0.516 ± 0.01 (12.17)	0.476 ± 0.01 (14.40)	0.496 ± 0.01 (12.65)
CD at p=0.05	0.025	0.040	0.026	0.040
Sem±	0.008	0.013	0.008	0.013

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.2.6.b: Effect of the treatment on total flavanol content (mg g⁻¹ FW) of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500μmol), T2= Cadaverine (1000μmol), T3= Putrescine (1500μmol) + Cadaverine (1000μmol), T4= Putrescine (3000μmol) + Cadaverine (500μmol), T5= Putrescine (750μmol) + Cadaverine (2000μmol), T6= Putrescine (750μmol) + Cadaverine (500μmol) and T7= Putrescine (3000μmol) + Cadaverine (2000μmol)

4.3. QUALITY PARAMETER

4.3.1. NPK content in the seed of black gram

The effect of foliar application of putrescine and cadaverine on NPK content in seeds of black gram was recorded during the summer and *Kharif* seasons of 2022 and 2023. Data are tabulated in Table 4.2.3.a and 4.2.3.b and showed in Figure 4.2.4.a. and 4.2.3.b.

In the summer season of 2022, treatment T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M) had the maximal nitrogen accumulation (3.79%), which was greater by 2.99% than the control (T0 – 3.68%). It was followed by T5 (3.77%) and T6 (3.76%), which also produced increases in nitrogen accumulation. This pattern carried over to summer 2023, with T3 again having the most nitrogen accumulation at 3.44%, followed closely by T5 (3.42%) and T6 (3.41%), compared to the control (3.31%).

In the *Kharif* 2022 season, treatment T3 had the highest nitrogen concentration (3.66%) relative to the control (3.55%), or a gain of 3.10%. Treatments T5 (3.64%) and T6 (3.63%) also produced notable increases. In *Kharif* 2023, T3 continued to have the highest nitrogen concentration of 3.34% compared to a control of 3.23%, or a gain of 3.41%. For phosphorus accumulation during the summer 2022 season, the treatment T3 (0.396%) exhibited the highest accumulation, which was 4.21% higher than the control (0.380%), as observed in T5 (0.390%) and T6 (0.388%). In summer 2023, T3 had the highest phosphorus accumulation (0.389%), which was 5.42% more than the control (0.369%), T5 (0.382%), and T6 (0.380%). In *Kharif* seasons, T3 had 0.391% and 0.388% in 2022 and 2023, respectively, and more significantly than the control (0.378% and 0.368%), which were gains of 3.43% and 5.43%. T3 was the best treatment for potassium in all growing seasons.

In summer 2022, T3 measured 0.989%, representing a 2.17% improvement over the control (0.968%), followed by T5 (0.985%) and T6 (0.981%). The same was seen during summer 2023 when T3 recorded a top score of 0.986%, again a 2.17% increase over the control (0.965%). In *Kharif* 2022 and 2023, T3 again outperformed the remaining treatments with potassium values of 0.987% and 0.985%, respectively, when compared to the controls on those dates (0.966 and 0.964), reflecting 2.17% and 2.18% increases.

The consistent increases in nitrogen, phosphorus, and potassium content in black gram seeds when influenced by putrescine and cadaverine, particularly in combination at moderate doses (T3: 1500 μ M + 1000 μ M), signify the beneficial effects of polyamines on nutrient assimilation.

It has been shown that polyamines influence nitrogen metabolism by stimulating enzymes that increase nitrate reductase, glutamine synthetase, and others. The observed increase in phosphorus content was most likely a result of increased root growth and membrane transport action due to polyamines' greater capacity to absorb phosphorus. Cadaverine and putrescine promote symplastic and apoplastic flows, and that might explain the increased phosphorus level here. The improvements in potassium uptake from polyamines were likely based on the effects of polyamines to maintain membrane stability, osmotic balance, and relieve stress. This allows for improved nutrient transport efficiency. Kumar *et al.* (2021) concluded that polyamines improve water relations in plants, and this may also improve K⁺ movement through plant tissues. Overall, the results highlight the synergistic effect of putrescine and cadaverine on seed nutrient quality, especially at moderate application concentrations, probably because of improved nutrient absorption, root morphology, and cellular metabolism.

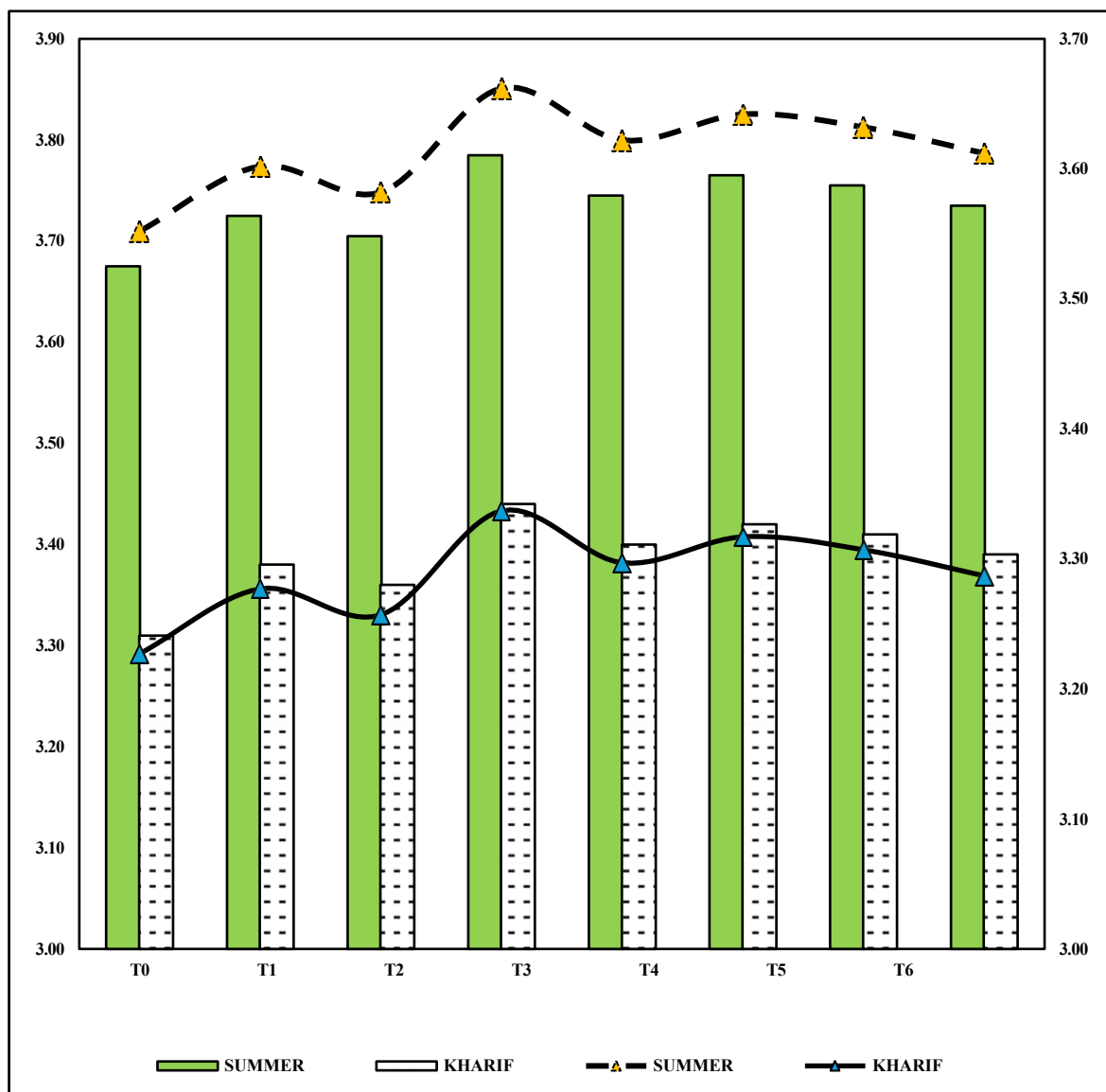
**Table 4.3.1.a: Effect of the polyamine on nitrogen content (%) of black gram, in
Summer
and *Kharif* season**

Treatment	<i>Summer 2022</i>	<i>Summer 2023</i>	<i>Kharif 2022</i>	<i>Kharif 2023</i>
T ₀	3.68	3.31	3.55	3.23
T ₁	3.73	3.38	3.60	3.28
T ₂	3.71	3.36	3.58	3.26
T ₃	3.79	3.44	3.66	3.34
T ₄	3.75	3.40	3.62	3.30
T ₅	3.77	3.42	3.64	3.32
T ₆	3.76	3.41	3.63	3.31
T ₇	3.74	3.39	3.61	3.29

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.3.1.a: Effect of the polyamine on nitrogen content (%) of black gram, in *Summer* and *Kharif* season



T0= Control, T1= Putrescine (1500µmol), T2= Cadaverine (1000µmol), T3= Putrescine (1500µmol) + Cadaverine (1000µmol), T4= Putrescine (3000µmol) + Cadaverine (500µmol), T5= Putrescine (750µmol) + Cadaverine (2000µmol), T6= Putrescine (750µmol) + Cadaverine (500µmol) and T7= Putrescine (3000µmol) + Cadaverine (2000µmol)

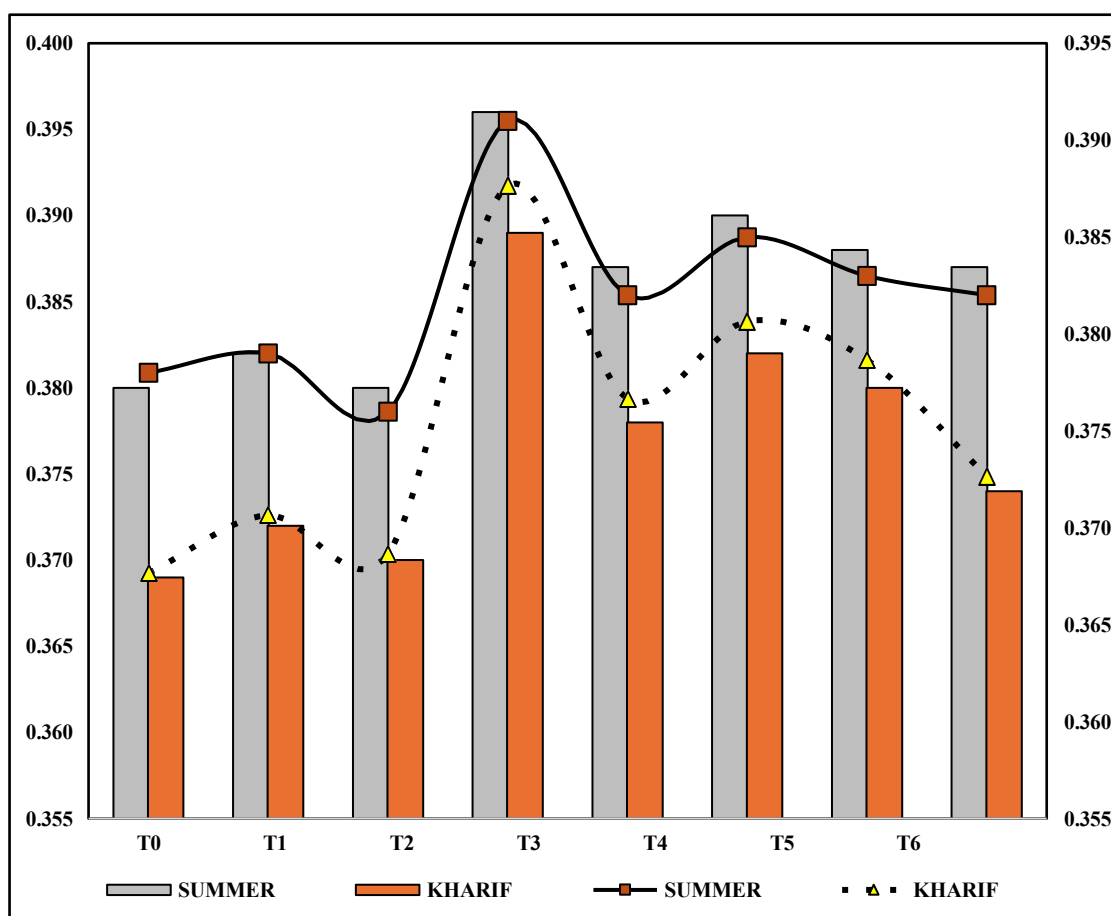
**Table 4.3.1.b: Effect of the polyamine on phosphorus content (%) of black gram, in
Summer and Kharif season**

Treatment	<i>Summer 2022</i>	<i>Summer 2023</i>	<i>Kharif 2022</i>	<i>Kharif 2023</i>
T ₀	0.380	0.369	0.378	0.368
T ₁	0.382	0.372	0.379	0.371
T ₂	0.380	0.370	0.376	0.369
T ₃	0.396	0.389	0.391	0.388
T ₄	0.387	0.378	0.382	0.377
T ₅	0.390	0.382	0.385	0.381
T ₆	0.388	0.380	0.383	0.379
T ₇	0.387	0.374	0.382	0.373

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

**Fig 4.3.1.b: Effect of the polyamine on phosphorus content (%) of black gram, in
Summer and Kharif season**



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

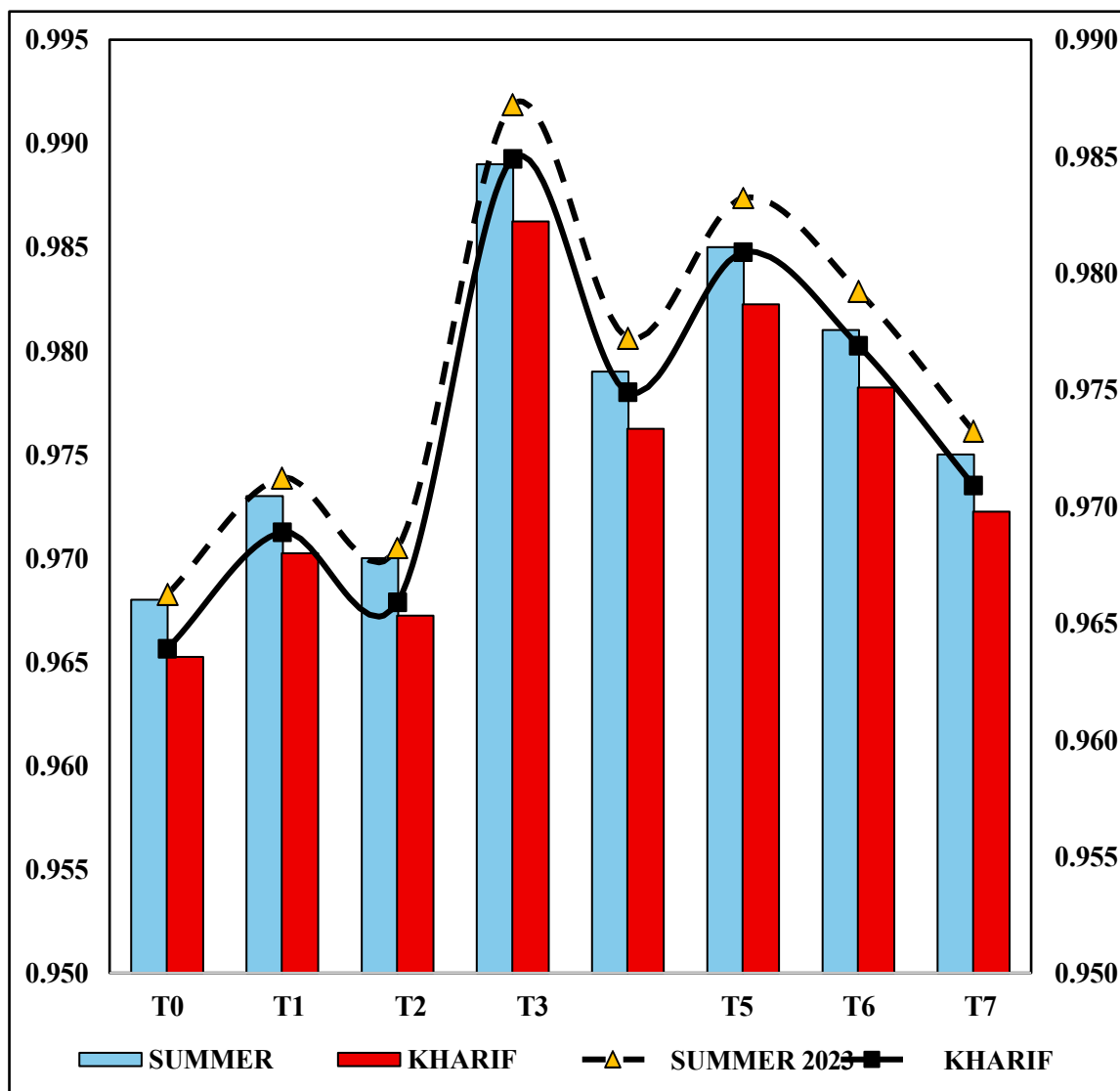
**Table 4.3.1.c: Effect of the polyamine on potassium content (%) of black gram, in
Summer and Kharif season**

Treatment	<i>Summer 2022</i>	<i>Summer 2023</i>	<i>Kharif 2022</i>	<i>Kharif 2023</i>
T ₀	0.968	0.965	0.966	0.964
T ₁	0.973	0.970	0.971	0.969
T ₂	0.970	0.967	0.968	0.966
T ₃	0.989	0.986	0.987	0.985
T ₄	0.979	0.976	0.977	0.975
T ₅	0.985	0.982	0.983	0.981
T ₆	0.981	0.978	0.979	0.977
T ₇	0.975	0.972	0.973	0.971

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.3.1.c: Effect of the polyamine on potassium content (%) of black gram, in *Summer* and *Kharif* season



T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.3.2. Tryptophan content in seed

The effect of foliar application of putrescine and cadaverine on tryptophan content in the seed of black gram during the summer and *Kharif* seasons of 2022 and 2023. Data are tabulated in Table 4.2.3.a and 4.2.3.b and illustrated in Figure 4.2.4.a. and 4.2.3.b.

Across the 2022 and 2023 summer seasons, the most enhanced tryptophan content was for treatment T3 (Putrescine 1500 μ M and Cadaverine 1000 μ M), which had the recorded tryptophan contents of 888.81 mg/100g and 853.21 mg/100g, respectively. This corresponded to increases of 23.90% and 25.08% over the control, T0 with 717.41 mg/100g and 681.81 mg/100g, respectively. The second lounge was T5 (Putrescine 750 μ M and Cadaverine 2000 μ M). The percentage increases for T5 were 18.25% and 19.22%, while T6 (750 μ M Putrescine and 500 μ M Cadaverine) corresponded to 12.81% to 13.46% over the control, in 2022 and 2023, respectively.

The lowest percentage increase was treatment T1 (1500 μ M Putrescine), which had increases of 2.90% in 2022 and 3.05% in 2023. For treatment T2 (Cadaverine 1000 μ M), the values were lower still than T1, with increases of 1.14% in 2022 and 1.19% in 2023 compared to the control.

During both the *Kharif* of 2022 and 2023, the trends were again similar. T3 still clearly showed the highest tryptophan content of 876.51 mg/100g and 843.39 mg/100g (corresponding to 24.31% and -25.46% increases over control values of 705.11 mg/100g and 671.99 mg/100g). Once again, followed by T5 and T6 (+18.55% and +13.03% in 2022, and +19.48% and 13.67% in 2023, respectively).

Tryptophan, an essential amino acid for protein biosynthesis, also leads to auxin synthesis and stress. In legumes like black gram, these processes are important. The data from the study indicate that polyamines applied to the leaves were positively correlated to the amount of tryptophan in the seed, and this relationship was particularly robust at intermediate levels of both treatments.

Using foliar application of polyamines, Treatment (T3) (Putrescine 1500 μ M + Cadaverine 1000 μ M) produced the most significant amount of tryptophan in both seasons and years of the study. Perhaps the interrelated functions of polyamines on unregulated pathways (as well as nitrogen uptake and amino acid biosynthesis) are responsible for this outcome. For example, Polyamines, like putrescine and cadaverine, may have increased the activity of enzymes

involved in nitrogen metabolism, e.g., glutamine synthetase and nitrate reductase (Hussain *et al.*, 2021). Therefore, with the polyamines applied together, there could have been more nitrogen available, which could provide an optimal environment for tryptophan biosynthesis. The argument that correctly applied polyamines improve amino acid metabolism while optimizing physiological stress is supported by the tryptophan concentration increases in treatments T5 and T6, which only had moderately elevated doses of the medicines.

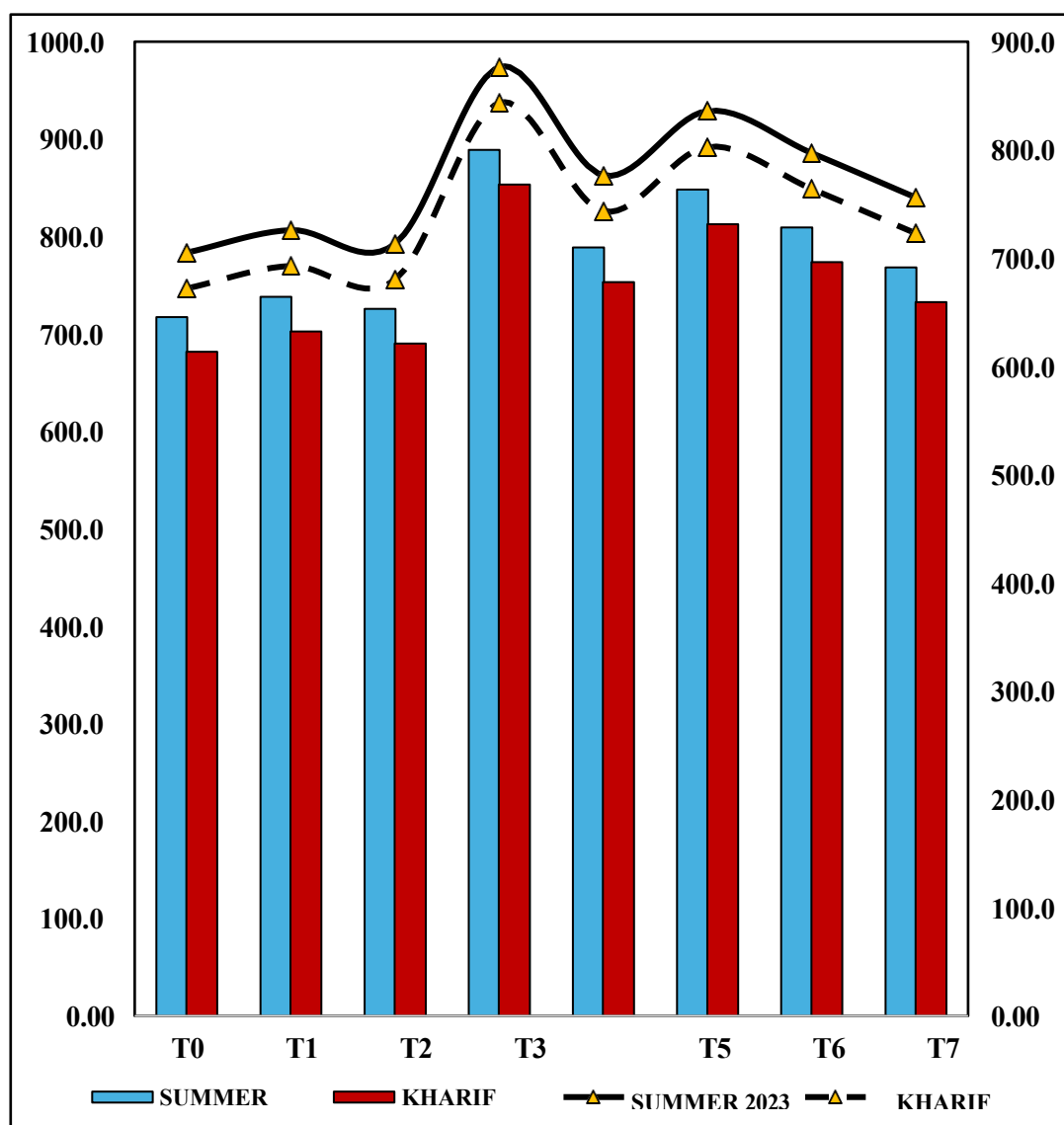
Table 4.3.2: Impact of the treatments on tryptophan (mg g⁻¹) content in the seed of black gram in the *Summer* and *Kharif* season

Treatment	<i>Summer 2022</i>	<i>Summer 2023</i>	<i>Kharif 2022</i>	<i>Kharif 2023</i>
T ₀	717.41	681.81	705.11	671.99
T ₁	738.21	702.61	725.91	692.79
T ₂	725.61	690.01	713.31	680.19
T ₃	888.81	853.21	876.51	843.39
T ₄	788.71	753.11	776.41	743.29
T ₅	848.31	812.71	836.01	802.89
T ₆	809.31	773.71	797.01	763.89
T ₇	768.21	732.61	755.91	722.79

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

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



T0= Control, T1= Putrescine (1500 μmol), T2= Cadaverine (1000 μmol), T3= Putrescine (1500 μmol) + Cadaverine (1000 μmol), T4= Putrescine (3000 μmol) + Cadaverine (500 μmol), T5= Putrescine (750 μmol) + Cadaverine (2000 μmol), T6= Putrescine (750 μmol) + Cadaverine (500 μmol) and T7= Putrescine (3000 μmol) + Cadaverine (2000 μmol)

4.4 Yield attributing parameters

4.4.1 Number of pods plant⁻¹

The effect of foliar application of putrescine and cadaverine on the Number of pods per plant during the summer and *Kharif* seasons of 2022 and 2023. Data are tabulated in Table 4.4.1.a. An illustration is shown in Figure 4.1.4. a and 4.1.4.b.

There were large treatment differences in summer 2022 ($CD_{0.05} = 2.562$), as T3 (Putrescine 1500 μM + Cadaverine 1000 μM) had the most pods per plant (36.7 pods plant⁻¹), which was 19.22% more than control (29.6 pods plant⁻¹), and the increase was completely above the CD value. T5 (35.2 pods) and T6 (34.9 pods) also had increased numbers of pods per plant with increases of 15.76% and 15.01%, respectively.

T3 again had the maximum number of pods per plant (34.5) in summer 2023 ($CD = 2.903$) with a 22.71% increase over control (26.7). T5 (33.8 pods) and T6 (32.9 pods) both had appreciable increases of 21.24% and 19.11%, respectively.

Interestingly, in kharif 2022 ($CD = 2.687$), T3 had the highest number of pods per plant (33.9 pods), which indicates a 21.20% increase over control (26.7 pods). T5 (32.5 pods) and T6 (32.0 pods) also had increases of 17.78% and 16.51%, respectively. Just as in kharif 2023 ($CD = 2.819$), T3 had the maximum number of pods per plant (31.6) with an increase of 24.15% over control (24.0), and the increase was significant. T5 (31.1 pods) and T6 (30.1 pods) also had significant increases in pods with 22.91% and 20.31%, respectively.

Overall, the combined use of putrescine and cadaverine (T3) produced consistently higher numbers of pods per plant ($\approx 19\text{--}24\%$ increase over control) over all seasons and years.

With the use of polyamines, especially putrescine (1500 μM) + cadaverine (1000 μM), the number of total pods per plant significantly increased. Improved floral initiation, flowering, and pollen viability due to polyamines appears to lead to increased pod production. Polyamines are known to stabilize membranes, modulate hormone-based signalling, and improve nutrient uptake, which all impact reproductive growth positively. The fact that moderate doses (750 μM putrescine + 2000 or 500 μM cadaverine) performed better than extremely high (3000 μM) or low doses shows a dose-dependent biphasic response (regulatory mechanisms) of polyamines

working in the plant system, which is typically the case when polyamines are applied. The combination of putrescine and cadaverine in optimum concentrations positively improved pod formation in black grams, with more pronounced effects noted during the *Kharif* season, possibly due to better soil moisture and favourable growing conditions for reproduction.

TABLE 4.1.4.a: Effect of the treatment on the total number of pods per plant of black gram in the *Summer* and *Kharif* season (2022 and 2023)

Treatments	<i>Summer</i>		<i>Kharif</i>	
	2022	2023	2022	2023
T ₀	29.6 ± 1.30	26.7 ± 0.84	26.7 ± 1.41	24.0 ± 0.84
T ₁	31.6 ± 0.24 (6.27)	29.9 ± 0.94 (10.82)	28.9 ± 0.24 (7.78)	27.2 ± 0.94 (11.88)
T ₂	30.5 ± 0.47 (2.99)	28.5 ± 0.78 (6.61)	27.9 ± 0.47 (4.25)	25.8 ± 0.77 (7.11)
T ₃	36.7 ± 0.17 (19.22)	34.5 ± 1.14 (22.71)	33.9 ± 1.19 (21.20)	31.6 ± 1.00 (24.15)
T ₄	33.6 ± 0.92 (11.84)	32.3 ± 1.17 (17.55)	30.9 ± 0.92 (13.74)	29.6 ± 1.16 (19.10)
T ₅	35.2 ± 0.59 (15.76)	33.8 ± 1.02 (21.24)	32.5 ± 0.70 (17.78)	31.1 ± 1.03 (22.91)
T ₆	34.9 ± 0.66 (15.01)	32.9 ± 0.35 (19.11)	32.0 ± 0.56 (16.51)	30.1 ± 0.31 (20.31)
T ₇	31.9 ± 0.49 (7.27)	30.6 ± 0.46 (12.85)	29.2 ± 0.53 (8.72)	27.8 ± 0.43 (13.88)
CD at p=0.05	2.562	2.903	2.687	2.819
Sem±	0.836	0.948	0.877	0.921

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.1.4.a: Effect of the treatment on the total number of pods per black gram plant in the *Summer* season

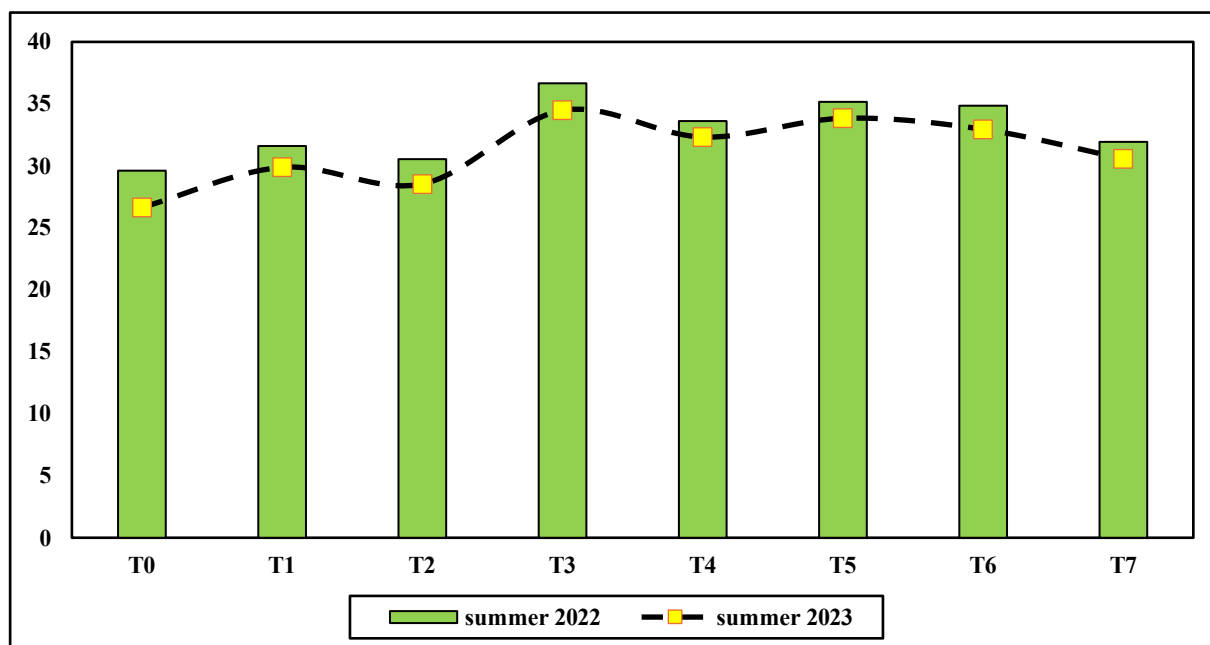
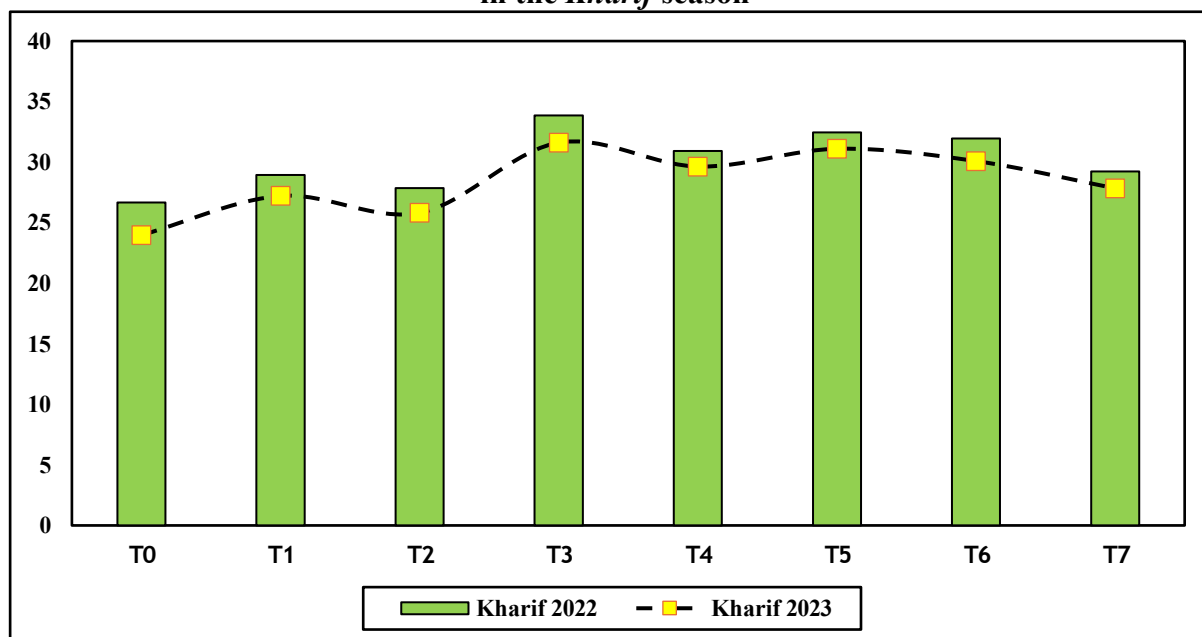


Fig 4.1.4.b: Effect of the treatment on the total number of pods per plant⁻¹ of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.4.2 Number of seed pod⁻¹

The effect of foliar application of putrescine and cadaverine on the number of seeds per pod during both seasons, summer and *Kharif* of 2022 and 2023. Data are tabulated in Table 4.4.2. a and showed in Figure 4.4.2.a. and 4.4.2.b.

There were significant differences between treatments in Summer 2022 ($CD_{0.05} = 0.295$). The highest number of seeds per pod was with T3 (Putrescine 1500 μ M+ Cadaverine 1000 μ M) which produced an average of 8.56 seeds pod⁻¹, 18.66% higher than the control (6.96 seeds pod⁻¹), with the differences exceeding the critical difference. T5 (7.84 seeds) and T6 (7.73 seeds) had important increases too, at 11.26% and 9.96% respectively over the control.

The significant treatment differences also continued in Summer 2023 ($CD = 0.341$), with T3 again producing the most seeds per pod (8.35) and a 19.25% increase over the control (6.74). T5 and T6 both demonstrated sizeable increases over the control (13.74% and 12.43%, respectively).

Significant differences in treatments were also seen in *Kharif* 2022 ($CD = 0.481$). T3 produced the highest number of seeds per pod (7.97) with a 16.98% increase over the control (6.62). T5 and T6 also had greater increases over the control (14.29% and 13.28%, respectively). *Kharif* 2023 treatment differences were also significant ($CD = 0.367$), with T3 producing the greatest number of seeds per pod (7.90) and had an increase of 17.18% over the control pod⁻¹ (6.54). T5 and T6 also had significant increases in seed numbers per pod compared to the control (14.47% and 13.15%, respectively).

In summary, the sole application of putrescine and cadaverine (T3) consistently produced the greatest number of seeds per pod (≈ 17 -19% greater than the control) regardless of season and/or year.

The data clearly show that application of polyamines, mainly the combined treatment of putrescine (1500 μ M) and cadaverine (1000 μ M), significantly promoted seed numbers per pod in all seasons and years. This suggests a synergistic effect from both polyamines at the optimal dose that favors reproductive efficiency. Polyamines are known to improve pollen viability, increase the likelihood of fertilization leading to seed development, and reduce oxidative stress through membrane stabilization, protection from oxidative damage, and regulation of gene expression associated with reproductive growth. The moderate combinations of putrescine and cadaverine also did well, revealing the importance of the right amount since exceedingly high

or low concentrations are unlikely to be as effective. The occurrence of the same pattern across summer and *Kharif*; the robustness of the treatment effect is confirmed and indicates that polyamines can be used to develop reproductive traits such as seed numbers per pod in black gram under changed climatic conditions.

TABLE 4.4.2: Effect of the treatment on the number of seeds pod⁻¹ of black gram in the *Summer* and *Kharif* (2022 and 2023)

Treatments	<i>Summer</i>		<i>Kharif</i>	
	2022	2023	2022	2023
T ₀	6.96 ± 0.08	6.74 ± 0.20	6.62 ± 0.27	6.54 ± 0.23
T ₁	7.19 ± 0.03 (3.20)	7.12 ± 0.06 (5.38)	7.09 ± 0.04 (6.69)	7.06 ± 0.03 (7.32)
T ₂	7.07 ± 0.07 (1.60)	7.07 ± 0.07 (4.71)	6.87 ± 0.25 (3.73)	6.83 ± 0.11 (4.25)
T ₃	8.56 ± 0.18 (18.66)	8.35 ± 0.18 (19.25)	7.97 ± 0.04 (16.98)	7.90 ± 0.06 (17.18)
T ₄	7.45 ± 0.06 (6.54)	7.45 ± 0.06 (9.49)	7.31 ± 0.08 (9.53)	7.30 ± 0.10 (10.41)
T ₅	7.84 ± 0.04 (11.26)	7.81 ± 0.03 (13.74)	7.72 ± 0.13 (14.29)	7.65 ± 0.03 (14.47)
T ₆	7.73 ± 0.13 (9.96)	7.70 ± 0.09 (12.43)	7.63 ± 0.04 (13.28)	7.53 ± 0.08 (13.15)
T ₇	7.38 ± 0.03 (5.63)	7.38 ± 0.03 (8.61)	7.24 ± 0.10 (8.63)	7.14 ± 0.03 (8.45)
CD at p=0.05	0.295	0.341	0.481	0.367
Sem±	0.096	0.111	0.157	0.120

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.4.2.a: Effect of the treatment on the number of seeds pod⁻¹ of black gram in the *Summer* season

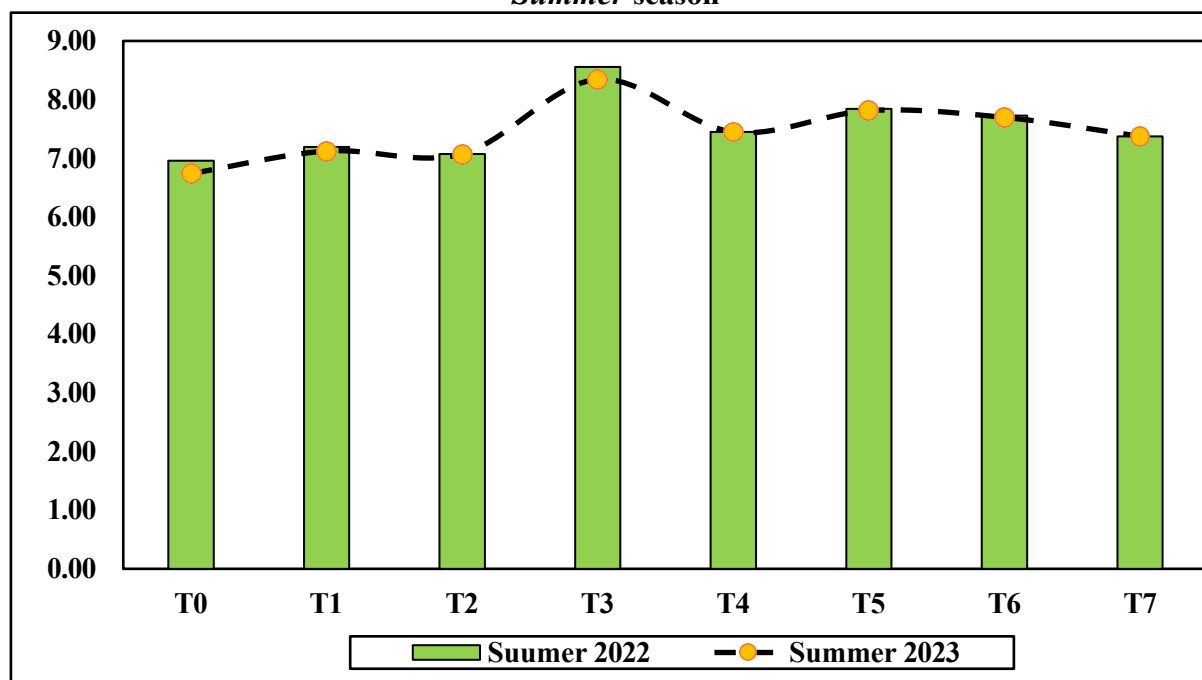
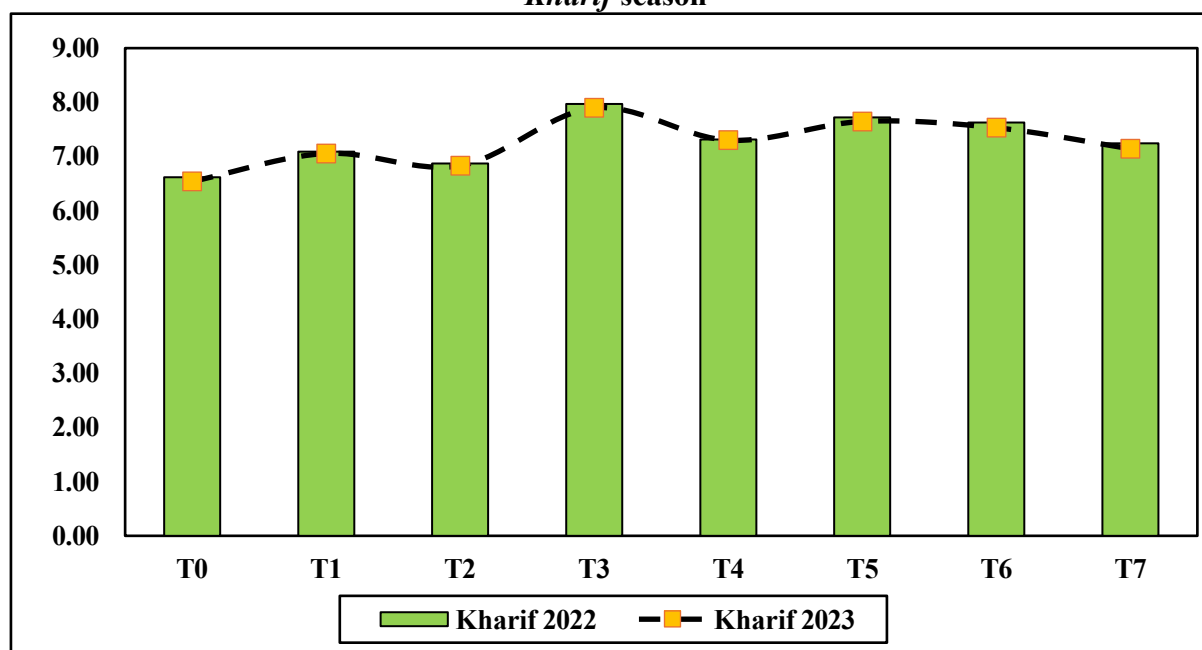


Fig 4.4.2.b: Effect of the treatment on the number of seeds pod⁻¹ of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.4.3 Seed weight pod⁻¹

The effect of foliar application of putrescine and cadaverine on total carbohydrate content in black gram leaves was recorded at 25 and 50 DAS during both seasons (summer and *Kharif*) of 2022 and 2023. Data are tabulated in Table 4.4.3, as shown in Figure 4.4.3.a and 4.4.3.b.

During Summer 2022, significant differences were observed among treatments ($CD_{0.05} = 0.025$). The highest seed weight per pod was recorded under T3 (Putrescine 1500 μM + Cadaverine 1000 μM) with 0.41 g, showing 17.87% increase over control (0.34 g) and the difference exceeded the CD value. Treatments T5 and T6 (0.40 g) also recorded higher values with 15.28% and 13.92% increase over control, respectively.

During Summer 2023, treatment differences remained significant ($CD = 0.024$). Treatment T3 recorded the maximum seed weight per pod (0.40 g) with 17.01% increase over control (0.33 g). Treatments T5 and T6 (0.39 g) also showed considerable increases with 14.02% and 15.25% improvement over control, respectively.

In *Kharif* 2022, significant variation among treatments was observed ($CD = 0.048$). Treatment T3 recorded the highest seed weight per pod (0.40 g) with 19.83% increase over control (0.32 g). Treatments T5 (0.39 g) and T4 (0.37 g) also recorded higher values with 16.02% and 13.39% increase over control, respectively. Similarly, during *Kharif* 2023, treatment differences were significant ($CD = 0.026$). T3 recorded the maximum seed weight per pod (0.39 g) showing 18.61% increase over control (0.31 g). Treatments T5 and T4 (0.37 g) also showed considerable increases with 16.07% improvement over control. Overall, the combined application of putrescine and cadaverine (T3) consistently recorded the highest seed weight per pod (≈ 17 – 20% increase over control) across seasons and years.

The increased seed weight per pod due to polyamine application, especially when applied in combination, confirms the beneficial impact of polyamines on reproductive development and seed filling in black gram. The application of Putrescine (1500 μM) + Cadaverine (1000 μM) was the best in each growing season across years and displayed a synergistic effect. Polyamines are known to enhance sink strength, improve translocation of nutrients, regulate the expression pattern of seed development genes, and may have resulted in increased seed weight. Moreover, they carry out the additional tasks of maintaining membrane stability and regulating enzyme activities involved in the seed-filling period of seed development. Polyamines generally

respond to moderate concentrations of, say, Putrescine 750 μM + Cadaverine 2000 or 500 μM , which may have also contributed to seed development, but still, it was significantly higher when compared to control treatments and high dose combinations, which indicates polyamines demonstrate common concentration-dependent responses in plant physiological processes.

TABLE 4.4.3: Effect of the treatment on the seed weight pod^{-1} of black gram in the Summer season

Treatments	<i>Summer</i>		<i>Kharif</i>	
	2022	2023	2022	2023
T ₀	0.34 $\pm$ 0.01	0.33 $\pm$ 0.00	0.32 $\pm$ 0.00	0.31 $\pm$ 0.00
T ₁	0.36 $\pm$ 0.00 (4.67)	0.35 $\pm$ 0.00 (5.66)	0.34 $\pm$ 0.01 (5.83)	0.34 $\pm$ 0.01 (7.84)
T ₂	0.36 $\pm$ 0.00 (6.42)	0.34 $\pm$ 0.00 (1.96)	0.34 $\pm$ 0.01 (4.90)	0.33 $\pm$ 0.00 (5.05)
T ₃	0.41 $\pm$ 0.01 (17.87)	0.40 $\pm$ 0.00 (17.01)	0.40 $\pm$ 0.01 (19.83)	0.39 $\pm$ 0.00 (18.61)
T ₄	0.38 $\pm$ 0.00 (11.30)	0.37 $\pm$ 0.00 (10.71)	0.37 $\pm$ 0.00 (13.39)	0.37 $\pm$ 0.00 (16.07)
T ₅	0.40 $\pm$ 0.00 (15.28)	0.39 $\pm$ 0.00 (14.02)	0.39 $\pm$ 0.00 (16.02)	0.37 $\pm$ 0.01 (16.07)
T ₆	0.40 $\pm$ 0.00 (13.92)	0.39 $\pm$ 0.00 (15.25)	0.37 $\pm$ 0.03 (12.22)	0.36 $\pm$ 0.01 (13.76)
T ₇	0.37 $\pm$ 0.00 (8.11)	0.37 $\pm$ 0.00 (10.71)	0.36 $\pm$ 0.01 (10.19)	0.35 $\pm$ 0.00 (11.32)
CD at p=0.05	0.025	0.024	0.048	0.026
Sem $\pm$	0.008	0.008	0.016	0.008

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500 μM , 1000 μM , 3000 μM , 500 μM , 750 μM , 2000 μM and 3000 μM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.4.3.a: Effect of the treatment on the seed weight pod⁻¹ of black gram in the *Summer* season

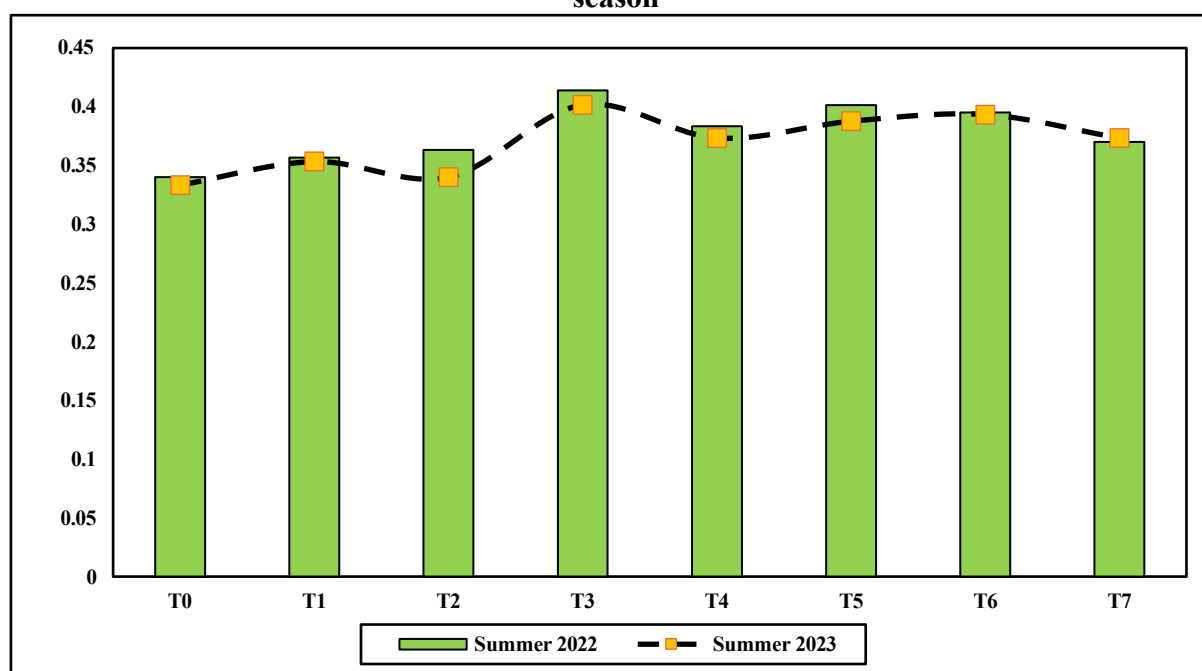
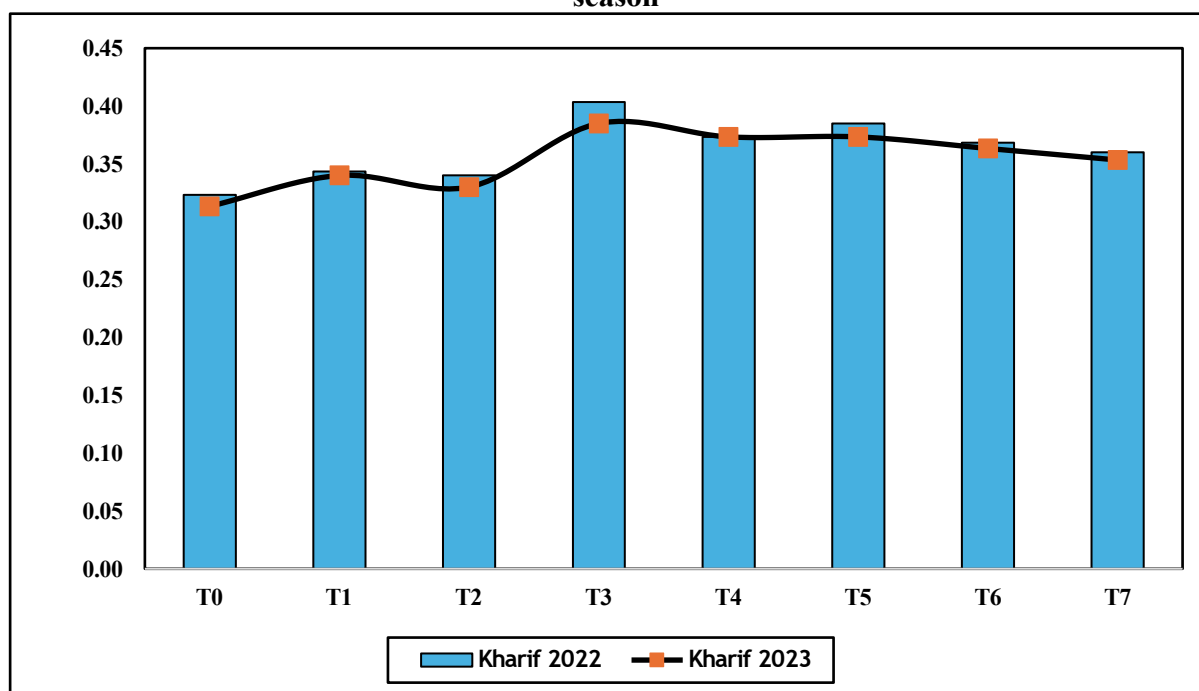


Fig 4.4.3.b: Effect of the treatment on the seed weight pod⁻¹ of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500μmol), T2= Cadaverine (1000μmol), T3= Putrescine (1500μmol) + Cadaverine (1000μmol), T4= Putrescine (3000μmol) + Cadaverine (500μmol), T5= Putrescine (750μmol) + Cadaverine (2000μmol), T6= Putrescine (750μmol) + Cadaverine (500μmol) and T7= Putrescine (3000μmol) + Cadaverine (2000μmol)

4.4.4 Seed weight plant⁻¹

The effect of foliar application of putrescine and cadaverine on Seed weight per plant during both seasons (summer and *Kharif*) of 2022 and 2023. Data are tabulated in Table 4.4.4. illustrated in Figure 4.4.4.a and 4.4.4.b.

In the summer of 2022, there were extremely large differences in the seed weights between some of the treatments ($CD_{0.05}$) with T3 giving the heaviest seed weight per plant (18.10 g) which represented a 32.39% increase compared to the control which had a seed weight of 12.24 g, absolutely exceeding the CD value. T5 and T6 were also noted to provide high seed weights per plant (17.02 g) with a 28.07% increase and (16.76 g) with a 28.97% increase over the control, respectively.

In the summer of 2023, the treatments continued to show significant differences ($CD = 1.342$). Also notable are that the T3 treatment produced the heaviest seed weight per plant (17.86 g) which represents a 32.47% increase over the control (12.06 g). Treatments T5 and T6 produced seed weights of 16.65 g and 16.31 g, respectively, which provided increases of 27.57% (T5) and 26.04% (T6) over the control.

In the *Kharif* season of 2022, all of the treatments produced significant differences in seed weight ($CD = 1.306$). Again, T3 produced the heaviest seed weight per plant (17.57 g) providing a 32.64% increase over the control (11.83 g) while T5 and T6 provided a seed weight per plant of 16.30 g and 15.97 g respectively, which represent a 27.40% and 25.89% increase over the control. In the *Kharif* season of 2023, T3 produced the highest seed weight per plant (17.30 g) and a 32.95% increase over the control (11.60 g). T5 and T6 produced a seed weight per plant of 16.00 g and 15.70 g respectively and represent a 27.50% and 26.11% increase over the control. Overall, the application of putrescine and cadaverine (T3) resulted in the highest increases in seed weight per plant across all seasons and years (approximately 32 to 33% increase).

The gradual increase in seed weight per plant exhibited by putrescine and cadaverine treatment combinations, namely putrescine (1500 μ M) and cadaverine (1000 μ M), provides evidence that their interaction increases plant production as a result of increased photosynthate partitioning into the reproductive organ. This occurred due to polyamines enhancing nutrient uptake and assimilation, increasing flower retention, increasing the chance of pod formation, and

enhancing seed development. The data also suggested that the response for Putrescine (750 μM) and Cadaverine (2000 and 500 μM) highlights that moderate doses of polyamines are beneficial while avoiding the potential inhibitive capacity of high concentrations. As well, cadaverine treatment or with both polyamines at higher concentrations was less effective than moderate combinations, suggesting dose-accelerated response capacity.

TABLE 4.4.4: Effect of the treatment on the seed weight plant⁻¹ of black gram in the *Summer and Kharif*

Treatments	<i>Summer</i>		<i>Kharif</i>	
	2022	2023	2022	2023
T ₀	12.24 $\pm$ 0.34	12.06 $\pm$ 0.32	11.83 $\pm$ 0.32	11.60 $\pm$ 0.30
T ₁	14.64 $\pm$ 0.35 (16.41)	14.31 $\pm$ 0.23 (15.74)	14.00 $\pm$ 0.20 (15.48)	13.70 $\pm$ 0.20 (15.33)
T ₂	12.94 $\pm$ 0.37 (5.41)	12.54 $\pm$ 0.38 (3.80)	12.30 $\pm$ 0.35 (3.79)	12.10 $\pm$ 0.35 (4.13)
T ₃	18.10 $\pm$ 0.33 (32.39)	17.86 $\pm$ 0.27 (32.47)	17.57 $\pm$ 0.24 (32.64)	17.30 $\pm$ 0.20 (32.95)
T ₄	16.24 $\pm$ 0.51 (24.62)	15.94 $\pm$ 0.57 (24.36)	15.67 $\pm$ 0.57 (24.47)	15.40 $\pm$ 0.55 (24.68)
T ₅	17.02 $\pm$ 0.03 (28.07)	16.65 $\pm$ 0.21 (27.57)	16.30 $\pm$ 0.20 (27.40)	16.00 $\pm$ 0.20 (27.50)
T ₆	16.76 $\pm$ 0.60 (28.97)	16.31 $\pm$ 0.51 (26.04)	15.97 $\pm$ 0.52 (25.89)	15.70 $\pm$ 0.49 (26.11)
T ₇	14.95 $\pm$ 0.75 (18.15)	14.66 $\pm$ 0.66 (17.72)	14.30 $\pm$ 0.65 (17.25)	14.03 $\pm$ 0.61 (17.34)
CD at p=0.05	1.513	1.342	1.306	1.244
Sem $\pm$	0.494	0.438	0.427	0.406

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500 μM , 1000 μM , 3000 μM , 500 μM , 750 μM , 2000 μM and 3000 μM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.4.4.a: Effect of the treatment on the seed weight plant⁻¹ of black gram in the *Summer* season

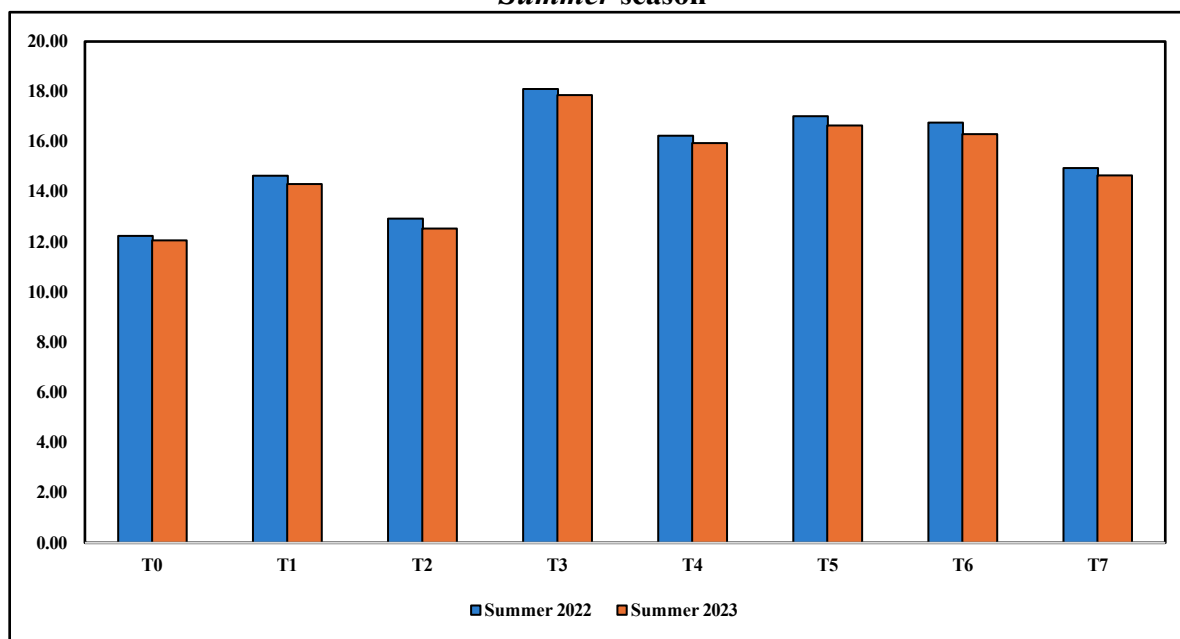
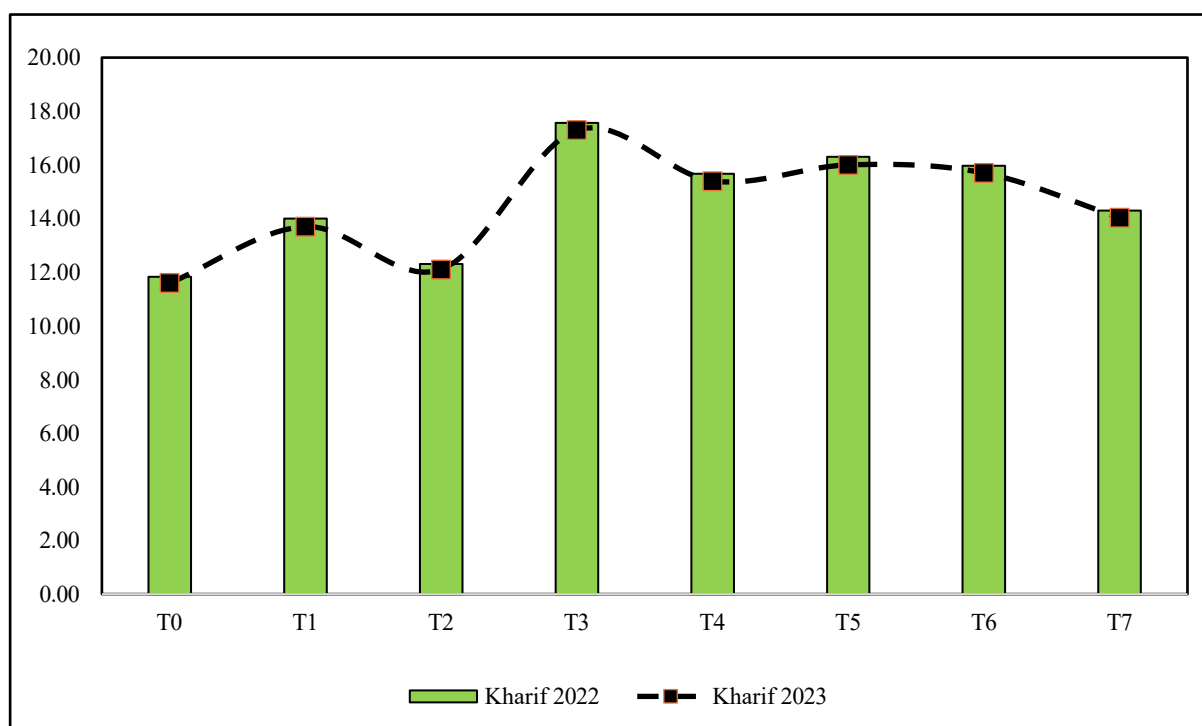


Fig 4.4.4.b: Effect of the treatment on the seed weight plant⁻¹ of black gram in the *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.4.5. TEST WEIGHT

The effect of foliar application of putrescine and cadaverine on test weight during the summer and *Kharif* seasons of 2022 and 2023. Data are tabulated in Table 4.2.3.a and 4.2.3.b and illustrated in Figure 4.2.4.a. and 4.2.3.b.

During Summer 2022, significant differences were observed among treatments ($CD_{0.05} = 1.685$). The highest test weight was recorded under T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M) with 45.12 g, showing 12.02% increase over control (39.69 g) and the difference exceeded the CD value. Treatments T5 (44.35 g) and T6 (43.84 g) also recorded higher values with 10.49% and 9.46% increase over control, respectively. During Summer 2023, treatment differences remained significant ($CD = 1.990$). Treatment T3 recorded the maximum test weight (44.72 g) with 16.60% increase over control (37.30 g). Treatments T5 (43.62 g) and T6 (42.78 g) also showed considerable improvement with 14.49% and 12.81% increase over control, respectively.

In Kharif 2022, significant variation among treatments was observed ($CD = 1.965$). Treatment T3 recorded the highest test weight (43.57 g) with 15.56% increase over control (36.55 g). Treatments T5 (42.63 g) and T6 (42.03 g) also recorded higher values with 13.71% and 12.48% increase over control, respectively. Similarly, during Kharif 2023, treatment differences were significant ($CD = 1.960$). T3 recorded the maximum test weight (42.70 g) showing 15.30% increase over control (36.17 g). Treatments T5 (41.77 g) and T6 (41.20 g) also showed considerable increases with 13.41% and 12.22% improvement over control, respectively. Overall, the combined application of putrescine and cadaverine (T3) consistently recorded the highest test weight (≈ 12 – 17% increase over control) across seasons and years.

The increase in test weight among all seasons with foliar applications of putrescine and cadaverine, particularly in T3, the combination Putrescine (1500 μ M) + Cadaverine (1000 μ M), suggests that polyamines can play significant roles in improving both seed development and seed filling. Furthermore, increases in seed test weight could have resulted from improved allocation of photosynthates, translocation of nutrients, and expansion of cells in developing seeds. Moreover, the moderate-dose combinations were particularly effective, such as Putrescine (750 μ M) + Cadaverine (2000 μ M) and Putrescine (750 μ M) + Cadaverine (500 μ M). Synergistic effects appeared to be dose-dependent. Treatments involving only one polyamine or both at higher concentrations were less effective, suggesting a lower threshold for responding to higher doses that will not lead to greater proportional benefit.

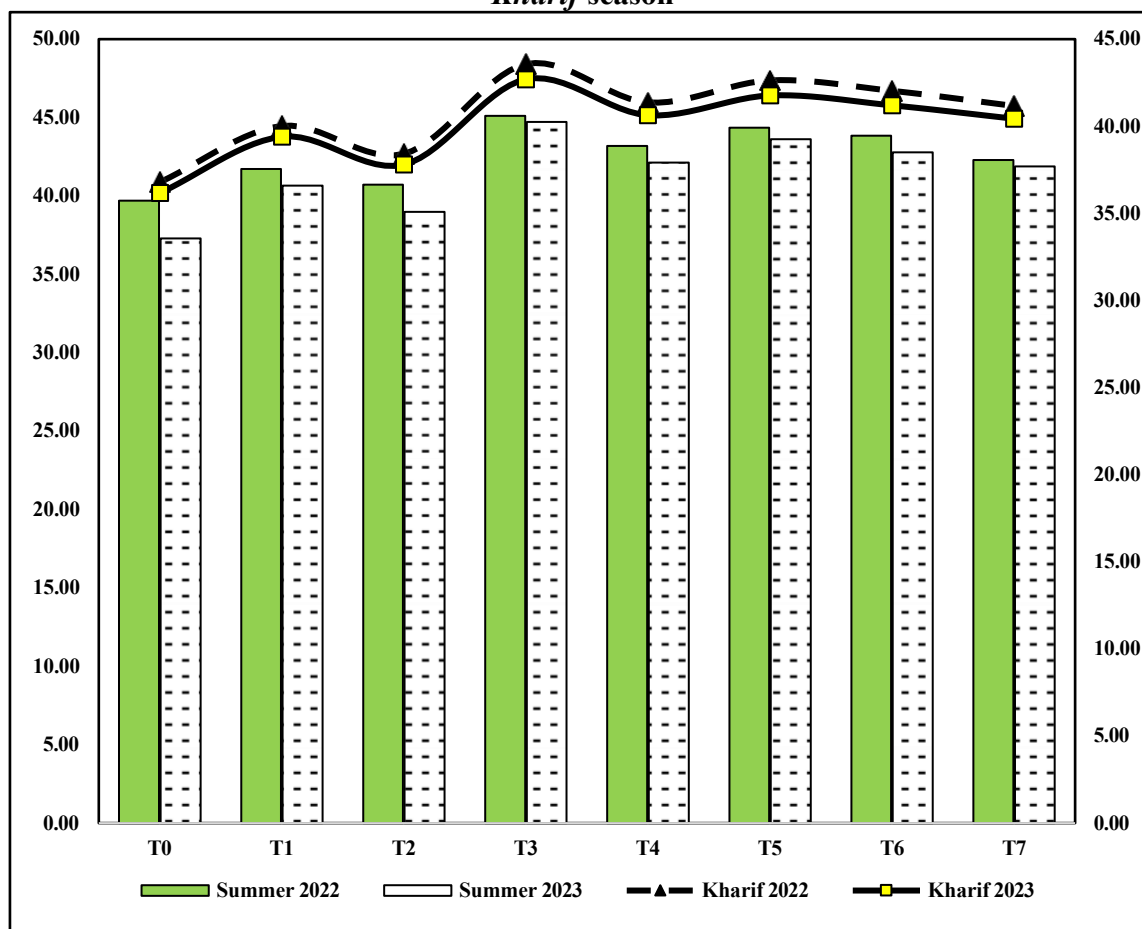
TABLE 4.4: Effect of the treatment on test weight (g) of black gram in the *Summer* and *Kharif* season

Treatments	<i>Summer</i>		<i>Kharif</i>	
	2022	2023	2022	2023
T ₀	39.69 ± 0.68	37.30 ± 0.98	36.55 ± 1.00	36.17 ± 1.01
T ₁	41.73 ± 0.36 (4.87)	40.66 ± 0.57 (8.28)	40.02 ± 0.56 (8.07)	39.40 ± 0.55 (8.21)
T ₂	40.72 ± 0.24 (2.52)	38.99 ± 0.33 (4.34)	38.42 ± 0.33 (4.24)	37.80 ± 0.32 (4.32)
T ₃	45.12 ± 1.52 (12.02)	44.72 ± 0.86 (16.60)	43.57 ± 0.82 (15.56)	42.70 ± 0.80 (15.30)
T ₄	43.18 ± 0.53 (8.08)	42.12 ± 0.41 (11.45)	41.37 ± 0.38 (11.07)	40.63 ± 0.35 (10.99)
T ₅	44.35 ± 0.28 (10.49)	43.62 ± 0.33 (14.49)	42.63 ± 0.32 (13.71)	41.77 ± 0.29 (13.41)
T ₆	43.84 ± 0.26 (9.46)	42.78 ± 0.07 (12.81)	42.03 ± 0.06 (12.48)	41.20 ± 0.05 (12.22)
T ₇	42.29 ± 0.17 (6.13)	41.89 ± 0.79 (10.97)	41.17 ± 0.79 (10.64)	40.43 ± 0.84 (10.55)
CD at p=0.05	1.685	1.990	1.965	1.960
Sem±	0.550	0.650	0.642	0.640

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.4.5: Effect of the treatment on test weight (g) of black gram in the *Summer* and *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.5. Yield

4.5.1 Biological yield

The effect of foliar application of putrescine and cadaverine on biological yield during both seasons (summer and *Kharif*) of 2022 and 2023. Data are tabulated in Table 4.5.1.a showed in Figure 4.2.4.a.

Treatment differences were recorded to have significantly different results in the biological yields of the plants used in the experiment from Summer 2022. The most yield achieved from the experiment under treatment number T3 (Putrescine 1500 μ M + Cadaverine 1000 μ M), organisms had biological yield of 3891.53 kg/ha, representing an increase of 20.70% compared to the control of 3085.90 kg/ha, which exceeded the critical difference in biological yield produced at that time. The yields produced with treatments T5 and T6 were 3732.53 kg/ha and 3653.33 kg/ha; thus, producing increases in yield of 17.32% and 15.53%, respectively. The results from Summer 2023 displayed no change in significant differences between treatments (T3, T5, and T6). T3 achieved the highest biological yield for the summer season at 3792.27 kg/ha, which is an increase of 20.38% from the control, which could produce 3019.23 kg/ha. T5 and T6 produced biological yields of 3632.51 kg/ha and 3520.74 kg/ha with increases of 16.88% and 14.24%, respectively.

With *Kharif* 2022, the treatments displayed significant differences in their biological yields (T3, T5, and T6) in accordance to the critical difference (CD = 233.599). T3 had the highest biological yield at 3673.33 kg/ha which was an increase of 20.12% from the 2934.33 kg/ha control. T5 had the second highest biological yield of 3535.00 kg/ha with a yield increase of 16.99% over the control; closely followed by T6 at 3422.33 kg/ha, representing a yield increase of 14.26% over the control.

Kharif 2023 displayed the same significant differences in biological yield from T3, T5, and T6. The maximum biological yield produced for *Kharif* 2023 was from T3 (3590.67 kg/ha) and increased 20.14% over the control of 2867.67 kg/ha. The next highest yield was again T5 (3451.67 kg/ha) with an increase of 16.92% over the control; T6 had the final yield with 3343.67 kg/ha with an increase of 14.24% over the control. Since the overall testing will continuously show the combined application of putrescine and cadaverine as the treatment with the maximum biological yield ($\approx$ 20% over the control), in all tested seasons/years, would confirm to an individual conducting experimentation that there is a relationship between the biological yield produced and the combined application of putrescine and cadaverine on the

same organism tested.

The increase in biological yield caused by this treatment stemmed from various physiological and biochemical improvements to plant metabolism, which were elicited by polyamines. They are a ubiquitous plant growth regulator known to have specific roles regulating cell division, elongation, and differentiation that influence plant height, leaf area, and branches to improve photosynthesis and assimilate production. Higher vegetative growth from polyamine treatment would have likely increased carbon fixation, improve dry matter accumulation, and resulted in a higher biological yield. An additional potential influence on biological yield would be the improved uptake and efficiency of nutrient assimilation, because of polyamines. Polyamines were found to regulate membrane stability and transport systems to enhance absorption and utilization of macro and micronutrients (potentially essential for sustained growth). Polyamines, in addition to their direct role in development, are involved in stress relief (such as drought and temperature fluctuations) by stabilizing proteins, membranes, and scavenging free radicals. This means metabolic activity can be sustained throughout the growth cycle, especially in the *Kharif* season, where the potential for climatic stress is higher than in subsequent growing phases.

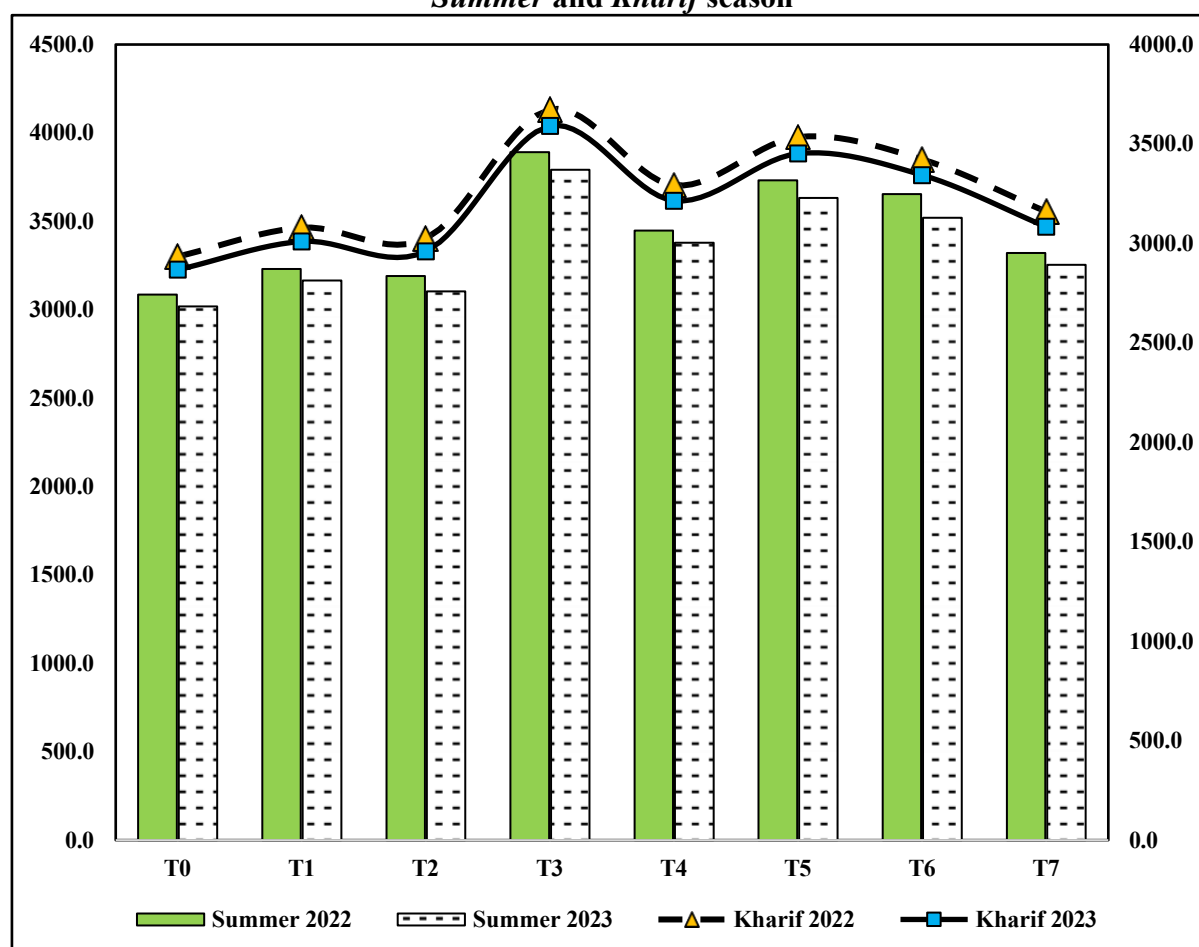
TABLE 4.5.1: Effect of the treatment on the biological yield (kg ha⁻¹) of black gram in the summer season and *Kharif* season

Treatments	SUMMER		<i>KHARIF</i>	
	2022	2023	2022	2023
T ₀	3085.90 ± 93.5	3019.23 ± 63.2	2934.33 ± 63.8	2867.67 ± 60.9
T ₁	3231.13 ± 9.42 (4.49)	3164.47 ± 61.6 (4.59)	3079.33 ± 60.0 (4.71)	3010.00 ± 59.5 (4.73)
T ₂	3191.07 ± 32.8 (3.30)	3104.40 ± 29.7 (2.74)	3024.67 ± 30.1 (2.99)	2960.33 ± 30.7 (3.13)
T ₃	3891.53 ± 20.3 (20.70)	3792.27 ± 111.3 (20.38)	3673.33 ± 113.4 (20.12)	3590.67 ± 111.7 (20.14)
T ₄	3447.03 ± 63.7 (10.48)	3379.37 ± 51.44 (10.66)	3292.33 ± 47.9 (10.87)	3215.00 ± 47.5 (10.80)
T ₅	3732.53 ± 90.9 (17.32)	3632.51 ± 123.7 (16.88)	3535.00 ± 123.9 (16.99)	3451.67 ± 121.1 (16.92)
T ₆	3653.33 ± 85.6 (15.53)	3520.74 ± 22.6 (14.24)	3422.33 ± 22.5 (14.26)	3343.67 ± 21.7 (14.24)
T ₇	3321.13 ± 107.3 (7.08)	3254.47 ± 90.1 (7.23)	3160.00 ± 89.9 (7.14)	3083.33 ± 86.9 (6.99)
CD at p=0.05	216.453	232.720	233.599	228.555
Sem±	70.677	75.988	107.870	74.628

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.5.1: Effect of the treatment on the biological yield (kg ha^{-1}) of black gram in the Summer and Kharif season



Note:

T0= Control, T1= Putrescine ($1500\mu\text{mol}$), T2= Cadaverine ($1000\mu\text{mol}$), T3= Putrescine ($1500\mu\text{mol}$) + Cadaverine ($1000\mu\text{mol}$), T4= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$), T5= Putrescine ($750\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$), T6= Putrescine ($750\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$) and T7= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$)

4.5.2 SEED YIELD

The effect of foliar application of putrescine and cadaverine on seed yield during the summer and *Kharif* seasons of 2022 and 2023. Data are tabulated in Table 4.5.2.a illustrated in Figure 4.5.2.a.

In summer 2022, the treatment with the maximum seed yield was T3 (putrescine 1500 μ M + cadaverine 1000 μ M), with 1294.4 kg/ha (significant increase of 37.06% over control at 814.7 kg/ha), exceeding the CD value (135.127). Additionally, T5 (1151.3 kg/ha) and T6 (1128.8 kg/ha) also were statistically different and had yields increased by 29.24% and 27.83%, respectively, over control. In summer 2023, the same results were achieved with significant treatment differences again (CD = 99.403). The maximum seed yield from T3 was 1231.0 kg/ha which was a significant increase of 36.26% over control (784.7 kg/ha). T5 (1118.0 kg/ha) and T6 (1095.5 kg/ha) both improved with increases of 29.81% and 28.37%, respectively, over control.

There were also significant differences in seed yield of treatments in *Kharif* 2022 (CD = 97.073). T3 had the maximum yield of 1206.0 kg/ha, a significant increase of 36.24% over control (769.0 kg/ha). Also, T5 and T6 had statistically superior yields at 1097.0 kg/ha and 1080.3 kg/ha, with yield improvements of 29.90% and 28.82%, respectively. *Kharif* 2023 also had significantly higher seed yields among treatments (CD = 95.868). Again, T3 had the maximum seed yield of 1181.3 kg/ha, a significant increase of 36.03% over the control (755.7 kg/ha). T5 and T6 also had higher yields at 1081.0 kg/ha and 1065.3 kg/ha with yield improvements of 30.10% and 29.07% over control, respectively.

Overall, the use of putrescine and cadaverine together (T3) provided the highest seed yield ($\approx$ 36–37% over control) throughout both years and for all growing seasons.

The continuous improvement in seed yield noted with the foliar application of putrescine and cadaverine, particularly in combination at moderate doses, highlights their advantageous contributions to improving reproductive efficiency and productivity of black gram. The combination of Putrescine (1500 μ M) + Cadaverine (1000 μ M) was the most effective of all treatments across all seasons. This treatment is likely to provide a more complete physiological stimulus in an appropriate balance that improves the source-sink relationship and seed filling. The considerable yield improvement experienced under this treatment is likely due to several physiological and biochemical benefits including, Improved nutrient assimilation and transport

due to metabolic activity and increased membrane permeability, Improved photosynthetic efficiency and carbohydrate partitioning to reproductive organs, Maintenance of flower and pod retention, thus increasing pod density per plant and the number of seeds per pod, Delayed senescence so that assimilates can accumulate later in the seed filling periods. In summary, the evidence provides a strong basis for the possibility that foliar-applied polyamines, especially at optimal balanced doses, may offer an economical approach to improve seed yield in black gram, whether grown in summer or *Kharif*.

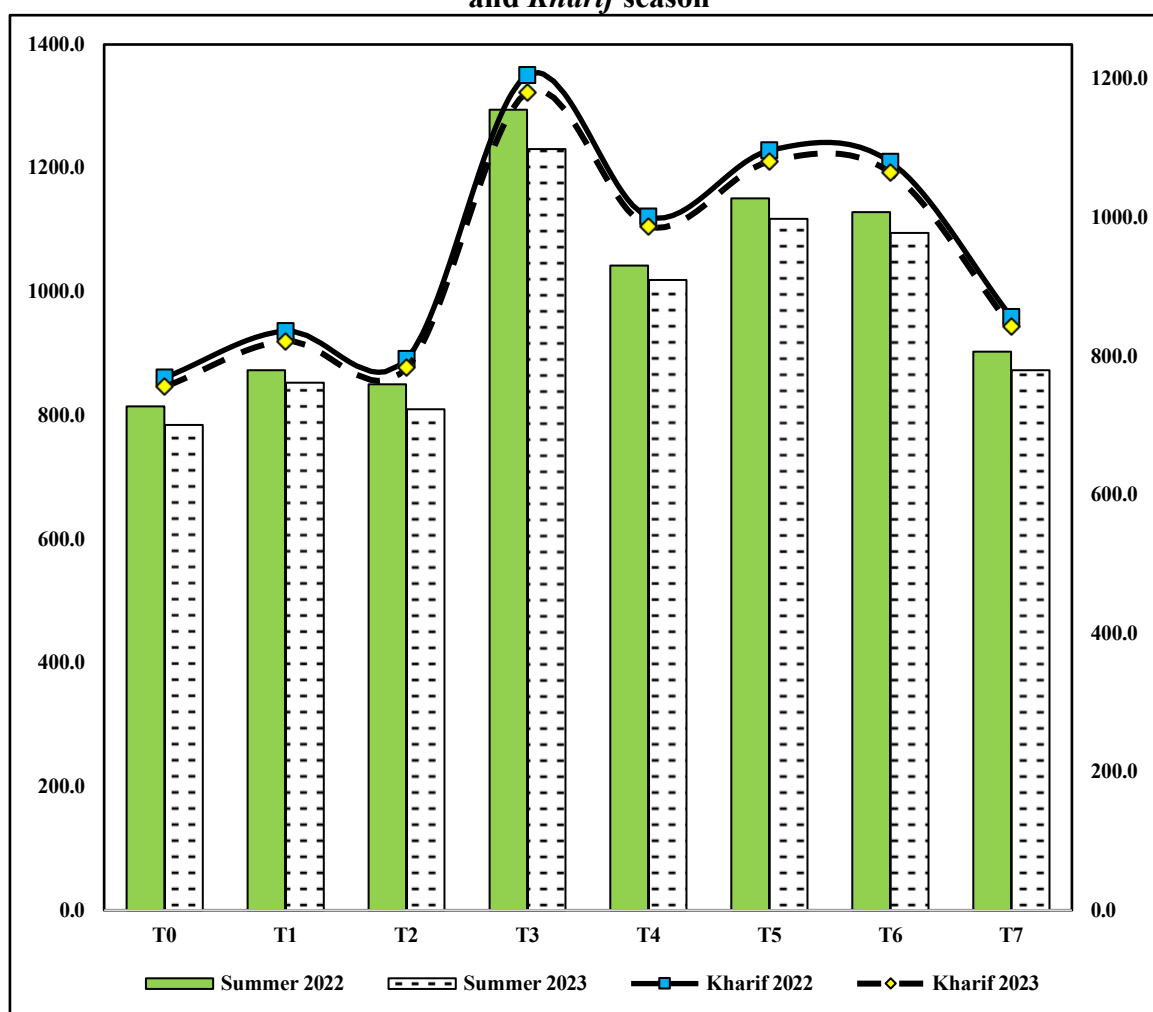
TABLE 4.5.2: Effect of the treatment on seed yield (kg ha⁻¹) of black gram in the *Summer and Kharif* season

Treatments	SUMMER		<i>KHARIF</i>	
	2022	2023	2022	2023
T ₀	814.7 ± 31.4	784.7 ± 28.1	769.0 ± 28.0	755.7 ± 27.9
T ₁	873.2 ± 64.0 (6.71)	853.2 ± 51.1 (8.04)	836.3 ± 50.6 (8.05)	822.0 ± 50.0 (8.07)
T ₂	850.4 ± 5.98 (4.20)	810.4 ± 17.8 (3.17)	796.0 ± 17.7 (3.39)	783.0 ± 17.7 (3.49)
T ₃	1294.4 ± 60.0 (37.06)	1231.0 ± 25.1 (36.26)	1206.0 ± 25.1 (36.24)	1181.3 ± 24.9 (36.03)
T ₄	1042.5 ± 31.4 (21.85)	1019.1 ± 23.5 (23.01)	1001.3 ± 22.0 (23.20)	987.3 ± 22.0 (23.46)
T ₅	1151.3 ± 40.0 (29.24)	1118.0 ± 41.6 (29.81)	1097.0 ± 39.7 (29.90)	1081.0 ± 38.6 (30.10)
T ₆	1128.8 ± 20.6 (27.83)	1095.5 ± 20.5 (28.37)	1080.3 ± 20.3 (28.82)	1065.3 ± 19.8 (29.07)
T ₇	903.2 ± 57.8 (9.81)	873.2 ± 43.2 (10.14)	856.3 ± 41.1 (10.20)	842.3 ^c ± 42.6 (10.29)
CD at p=0.05	135.127	99.403	97.073	95.868
Sem±	44.122	32.457	31.697	31.303

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.5.2.a: Effect of the treatment on seed yield (kg ha⁻¹) of black gram in the *Summer* and *Kharif* season



Note:

T0= Control, T1= Putrescine (1500μmol), T2= Cadaverine (1000μmol), T3= Putrescine (1500μmol) + Cadaverine (1000μmol), T4= Putrescine (3000μmol) + Cadaverine (500μmol), T5= Putrescine (750μmol) + Cadaverine (2000μmol), T6= Putrescine (750μmol) + Cadaverine (500μmol) and T7= Putrescine (3000μmol) + Cadaverine (2000μmol)

4.5.3 Stover yield

The effect of foliar application of putrescine and cadaverine on stover yield during the summer and *Kharif* seasons of 2022 and 2023. Data are tabulated in Table 4.5.3 as illustrated in Figure 4.5.3.a.

In Summer 2022, the highest stover yield (2597.16 kg/ha) was recorded in the Putrescine (1500 μM) + Cadaverine (1000 μM) treatment, followed close behind by the Putrescine (750 μM) + Cadaverine (2000 μM) treatment (2581.56 kg/ha), the Putrescine (750 μM) + Cadaverine (500 μM) treatment (2525.23 kg/ha) and lastly, the control (2271.23 kg/ha).

In Summer 2023, a similar trend continued, with a combination of Putrescine (1500 μ M) + Cadaverine (1000 μ M) treatment yielding 2561.23 kg/ha, followed by the 750 + 2000 μ M treatment at 2515.53 kg/ha and a 750 + 500 μ M treatment yielding 2425.28 kg/ha (also significantly higher than the control (2235.56 kg/ha)).

In *Kharif* 2022 and 2023, again, the Putrescine + Cadaverine (1500 + 1000 μ M) treatment produced the highest stover yields (2467.33 and 2409.33 kg/ha, respectively), with other good yields under the Putrescine (750 μ M) + Cadaverine (2000 μ M) and 750 μ M + 500 μ M combinations. The lowest yields were once again the control treatments (2165.33 and 2112.00 kg/ha for *Kharif* 2022 and 2023, respectively).

The significant increase in stover yield under the putrescine and cadaverine treatments, particularly in combination, shows that both polyamines have positively affected the accumulation of vegetative biomass in black gram. Overall, Putrescine (1500 μ M) + Cadaverine (1000 μ M) had superior stover across both seasons and years of growth, jealously regards the optimum physiological activity these two polyamines produce when applied at moderate concentrations. Polyamines have been reported to enhance vegetative growth and increase stover biomass yield. The enhancement of cell division, expansion, and differentiation of cells in stover facilitates this. Treated plants expressed higher stover yield because of greater photosynthetic activity (which would lead to an increase in yield), superior nutrient uptake and distribution of reproductive biomass, and vegetative biomass tissue distribution.

In summary, foliar-applied putrescine and cadaverine, especially at moderate levels and in non-additive concentrations, can potentially enhance stover yield, allowing for improved crop productivity. This suggests even greater economic value through fodder or residue returns, something of key importance in an integrated farming system.

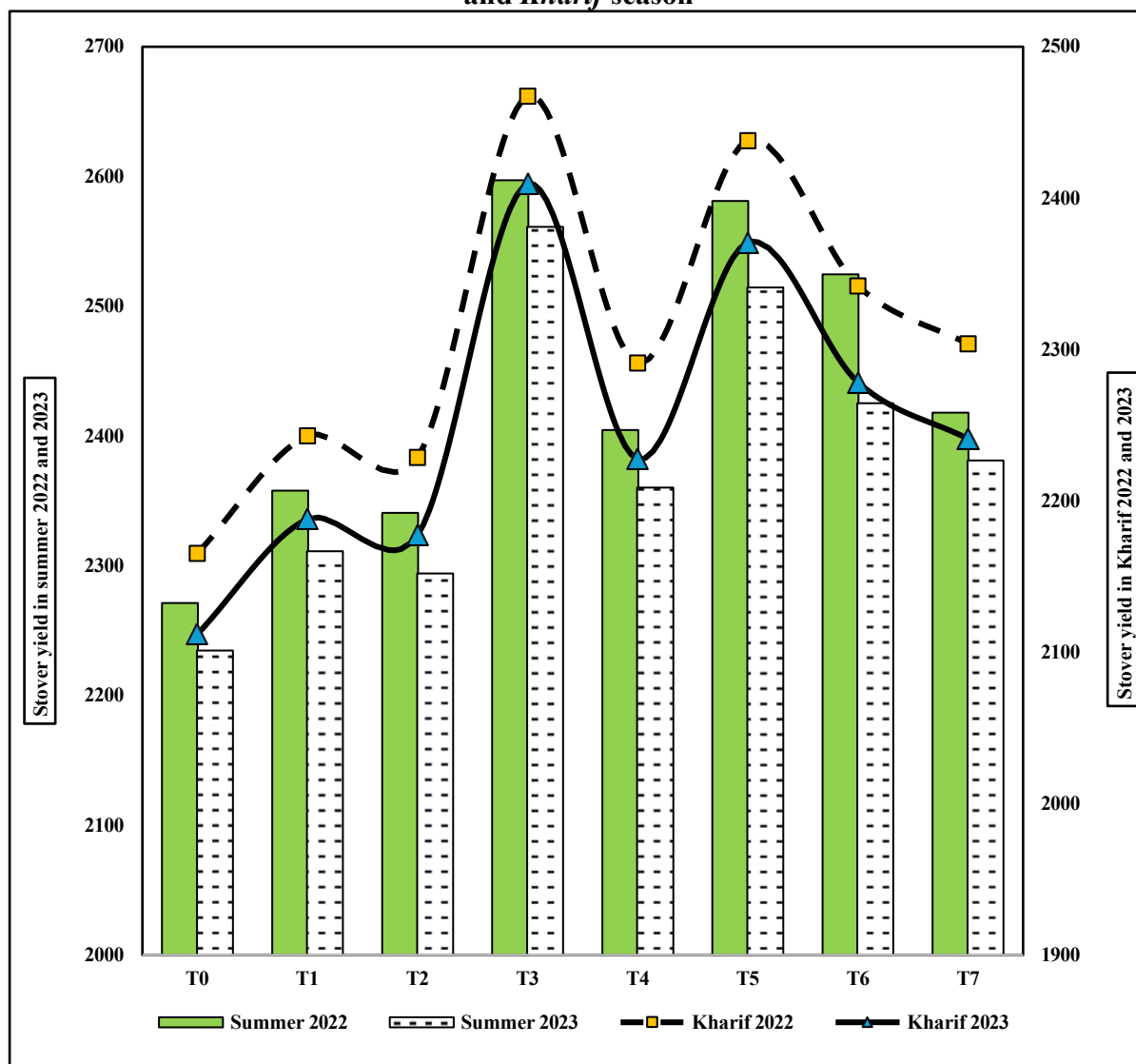
TABLE 4.5.3: Effect of the treatment on stover yield (kg ha⁻¹) of black gram in the *Summer* and *Kharif* season

Treatments	<i>Summer</i>		<i>Kharif</i>	
	2022	2023	2022	2023
T ₀	2271.23 ± 75.7	2235.56 ± 39.3	2165.33 ± 39.7	2112.00 ± 37.4
T ₁	2358.89 ± 67.7	2311.23 ± 11.5	2243.00 ± 10.3	2188.00 ± 11.0
T ₂	2341.67 ± 35.6	2294.01 ± 14.3	2229.66 ± 42.1	2177.33 ± 42.8
T ₃	2597.16 ± 40.8	2561.23 ± 95.0	2467.33 ± 96.6	2409.33 ± 95.1
T ₄	2405.16 ± 73.1	2360.23 ± 72.3	2291.00 ± 67.6	2228.66 ± 67.3
T ₅	2581.56 ± 127.0	2515.53 ± 143.4	2438.00 ± 144.0	2371.63 ± 140.1
T ₆	2525.23 ± 93.3	2425.28 ± 37.3	2342.00 ± 37.8	2278.32 ± 36.5
T ₇	2418.87 ± 145.2	2381.23 ± 19.7	2304.66 ± 89.6	2241.01 ± 87.0
CD at p=0.05	N/A	N/A	N/A	N/A
Sem±	94.667	80.036	80.024	78.278

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

Fig 4.5.3.b: Effect of the treatment on stover yield (kg ha^{-1}) of black gram in the *Summer* and *Kharif* season



Note:

T0= Control, T1= Putrescine ($1500\mu\text{mol}$), T2= Cadaverine ($1000\mu\text{mol}$), T3= Putrescine ($1500\mu\text{mol}$) + Cadaverine ($1000\mu\text{mol}$), T4= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$), T5= Putrescine ($750\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$), T6= Putrescine ($750\mu\text{mol}$) + Cadaverine ($500\mu\text{mol}$) and T7= Putrescine ($3000\mu\text{mol}$) + Cadaverine ($2000\mu\text{mol}$)

4.5.4 HARVEST INDEX

The effect of foliar application of putrescine and cadaverine on Harvest index during the summer and *Kharif* seasons of 2022 and 2023. Data are tabulated in Table 4.5.4, as illustrated in Figure 4.5.4.a

During Summer 2022, the use of Putrescine (1500 μ M) and Cadaverine (1000 μ M) resulted in the highest harvest index (33.25%), a 20.80% increase over the control (26.41%). This was followed by Putrescine (750 μ M) and Cadaverine (500 μ M) (30.94%), and Putrescine (750 μ M) + Cadaverine (2000 μ M) (30.93%). The control had the lowest harvest index.

In Summer 2023, and continued this trend where Putrescine + Cadaverine (1500 + 1000 μ M) again had the maximum ve (32.49%), with a 20.05% increase over control (25.98%), followed closely by moderate combinations of 750 + 500 μ M (31.12%) and 750 + 2000 μ M (30.87%) and which all showed significant improvement over the untreated check.

In the *Kharif* 2022 season, the highest harvest index was also attributable to Putrescine + Cadaverine (1500 + 1000 μ M) (32.86%), with a value that was 20.30% higher than the control (26.19%). Relative to the *Kharif* 2022 control, the harvest indices recorded for treatments that included Putrescine (750 μ M) and Cadaverine (2000 or 500 μ M) also had statistically similar harvest indices (30.44-31.57%) that were significantly lower than 32.86; however, they were still 20% higher. *Kharif* 2023 showed a significant increase in Putrescine + Cadaverine (1500 + 1000 μ M), reaching a maximum value of 32.93%, with a 20.03% gain over the control (26.34%). The 750 + 500 μ M and 750 + 2000 μ M combinations followed closely, with values of 31.42-31.87%, significantly differentiating them from the control (26.34%).

The term harvest index (HI) is an essential indicator in that it is a measure of the overall efficiency of a plant to convert total biomass into an economic yield (i.e., seeds). The greater the HI, the better the plant is at partitioning photosynthates into reproductive organs as opposed to vegetative organs. In all four seasons, a consistent pattern emerged, where putrescine and cadaverine, particularly at moderate and synergistic concentrations such as Putrescine 1500 μ M + Cadaverine 1000 μ M, significantly affected HI in black gram. This indicates that those treatments improved the translocation of assimilates to sink organs (e.g. pods and seeds), possibly due to an enhanced source-sink relationship under polyamine signalling.

Putrescine and cadaverine, as polyamines, likely promote some regulatory processes involved in flowering, pod set, seed filling, and dry matter partitioning, thus supporting our assertion that

the application of polyamines enhances enzymatic and hormonal functions that orchestrate nutrient distribution toward the reproductive parts. This explains the elevated HI of the combined treatments. Overall, these results reinforce the hypothesis that balanced foliar application of putrescine and cadaverine promotes physiological traits important for optimal biomass allocation, which led to a higher harvest index and better economic return.

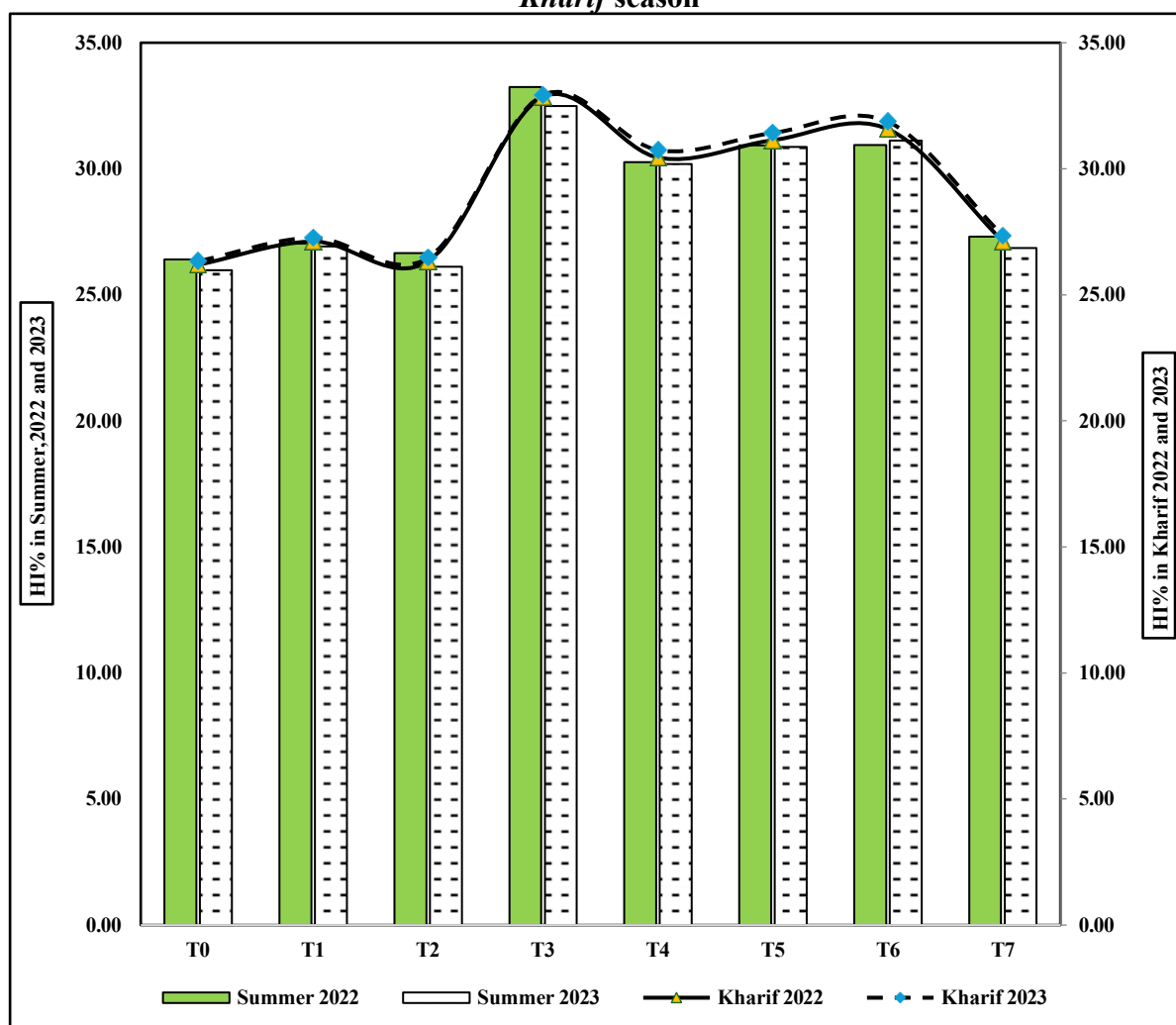
TABLE 4.5.4: Effect of the treatment on harvest index (%) of black gram in the *Summer* and *Kharif* season

Treatments	<i>Summer</i>		<i>Kharif</i>	
	2022	2023	2022	2023
T ₀	26.41 ± 0.73	25.98 ± 0.49	26.19 ± 0.50	26.34 ± 0.53
T ₁	27.03 ± 2.00 (2.31)	26.92 ± 1.11 (3.51)	27.12 ± 1.14 (3.40)	27.26 ± 1.14 (3.40)
T ₂	26.66 ± 0.38 (0.94)	26.11 ± 0.72 (0.52)	26.33 ± 0.75 (0.51)	26.46 ± 0.77 (0.47)
T ₃	33.25 ± 1.37 (20.58)	32.49 ± 0.67 (20.05)	32.86 ± 0.71 (20.30)	32.93 ± 0.72 (20.03)
T ₄	30.27 ± 1.11 (12.76)	30.19 ± 1.10 (13.96)	30.44 ± 1.06 (13.96)	30.74 ± 1.08 (14.32)
T ₅	30.93 ± 1.75 (14.62)	30.87 ± 1.75 (15.86)	31.14 ± 1.78 (15.87)	31.42 ± 1.78 (16.18)
T ₆	30.94 ± 1.03 (14.65)	31.12 ± 0.70 (16.53)	31.57 ± 0.73 (17.04)	31.87 ± 0.73 (17.36)
T ₇	27.31 ± 2.31 (3.33)	26.86 ± 1.39 (3.29)	27.13 ± 1.42 (3.44)	27.35 ± 1.44 (3.69)
CD at p=0.05	NS	3.433	3.487	3.521
Sem±	1.568	1.121	1.138	1.150

Note:

- 1- Putrescine and Cadaverine were used at a concentration 1500µM, 1000µM, 3000µM, 500µM, 750µM, 2000µM and 3000µM
- 2- Values given in parentheses indicate the percent change over the control.

4.5.4: Effect of the treatment on harvest index (%) of black gram in the *Summer* and *Kharif* season



Note:

T0= Control, T1= Putrescine (1500 μ mol), T2= Cadaverine (1000 μ mol), T3= Putrescine (1500 μ mol) + Cadaverine (1000 μ mol), T4= Putrescine (3000 μ mol) + Cadaverine (500 μ mol), T5= Putrescine (750 μ mol) + Cadaverine (2000 μ mol), T6= Putrescine (750 μ mol) + Cadaverine (500 μ mol) and T7= Putrescine (3000 μ mol) + Cadaverine (2000 μ mol)

4.6. Economics

During the summer and Kharif seasons (2022 and 2023), different treatments (T_0 to T_7) of putrescine and cadaverine were assessed for the economics of black gram cultivation. Data on cost of cultivation (COC), gross return (GR), net return (NR), and B:C ratio are presented in Tables 4.6.a, 4.6.b, 4.6.c, 4.6.d, and Appendix II.

In summer 2022, treatment T_3 (Putrescine 1500 μM + Cadaverine 1000 μM) provided the maximum economic returns with a gross return of Rs. 94,167.60, net return of Rs. 56,185.60, and a B:C ratio of 2.48. The T_5 (Putrescine 750 μM + Cadaverine 2000 μM) treatment ranked second, showing a gross return of Rs. 83,757.08, net return of Rs. 45,484.08, and B:C ratio of 2.19. The lowest returns were recorded in the control (T_0), with gross return Rs. 59,267.24, net return Rs. 21,585.24, and B:C ratio of 1.57.

In summer 2023, T_3 again demonstrated the highest economic return, with gross return of Rs. 94,167.60, net return of Rs. 54,685.60, and B:C ratio of 2.38. The next best was T_5 , showing gross and net returns of Rs. 83,757.08 and Rs. 43,984.08, respectively, and a B:C ratio of 2.11. The control (T_0) recorded the lowest returns with gross Rs. 59,267.24, net Rs. 20,085.24, and B:C ratio of 1.51.

During the Kharif 2022 season, T_3 again emerged as the most profitable treatment, with a gross return of Rs. 94,167.60, net return Rs. 56,685.60, and B:C ratio 2.51. The second-best treatment was T_5 , with gross Rs. 83,757.08, net Rs. 45,984.08, and B:C ratio 2.22. The lowest economic return was recorded in the control (T_0) with gross Rs. 59,267.24, net Rs. 22,085.24, and B:C ratio 1.59.

In Kharif 2023, T_3 again showed the best economic performance, with gross return Rs. 94,167.60, net return Rs. 55,185.60, and B:C ratio 2.41. The following best treatment was T_5 , providing gross Rs. 83,757.08, net Rs. 44,484.08, and B:C ratio 2.13. The control (T_0) had the lowest returns with gross Rs. 59,267.24, net Rs. 20,585.24, and B:C ratio 1.53.

Thus, across both seasons and years, T_3 (Putrescine 1500 μM + Cadaverine 1000 μM) consistently exhibited the highest economic returns, making it the most profitable and efficient treatment combination for black gram cultivation under the given conditions. T_5 consistently ranked second, while the control treatment (T_0) gave the lowest returns in all cases.

4.6.a Economics of the Summer season 2022

Treatments	COC (Rs.)	GROSS RETURN (Rs.)	NET RETURN (Rs.)	B: C RATIO
T ₀	37,682	59,267.24	21,585.24	1.57
T ₁	37,689	63,528.21	25,839.21	1.69
T ₂	37,976	61,865.87	23,889.87	1.63
T ₃	37,982	94,167.60	56,185.60	2.48
T ₄	37,842	75,841.88	37,999.88	2
T ₅	38,273	83,757.08	45,484.08	2.19
T ₆	37,832	82,120.20	44,288.20	2.17
T ₇	38,283	65,710.71	27,427.71	1.72

4.6.b Economics of the Summer season 2023

Treatments	COC (Rs.)	GROSS RETURN (Rs.)	NET RETURN (Rs.)	B: C RATIO
T ₀	39,182	59,267.24	20,085.24	1.51
T ₁	39,189	63,528.21	24,339.21	1.62
T ₂	39,476	61,865.87	22,389.87	1.57
T ₃	39,482	94,167.60	54,685.60	2.38
T ₄	39,342	75,841.88	36,499.88	1.93
T ₅	39,773	83,757.08	43,984.08	2.11
T ₆	39,332	82,120.20	42,788.20	2.09
T ₇	39,783	65,710.71	25,927.71	1.65

4.6.c Economics of the *Kharif* season 2022

Treatments	COC (Rs.)	GROSS RETURN (Rs.)	NET RETURN (Rs.)	B: C RATIO
T ₀	37,182	59,267.24	22,085.24	1.59
T ₁	37,189	63,528.21	26,339.21	1.71
T ₂	37,476	61,865.87	24,389.87	1.65
T ₃	37,482	94,167.60	56,685.60	2.51
T ₄	37,342	75,841.88	38,499.88	2.03
T ₅	37,773	83,757.08	45,984.08	2.22
T ₆	37,332	82,120.20	44,788.20	2.2
T ₇	37,783	65,710.71	27,927.71	1.74

4.6.d Economics of the *Kharif* season 2023

Treatments	COC (Rs.)	GROSS RETURN (Rs.)	NET RETURN (Rs.)	B: C RATIO
T ₀	38,682	59,267.24	20,585.24	1.53
T ₁	38,689	63,528.21	24,839.21	1.64
T ₂	38,976	61,865.87	22,889.87	1.59
T ₃	38,982	94,167.60	55,185.60	2.41
T ₄	38,842	75,841.88	36,999.88	1.95
T ₅	39,273	83,757.08	44,484.08	2.13
T ₆	38,832	82,120.20	43,288.20	2.12
T ₇	39,283	65,710.71	26,427.71	1.67

SUMMARY AND CONCLUSION

The ongoing part of the research project named "**Studies on the Effect of Polyamines on the Growth, Yield and Quality of Black Gram *Vigna mungo* (L.) Hepper**" was conducted at the farm of Lovely Professional University, Phagwara, Punjab, during the summer and *Kharif* seasons of 2022 and 2023. During the experiment, we have used leafy application of polyamines, namely putrescine and cadaverine, at different stages of growth. The effect of treatment on morphological, biochemical, yield attributing characters, and yield of black gram is studied. The experimental design was a Randomized Block Design (RBD) with eight treatments (T₀–T₇) with three replications. Eight treatments were T₀ = control, T₁ = Putrescine (1500 µM), T₂ = Cadaverine (1000 µM), T₃ = Putrescine (1500 µM) + Cadaverine (1000 µM), T₄ = Putrescine (3000 µM) + Cadaverine (500 µM), T₅ = Putrescine (750 µM) + Cadaverine (2000 µM), T₆ = Putrescine (750 µM) + Cadaverine (500 µM), T₇ = Putrescine (3000 µM) + Cadaverine (2000 µM) with replications R₁, R₂ and R₃. The variety of black gram used in both seasons was MASH-1008. The recommended dose of fertilizers was applied at the time of sowing. All other agronomic practices were uniformly observed and followed for all the treatments. Observations related to morphological and biochemical characteristics were recorded at 25 and 50 days after sowing (DAS). Fertilizer application is conducted at the time of sowing. Observations relating to morphology and biochemistry are taken at 25 and 50DAS, whereas metrics relating to yield are taken at harvest.

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

Growth parameter

- a. The highest plant height was recorded under T₃ during both summer and kharif seasons. T₃ recorded 38.44 cm and 64.76 cm in summer 2022, 36.77 cm and 62.76 cm in summer 2023, and 36.10 cm and 61.82 cm in kharif 2022, and 34.16 cm and 59.50 cm in kharif 2023 at 25 and 50 DAS, respectively. This represented an increase of about 24–32% over the control. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.
- b. The highest number of leaves plant⁻¹ was recorded under T₃ during both summer and kharif seasons. T₃ recorded 19.40 and 36.30 leaves plant⁻¹ in summer 2022, 19.32 and 34.38 in summer 2023, 16.51 and 32.68 in kharif 2022, and 15.74 and 32.10 in kharif 2023 at 25 and 50 DAS, respectively. This represented an increase of about 33–40% at 25 DAS and 36–41% at 50

DAS over the control. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

c. The highest number of branches plant^{-1} was recorded under T3 during both summer and kharif seasons. T3 recorded 5.07 and 9.17 branches plant^{-1} in summer 2022, 4.53 and 8.89 in summer 2023, 4.30 and 8.39 in kharif 2022, and 4.21 and 8.09 in kharif 2023 at 25 and 50 DAS, respectively. This represented an increase of about 44–49% at 25 DAS and 24–27% at 50 DAS over the control. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

d. The highest leaf area was recorded under T3 during both summer and kharif seasons. T3 recorded 414.97 and 715.69 $\text{cm}^2 \text{plant}^{-1}$ in summer 2022, 405.41 and 703.22 $\text{cm}^2 \text{plant}^{-1}$ in summer 2023, 401.88 and 689.06 $\text{cm}^2 \text{plant}^{-1}$ in kharif 2022, and 387.71 and 661.96 $\text{cm}^2 \text{plant}^{-1}$ in kharif 2023 at 25 and 50 DAS, respectively. This represented an increase of about 27–29% at 25 DAS and 25–27% at 50 DAS over the control. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

e. The highest leaf area index was recorded under T3 during both summer and kharif seasons. T3 recorded 1.38 and 2.39 in summer 2022, 1.35 and 2.34 in summer 2023, 1.34 and 2.30 in kharif 2022, and 1.29 and 2.21 in kharif 2023 at 25 and 50 DAS, respectively. This represented an increase of about 27–29% at 25 DAS and 25–27% at 50 DAS over the control. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

f. The highest leaf area duration was recorded under T3 during both summer and kharif seasons. T3 recorded 42.4 and 41.7 days in summer 2022 and summer 2023, and 40.7 and 39.0 days in kharif 2022 and kharif 2023, respectively. This represented an increase of about 24–26% over the control. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

g. The minimum days taken for 50% flowering were recorded under T3 during both summer and kharif seasons. T3 recorded 40.35 and 41.30 days in summer 2022 and summer 2023, and 41.58 and 42.47 days in kharif 2022 and kharif 2023, respectively. This represented a reduction of about 12–13% over the control, indicating earlier flowering. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

h. The minimum days taken for maturity were recorded under T3 during both summer and kharif seasons. T3 recorded 60.48 and 61.00 days in summer 2022 and summer 2023, and 61.47

and 61.83 days in kharif 2022 and kharif 2023, respectively. This showed a reduction of about 8–9% over the control, indicating earlier crop maturity. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

i. The highest root length was recorded under T3 during both summer and kharif seasons. T3 recorded 15.08 and 22.19 cm in summer 2022, 14.27 and 21.03 cm in summer 2023, 14.05 and 20.62 cm in kharif 2022, and 13.80 and 20.32 cm in kharif 2023 at 25 and 50 DAS, respectively. This represented an increase of about 41–43% at 25 DAS and 28–32% at 50 DAS over the control. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

j. The highest number of root nodules plant^{-1} was recorded under T3 during both summer and kharif seasons. T3 recorded 63.20 and 72.90 nodules plant^{-1} in summer 2022, 61.72 and 69.96 in summer 2023, 60.86 and 68.65 in kharif 2022, and 60.00 and 67.60 in kharif 2023 at 25 and 50 DAS, respectively. This represented an increase of about 50–52% at 25 DAS and 37–38% at 50 DAS over the control. The differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.

Biochemical parameter

a. The highest chlorophyll index was recorded under T3 during both summer and kharif seasons. T3 recorded 40.55 and 60.97 in summer 2022 and 39.40 and 58.67 in summer 2023 at 25 and 50 DAS, respectively. Similarly, during kharif seasons, T3 recorded 38.17 and 57.27 in 2022 and 37.08 and 56.10 in 2023. This represented an increase of about 18–24% over the control. The differences among treatments were statistically significant at $p = 0.05$.

b. The highest chlorophyll content was observed under T3 during both seasons. T3 recorded 2.10 and 2.40 mg g^{-1} FW in summer 2022 and 1.89 and 2.15 mg g^{-1} FW in summer 2023 at 25 and 50 DAS, respectively. During kharif seasons, T3 recorded 1.69 and 1.97 mg g^{-1} FW in 2022 and 1.61 and 1.94 mg g^{-1} FW in 2023. This indicated an increase of about 40–47% over the control.

c. The highest total soluble protein content was recorded under T3 during both seasons. T3 recorded 22.09 and 24.14 mg g^{-1} FW in summer 2022 and 20.63 and 22.44 mg g^{-1} FW in summer 2023 at 25 and 50 DAS, respectively. In kharif seasons, T3 recorded 19.13 and 20.94 mg g^{-1} FW in 2022 and 18.32 and 19.99 mg g^{-1} FW in 2023. This represented an increase of about 19–26% over the control.

d. The highest total carbohydrate content was recorded under T3 during both seasons. T3 recorded 8.41 and 9.61 mg g^{-1} FW in summer 2022 and 8.13 and 9.05 mg g^{-1} FW in summer

2023 at 25 and 50 DAS, respectively. Similarly, during kharif seasons, T3 recorded 7.94 and 8.84 mg g⁻¹ FW in 2022 and 7.84 and 8.76 mg g⁻¹ FW in 2023. This represented an increase of about 7–10% over the control.

e. The highest total phenol content was recorded under T3 during both seasons. T3 recorded 0.287 and 0.329 mg g⁻¹ FW in summer 2022 and 0.278 and 0.320 mg g⁻¹ FW in summer 2023 at 25 and 50 DAS, respectively. Similarly, in kharif seasons T3 recorded 0.274 and 0.318 mg g⁻¹ FW in 2022 and 0.264 and 0.307 mg g⁻¹ FW in 2023. The increase was about 15–26% over the control.

f. The highest total flavanol content was recorded under T3 during both seasons. T3 recorded 0.567 and 0.645 mg g⁻¹ FW in summer 2022 and 0.537 and 0.591 mg g⁻¹ FW in summer 2023 at 25 and 50 DAS, respectively. During kharif seasons, T3 recorded 0.512 and 0.568 mg g⁻¹ FW in 2022 and 0.495 and 0.548 mg g⁻¹ FW in 2023. This showed an increase of about 16–21% over the control, and the treatment differences were statistically significant at $p = 0.05$.

Quality parameter

a. The highest nitrogen content was recorded under T3 during both summer and kharif seasons. T3 recorded 3.79% and 3.44% in summer 2022 and summer 2023, and 3.66% and 3.34% in kharif 2022 and kharif 2023, respectively. This represented an increase of about 3–4% over the control, indicating improved nitrogen accumulation under combined polyamine treatment.

b. The highest phosphorus content was recorded under T3 during both summer and kharif seasons. T3 recorded 0.396% and 0.389% in summer 2022 and summer 2023, and 0.391% and 0.388% in kharif 2022 and kharif 2023, respectively. This showed an increase of about 5–6% over the control, indicating enhanced phosphorus uptake.

c. The highest potassium content was recorded under T3 during both summer and kharif seasons. T3 recorded 0.989% and 0.986% in summer 2022 and summer 2023, and 0.987% and 0.985% in kharif 2022 and kharif 2023, respectively. This represented an increase of about 2% over the control, showing improved potassium accumulation under the treatment.

d. The highest tryptophan content in seed was recorded under T3 during both summer and kharif seasons. T3 recorded 888.81 and 853.21 mg g⁻¹ in summer 2022 and summer 2023, and 876.51 and 843.39 mg g⁻¹ in kharif 2022 and kharif 2023, respectively. This represented an increase of about 24% over the control, indicating enhanced tryptophan accumulation under the combined polyamine treatment.

Yield attributing parameters

- a. The highest number of pods per plant was recorded under T3 during both summer and kharif seasons. T3 recorded 36.7 and 34.5 pods plant⁻¹ in summer 2022 and summer 2023, and 33.9 and 31.6 pods plant⁻¹ in kharif 2022 and kharif 2023, respectively. This represented an increase of about 19–24% over the control, and the differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.
- b. The highest number of seeds per pod was recorded under T3 during both summer and kharif seasons. T3 recorded 8.56 and 8.35 seeds pod⁻¹ in summer 2022 and summer 2023, and 7.97 and 7.90 seeds pod⁻¹ in kharif 2022 and kharif 2023, respectively. This showed an increase of about 17–19% over the control, with statistically significant differences among treatments at $p = 0.05$.
- c. The highest seed weight per pod was recorded under T3 during both summer and kharif seasons. T3 recorded 0.41 and 0.40 g in summer 2022 and summer 2023, and 0.40 and 0.39 g in kharif 2022 and kharif 2023, respectively. This represented an increase of about 17–20% over the control, and the treatment differences were statistically significant at $p = 0.05$.
- d. The highest seed weight per plant was recorded under T3 during both summer and kharif seasons. T3 recorded 18.10 and 17.86 g plant⁻¹ in summer 2022 and summer 2023, and 17.57 and 17.30 g plant⁻¹ in kharif 2022 and kharif 2023, respectively. This represented an increase of about 32–33% over the control, and the differences among treatments were statistically significant at $p = 0.05$.

Yield

- a. The highest biological yield was recorded under T3 during both summer and kharif seasons. T3 recorded 3891.53 and 3792.27 kg ha⁻¹ in summer 2022 and summer 2023, and 3673.33 and 3590.67 kg ha⁻¹ in kharif 2022 and kharif 2023, respectively. This represented an increase of about 20% over the control, and the differences among treatments were statistically significant as the treatment effects exceeded the CD values at $p = 0.05$.
- b. The highest seed yield was recorded under T3 during both summer and kharif seasons. T3 recorded 1294.4 and 1231.0 kg ha⁻¹ in summer 2022 and summer 2023, and 1206.0 and 1181.3 kg ha⁻¹ in kharif 2022 and kharif 2023, respectively. This represented an increase of about 36–37% over the control, and the differences among treatments were statistically significant at $p = 0.05$.

c. The highest stover yield was recorded under T3 during both summer and kharif seasons. T3 recorded 2597.16 and 2561.23 kg ha⁻¹ in summer 2022 and summer 2023, and 2467.33 and 2409.33 kg ha⁻¹ in kharif 2022 and kharif 2023, respectively. This represented an increase of about 14–15% over the control.

d. The highest harvest index was recorded under T3 during both summer and kharif seasons. T3 recorded 33.25%, 32.49%, 32.86% and 32.93% during summer 2022, summer 2023, kharif 2022 and kharif 2023, respectively, showing an increase of about 20% over the control. The differences were statistically significant during summer 2023 and kharif seasons, while summer 2022 showed non-significant variation.

Economics

The highest economic return was obtained under T3 during both summer and kharif seasons. T3 recorded gross returns of ₹94,167.60 ha⁻¹, with net returns of ₹56,185.60, ₹54,685.60, ₹56,685.60 and ₹55,185.60 ha⁻¹ during summer 2022, summer 2023, kharif 2022 and kharif 2023, respectively. The benefit:cost ratio was also highest under T3 (2.48, 2.38, 2.51 and 2.41) compared with the control.

The results of this experiment, entitled "**Studies on the Effect of Polyamines on the Growth, Yield and Quality of Black Gram *Vigna mungo* (L.) Hepper,**" concluded that.

Objective 1: To study the effect of Putrescine and Cadaverine on the growth, yield, and quality of *Vigna mungo*.

The use of polyamines is seen to have a significant effect on the growth and yield of *Vigna mungo*, as evidenced by their increased growth and maximum yield achieved when treated with T3 (Putrescine 1500µM + Cadaverine 1000µM). The average height of the maturity plant (2022-2023) was 36.7 cm, while the average root length and average number of nodules were 34.5 cm and 8.56 cm respectively. T3 treated *Vigna mungo* produced the highest pods per plant (7.0 ppts), seeds per pod (8.56, 8.35) and seed weight per pod (0.41 g, 0.40 g). The T3 treatment also gave the maximum biological yield (3891.53 kg ha⁻¹) and seed yield (1294.4 kg ha⁻¹) which were significantly greater than the untreated control by 20-37% for biological yield and both seed and pod yield, respectively, in 2022 and 2023. In addition, all quality parameters, including nitrogen (N), phosphorus (P), and potassium (K), were found to be higher in T3 treatment. Therefore, the use of Putrescine and Cadaverine together will enhance the overall growth, yield and nutrient accumulation of black gram.

Objective 2: To study the impact of different treatments on the physiological responses of

Vigna mungo.

Polyamine has a positive effect on the physiological and biochemical characteristics of the plant. T3 had the highest rating for chlorophyll index, chlorophyll amount (total), total amino acids, total carbohydrates, total phenols and total flavonoids from both first and second growth times. These moving up in rating states that the crop is more energy efficient and able to produce more food under stressed conditions by having increased metabolic activity with higher energy levels as a result of differently applied polyamines. Consequently, the use of both putrescine and cadaverine together created positive impacts on the physiology of black gram by increasing photosynthesis efficiency.

Objective 3: To study the evaluation of different treatments on tryptophan analysis in seeds.

The polyamine treatments had a dramatic influence on the amount of tryptophan in the seeds. The highest amount of tryptophan recorded was for T3 during summer 2022, summer 2023, kharif 2022 and kharif 2023 with values of 888.81, 853.21, 876.51 and 843.39 mg g⁻¹, respectively. This was an increase of roughly 24% relative to control, showing that the use of Putrescine and Cadaverine together led to an increase in the nutritional quality of black gram seeds.

Objective 4: Assessment of economics in *Vigna mungo*.

Economic analysis revealed that T3 (Putrescine 1500 µM + Cadaverine 1000 µM) was the most profitable treatment. It recorded the highest gross return (₹94,167.60 ha⁻¹), net return (up to ₹56,685.60 ha⁻¹) and benefit–cost ratio (2.48, 2.38, 2.51 and 2.41) during different seasons. Therefore, the combined application of Putrescine and Cadaverine proved to be economically viable for enhancing productivity and profitability of black gram cultivation.

Limitation

Polyamine treatments showed benefits in these experiments, but there are factors that limit the usefulness of the experimental data. The experiments were done under a specific agroclimatic condition, and only during a limited time period, which limits the application of the results to other environmental conditions. Furthermore, the economic viability of polyamines for farmers requires further assessment in other locations and when applied to larger fields.

Practical Implication

The results suggest that the combined application of polyamines, particularly treatment T3, can be an effective strategy for improving the productivity of black gram. The use of polyamines may help in enhancing crop performance under varying environmental conditions by improving physiological efficiency and stress tolerance.

Future Scope

- Evaluating polyamine application under different agro-climatic regions and soil types.
- Studying the molecular and biochemical mechanisms associated with polyamine-mediated stress tolerance.
- Assessing the economic feasibility and cost-benefit ratio of polyamine application under farmer field conditions.
- Investigating the interaction of polyamines with other plant growth regulators and nutrient management strategies to optimize crop productivity.

Linkage with Sustainable Development Goals (SDGs)

The results of this study will help develop sustainable agricultural production by increasing crop yield/production via physiological improvement. The use of polyamines to improve black gram yield/production helps achieve Sustainable Development Goals SDG 2 (Zero Hunger) through an increase in food production, and SDG 12 (Responsible Consumption and Production) via the use of efficient crop management practices.

Final Recommendation

Based on the results of the present investigation, treatment T3 (combined application of polyamines) may be recommended for improving growth, physiological performance, and yield of black gram under similar agro-climatic conditions.

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APPENDIX-i

Meteorologica dataofthe year2022

Date	Temperature (Max.) °C	Temperature (Min.) °C	Relative humidity (Max.) %	Relative humidity (Min.) %	Wind speed (km/hr)	Rain falls (mm)
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- 16-07-2022	36.57	31.43	59.57	60.29	12.00	0.89
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

Meteorological data for the year 2023

Date	Temperature (Max.) °C	Temperature (Min.) °C	Relative humidity (Max.) %	Relative humidity (Min.) %	Wind speed (km/hr)	Rainfall (mm)
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- 13-05-2023	40.5	23.2	49.6	38.6	10.9	0.0
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

Appendix-ii

ECONOMICS OF SUMMER 2022

SUMMER 2022

FIXED COST

S.no	Operation	Name	Quantity	Cost per quantity	Total
1	Land preparation (Tractor ploughing and bunds)	10 labour + 2 hours tractor		500+ 3000	8000
2	Layout preparation	6 labours		500 per day	3000
3	Seed	30kg/hectare		160 per kg	4800
4	Sowing and basal dose fertilizer application	7 labours		500 per day	3500
5	Fertilizer				
	N	Urea	5 kg/acre	268 per 50 kg/bag	1340
	P	SSP	16kg/acre	362 per 50 kg/bag	5792
6	Intercultural operations				
	Hand weeding		4 Labours	500 per day	2000
	Gapfilling		2Labours	500 per day	1000
	Spraying	Herbicide	1 Labour	500 per day	500
7	Herbicides and plant protection chemicals		1 herbicide	350	350
			1 plant protection chemical	400	400
8	Irrigation for the cropping season	2 labour*3 times		500per day	3000
9	Harvesting	4labours		500 per day	2000
10	Land lease and miscellaneous for the cropping season				2000
				TOTAL	37,682

TREATMENT WISE COST

S.No.	Treatment	Dose	RATE	DOSE COST	TOTAL
T ₀	Control	0	-	0	0
T ₁	Putrescine (1500μM)	1500μM	₹0.10/g	6.61	7
T ₂	Cadaverine (1000μM)	1000μM	₹2.88/g	293.76	294
T ₃	Putrescine (1500μM) + Cadaverine (1000μM)	1500 μM+1000 μM	₹0.10/g + ₹2.88/g	6.61 + 293.76	300
T ₄	Putrescine(3000μM) + Cadaverine(500μM)	3000 μM+500 μM	₹0.10/g + ₹2.88/g	13.22 + 146.88	160
T ₅	Putrescine(750μM) + Cadaverine(2000μM)	750 μM+2000 μM	₹0.10/g + ₹2.88/g	3.31 + 587.95	591
T ₆	Putrescine(750μM) + Cadaverine(500μM)	750 μM+500 μM	₹0.10/g + ₹2.88/g	3.31 + 146.88	150
T ₇	Putrescine(3000μM) + Cadaverine(2000μM)	3000 μM+2000 μM	₹0.10/g + ₹2.88/g	13.22 + 587.95	601

COST OF CULTIVATION SUMMER 2022

S.The number	Treatment	Fixed cost	Variable cost	Total
T ₀	Control	37,682	0	37,682
T ₁	Putrescine (1500μM)	37,682	7	37,689
T ₂	Cadaverine (1000μM)	37,682	294	37,976
T ₃	Putrescine (1500μM) + Cadaverine (1000μM)	37,682	300	37,982
T ₄	Putrescine(3000μM) + Cadaverine(500μM)	37,682	160	37,842
T ₅	Putrescine(750μM) + Cadaverine(2000μM)	37,682	591	38,273
T ₆	Putrescine(750μM) + Cadaverine(500μM)	37,682	150	37,832
T ₇	Putrescine(3000μM) + Cadaverine(2000μM)	37,682	601	38,283

SUMMER 2022

Treatments	COC (Rs.)	GROSS RETURN (Rs.)	NET RETURN (Rs.)	B: C RATIO
T ₀	37,682	59,267.24	21,585.24	1.57
T ₁	37,689	63,528.21	25,839.21	1.69
T ₂	37,976	61,865.87	23,889.87	1.63
T ₃	37,982	94,167.60	56,185.60	2.48
T ₄	37,842	75,841.88	37,999.88	2
T ₅	38,273	83,757.08	45,484.08	2.19
T ₆	37,832	82,120.20	44,288.20	2.17
T ₇	38,283	65,710.71	27,427.71	1.72

SUMMER 2023

S.no	Operation	Name	Quantity	Cost per quantity	Total
1	Land preparation (Tractor ploughing and bunds)	10 labour + 2 hours tractor		500+ 3000	8000
2	Layout preparation	8 labours		500 per day	4500
3	Seed	30kg/hectare		160 per kg	4800
4	Sowing and basal dose fertilizer application	6 labours		500 per day	3500
5	Fertilizer				
	N	Urea	5 kg/acre	268 per 50 kg/bag	1340
	P	SSP	16kg/acre	362 per 50 kg/bag	5792
6	Intercultural operations				
	Hand weeding		4 Labours	500 per day	2000
	Gapfilling		2Labours	500 per day	1000
	Spraying	Herbicide	1 Labour	500 per day	500
7	Herbicides and plant protection chemicals		1 herbicide	350	350
			1 plant protection chemical	400	400
8	Irrigation for the cropping season	2 labour*3 times		500per day	3000
9	Harvesting	4labours		500 per day	2000
10	Land lease and miscellaneous for the cropping season				2000
				TOTAL	39,182

TREATMENT WISE COST

S.No.	Treatment	Dose	RATE	DOSE COST	TOTAL
T ₀	Control	0	-	0	0
T ₁	Putrescine (1500μM)	1500μM	₹0.10/g	6.61	7
T ₂	Cadaverine (1000μM)	1000μM	₹2.88/g	293.76	294
T ₃	Putrescine (1500μM) + Cadaverine (1000μM)	1500 μM+1000 μM	₹0.10/g + ₹2.88/g	6.61 + 293.76	300
T ₄	Putrescine(3000μM) + Cadaverine(500μM)	3000 μM+500 μM	₹0.10/g + ₹2.88/g	13.22 + 146.88	160
T ₅	Putrescine(750μM) + Cadaverine(2000μM)	750 μM+2000 μM	₹0.10/g + ₹2.88/g	3.31 + 587.95	591
T ₆	Putrescine(750μM) + Cadaverine(500μM)	750 μM+500 μM	₹0.10/g + ₹2.88/g	3.31 + 146.88	150
T ₇	Putrescine(3000μM) + Cadaverine(2000μM)	3000 μM+2000 μM	₹0.10/g + ₹2.88/g	13.22 + 587.95	601

COST OF CULTIVATION SUMMER 2023

S.The number	Treatment	Fixed cost	Variable cost	Total
T ₀	Control	39,182	0	39,182
T ₁	Putrescine (1500μM)	39,182	7	39,189
T ₂	Cadaverine (1000μM)	39,182	294	39,476
T ₃	Putrescine (1500μM) + Cadaverine (1000μM)	39,182	300	39,482
T ₄	Putrescine(3000μM) + Cadaverine(500μM)	39,182	160	39,342
T ₅	Putrescine(750μM) + Cadaverine(2000μM)	39,182	591	39,773
T ₆	Putrescine(750μM) + Cadaverine(500μM)	39,182	150	39,332
T ₇	Putrescine(3000μM) + Cadaverine(2000μM)	39,182	601	39,783

SUMMER 2023

Treatments	COC (Rs.)	GROSS RETURN (Rs.)	NET RETURN (Rs.)	B: C RATIO
T ₀	39,182	59,267.24	20,085.24	1.51
T ₁	39,189	63,528.21	24,339.21	1.62
T ₂	39,476	61,865.87	22,389.87	1.57
T ₃	39,482	94,167.60	54,685.60	2.38
T ₄	39,342	75,841.88	36,499.88	1.93
T ₅	39,773	83,757.08	43,984.08	2.11
T ₆	39,332	82,120.20	42,788.20	2.09
T ₇	39,783	65,710.71	25,927.71	1.65

KHARIF 2022

S.no	Operation	Name	Quantity	Cost per quantity	Total
1	Land preparation (Tractor ploughing and bunds)	10 labour + 2 hours tractor		500+ 3000	8000
2	Layout preparation	7 labours		500 per day	3500
3	Seed	30kg/hectare		160 per kg	4800
4	Sowing and basal dose fertilizer application	5 labours		500 per day	2500
5	Fertilizer				
N		Urea	5 kg/acre	268 per 50 kg/bag	1340
P		SSP	16kg/acre	362 per 50 kg/bag	5792
6	Intercultural operations				
Hand weeding			4 Labours	500 per day	2000
Gapfilling			2Labours	500 per day	1000
Spraying		Herbicide	1 Labour	500 per day	500
7	Herbicides and plant protection chemicals		1 herbicide	350	350
			1 plant protection chemical	400	400
8	Irrigation for the cropping season	2 labour*3 times		500per day	3000
9	Harvesting	4 labours		500 per day	2000
10	Land lease and miscellaneous for the cropping season				2000
				TOTAL	37,182

KHARIF 2022 TREATMENT WISE COST

S.No.	Treatment	Dose	RATE	DOSE COST	TOTAL
T ₀	Control	0	-	0	0
T ₁	Putrescine (1500μM)	1500μM	₹0.10/g	6.61	7
T ₂	Cadaverine (1000μM)	1000μM	₹2.88/g	293.76	294
T ₃	Putrescine (1500μM) + Cadaverine (1000μM)	1500 μM+1000 μM	₹0.10/g + ₹2.88/g	6.61 + 293.76	300
T ₄	Putrescine(3000μM) + Cadaverine(500μM)	3000 μM+500 μM	₹0.10/g + ₹2.88/g	13.22 + 146.88	160
T ₅	Putrescine(750μM) + Cadaverine(2000μM)	750 μM+2000 μM	₹0.10/g + ₹2.88/g	3.31 + 587.95	591
T ₆	Putrescine(750μM) + Cadaverine(500μM)	750 μM+500 μM	₹0.10/g + ₹2.88/g	3.31 + 146.88	150
T ₇	Putrescine(3000μM) + Cadaverine(2000μM)	3000 μM+2000 μM	₹0.10/g + ₹2.88/g	13.22 + 587.95	601

COST OF CULTIVATION KHARIF 2022

S.The number	Treatment	Fixed cost	Variable cost	Total
T ₀	Control	37,182	0	37,182
T ₁	Putrescine (1500µM)	37,182	7	37,189
T ₂	Cadaverine (1000µM)	37,182	294	37,476
T ₃	Putrescine (1500µM) + Cadaverine (1000µM)	37,182	300	37,482
T ₄	Putrescine(3000µM) + Cadaverine(500µM)	37,182	160	37,342
T ₅	Putrescine(750µM) + Cadaverine(2000µM)	37,182	591	37,773
T ₆	Putrescine(750µM) + Cadaverine(500µM)	37,182	150	37,332
T ₇	Putrescine(3000µM) + Cadaverine(2000µM)	37,182	601	37,783

KHARIF 2022

Treatments	COC (Rs.)	GROSS RETURN (Rs.)	NET RETURN (Rs.)	B: C RATIO
T ₀	37,182	59,267.24	22,085.24	1.59
T ₁	37,189	63,528.21	26,339.21	1.71
T ₂	37,476	61,865.87	24,389.87	1.65
T ₃	37,482	94,167.60	56,685.60	2.51
T ₄	37,342	75,841.88	38,499.88	2.03
T ₅	37,773	83,757.08	45,984.08	2.22
T ₆	37,332	82,120.20	44,788.20	2.2
T ₇	37,783	65,710.71	27,927.71	1.74

KHARIF 2023

S.no	Operation	Name	Quantity	Cost per quantity	Total
1	Land preparation (Tractor ploughing and bunds)	10 labour + 2 hours tractor		500+ 3000	8000
2	Layout preparation	7 labours		500 per day	3500
3	Seed	30kg/hectare		160 per kg	4800
4	Sowing and basal dose fertilizer application	5 labours		500 per day	2500
5	Fertilizer				
	N	Urea	5 kg/acre	268 per 50 kg/bag	1340
	P	SSP	16kg/acre	362 per 50 kg/bag	5792
6	Intercultural operations				
	Hand weeding		4 Labours	500 per day	2000
	Gapfilling		2Labours	500 per day	1000
	Spraying	Herbicide	1 Labour	500 per day	500
7	Herbicides and plant protection chemicals		1 herbicide	350	350
			1 plant protection chemical	400	400
8	Irrigation for the cropping season	3 labour*3 times		500per day	4500
9	Harvesting	4 labours		500 per day	2000
10	Land lease and miscellaneous for the cropping season				2000
				TOTAL	38,682

KHARIF 2023 TREATMENT WISE COST

S.No.	Treatment	Dose	RATE	DOSE COST	TOTAL
T ₀	Control	0	-	0	0
T ₁	Putrescine (1500μM)	1500μM	₹0.10/g	6.61	7
T ₂	Cadaverine (1000μM)	1000μM	₹2.88/g	293.76	294
T ₃	Putrescine (1500μM) + Cadaverine (1000μM)	1500 μM+1000 μM	₹0.10/g + ₹2.88/g	6.61 + 293.76	300
T ₄	Putrescine(3000μM) + Cadaverine(500μM)	3000 μM+500 μM	₹0.10/g + ₹2.88/g	13.22 + 146.88	160
T ₅	Putrescine(750μM) + Cadaverine(2000μM)	750 μM+2000 μM	₹0.10/g + ₹2.88/g	3.31 + 587.95	591
T ₆	Putrescine(750μM) + Cadaverine(500μM)	750 μM+500 μM	₹0.10/g + ₹2.88/g	3.31 + 146.88	150
T ₇	Putrescine(3000μM) + Cadaverine(2000μM)	3000 μM+2000 μM	₹0.10/g + ₹2.88/g	13.22 + 587.95	601

COST OF CULTIVATION KHARIF 2023

S.The number	Treatment	Fixed cost	Variable cost	Total
T ₀	Control	38,682	0	38,682
T ₁	Putrescine (1500μM)	38,682	7	38,689
T ₂	Cadaverine (1000μM)	38,682	294	38,976
T ₃	Putrescine (1500μM) + Cadaverine (1000μM)	38,682	300	38,982
T ₄	Putrescine(3000μM) + Cadaverine(500μM)	38,682	160	38,842
T ₅	Putrescine(750μM) + Cadaverine(2000μM)	38,682	591	39,273
T ₆	Putrescine(750μM) + Cadaverine(500μM)	38,682	150	38,832
T ₇	Putrescine(3000μM) + Cadaverine(2000μM)	38,682	601	39,283

KHARIF 2023

Treatments	COC (Rs.)	GROSS RETURN (Rs.)	NET RETURN (Rs.)	B: C RATIO
T ₀	38,682	59,267.24	20,585.24	1.53
T ₁	38,689	63,528.21	24,839.21	1.64
T ₂	38,976	61,865.87	22,889.87	1.59
T ₃	38,982	94,167.60	55,185.60	2.41
T ₄	38,842	75,841.88	36,999.88	1.95
T ₅	39,273	83,757.08	44,484.08	2.13
T ₆	38,832	82,120.20	43,288.20	2.12
T ₇	39,283	65,710.71	26,427.71	1.67