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Enhancing Biochemical Attributes and Yield of Black Gram (*Vigna mungo* L.) Through Exogenous Application of Polyamines

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ABSTRACT

Background: Polyamines act as growth regulators in crops and enhance their growth and development. Despite its adaptability and numerous benefits, black gram suffers from low productivity and vulnerability to various abiotic and biotic stresses. Hence, the exogenous application of polyamines (putrescine and cadaverine) could enhance their metabolism and productivity.

Methods: The experiment consists of 8 treatments and 3 replications, in which putrescine and cadaverine are applied using foliar application in both individual and combined forms. Putrescine (0.75 mM, 1.5 mM, 3 mM) and cadaverine (0.5 mM, 1 mM, 2 mM) were applied in the field by foliar application through a knapsack sprayer.

Result: The results indicated that applying putrescine and cadaverine (1.5 mM and 1 mM, respectively) significantly affects biochemical and yield attributes. It was revealed that at 50 and harvest T_3 (putrescine 1.5 mM combined with cadaverine 1 mM) increased the total amino acid content by (10.6 and 11.61%, respectively), total protein content by (36.29 and 32.58% respectively), total carbohydrate by (12.17% and 23.24% respectively), bound phenol by (15.04 and 26.95% respectively) and total flavanol by (19.75% and 10.94% respectively). Out of all the biochemical attributes, only bound phenol was recorded maximum at harvest compared to 50 DAS. A similar result was also obtained in the yield parameter; T_5 shows the maximum percent increase over control in biological and grain yield and harvest index (HI) by 17.7%, 10.5% and 8.0%, respectively. These findings strongly suggest that the use of putrescine 1.5 mM combined with cadaverine 1 mM as a foliar application can significantly trigger the synthesis of biochemical and yield attributes of black gram.

Key words: Biochemical, Black gram, Cadaverine, Foliar spray, Polyamines, Putrescine.

INTRODUCTION

One of the major food grain categories in our country is pulses. They provide three times more high-quality protein per serving than cereals. Pulses are considered a “marvel of nature” as they withstand drought and can prevent soil erosion due to their deep roots and tall, lush vegetation (Roy and Roy, 2022).

Pulses should be part of an individual’s diet for health reasons, as they contain vitamin B, minerals and a specific type of fiber. Pulse crops fix atmospheric nitrogen through symbiotic nitrogen fixation, rejuvenating soils (Harika *et al.,* 2023). They are an excellent protein source, supporting organic farming by keeping the soil fertile through biological nitrogen fixation. Black gram (*Vigna mungo* L.), the third-largest pulse crop, matures in 90-100 days and is a good nitrogen fixer. It is cultivated mainly under rain-fed, rice fallow and irrigated conditions during the kharif, rabi and summer seasons. It is a native crop of India, produced and consumed and used to cook many dishes with rice flour (Kanimozhi *et al.,* 2021).

Black gram is rich in calcium, phosphorus, iron and small amounts of vitamin B complex, along with a good source of protein, carbohydrates, fat, minerals and fiber. It is also a rich in vitamins A, B1 and B3 but low in thiamine, riboflavin, niacin and vitamin C (Kanth *et al.,* 2021 and Meena *et al.,* 2021). Black gram is commonly grown in Asian countries such as India, Myanmar, Pakistan and other Southern Asian regions. India is the largest producer and consumer of black gram, annually producing 23.4 lakh tonnes from 46.7 lakh hectares, with an average yield of 501 kg per hectare during 2020-21. In India’s total pulse production, black gram accounts for 15.7% of the area and 9.09% of the total output (Selvaraj *et al.,* 2020). The most significant contributor is Uttar Pradesh, with 6.99 lakh hectares of *kharif* sowing (Nithila *et al.,* 2020). Despite these qualities, black gram production remains below average due to several constraints. The existing natural boundaries and other limitations are primarily due to an imbalance between nutrient supply and utilization (Parashar and Tripathi, 2020). Despite the adaptability and

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many virtues of black gram, low productivity and susceptibility to various abiotic and biotic stresses necessitate physio-molecular approaches to improve stress tolerance. One such area of interest in recent times is polyamines, small organic molecules involved in plant growth, development and stress tolerance (Lakshmi *et al.,* 2023).

Polyamines, such as putrescine (Put) and cadaverine (Cad), are naturally occurring aliphatic amines present in all living organisms and are involved in various cellular functions, including growth, differentiation and stress adaptation (Blázquez, 2024). Polyamines also influence plant physiology by modulating seed germination, root development, flower primordia formation, fruit set and senescence (Priyanka *et al.,* 2025). Polyamines play a crucial role in black gram by enhancing metabolic pathways such as chlorophyll content, nitrogen assimilation and protein synthesis (Chen *et al.,* 2019). Similarly, polyamines may regulate the partitioning of assimilates and resource allocation in plants, favoring more excellent biomass production and yield. This study investigates the effects of exogenously applied putrescine and cadaverine on black gram's biochemical traits and yield performance (Shao *et al.,* 2022). The results will enhance our understanding of polyamines in plants and provide insights for improving the productivity and resilience of black gram under both normal and stressful conditions.

MATERIALS AND METHODS

The field experiment was conducted at the Agricultural Research Farm of Lovely Professional University, Phagwara, Punjab, India to "Enhancing Biochemical Attributes and Yield of Black Gram (*Vigna mungo* L.) Through Exogenous Application of Polyamines" mediated by putrescine and cadaverine to improve biochemical parameters and yield attributing characteristics in black gram during summer seasons of 2023 and 2024. The experiment was laid out in a randomized block design (RBD) with three replications. The experimental field was divided into 24 plots, with a spacing of 30×10 cm between rows and plants. The black gram variety used in this study was Mash 1008 procured from the Punjab Agriculture University, Ludhiana. The experiment involved applying polyamines both individually and in combination. Putrescine was applied at concentrations of 0.75 mM, 1.5 mM and 3 mM, while cadaverine was used at 0.5 mM, 1 mM and 2 mM, which was applied as a foliar application using a knapsack sprayer.

The total free amino acid was estimated following the method given by Moore and Stein (1948). A 500 mg of the leaf sample was extracted with 10 ml of 80% ethanol. The extract was centrifuged at 3000 rpm for 10 minutes and an aliquot was collected. An aliquot of 1 ml was then Pipette out and mixed with 1 ml of ninhydrin reagent. Thereafter, the sample was placed in a boiling water bath for 15 minutes to allow the reaction and color development. After cooling the sample to room temperature, it was diluted by adding 5 ml of distilled water. The OD was measured at 570 nm while the amount of amino acid was calculated by using the standard curve made with L-Leucine.

The total protein content in the leaf of black gram was analyzed as per Lowry *et al.* (1951), wherein 0.5 g of leaf sample was homogenized with phosphate buffer (pH 7.5). After centrifugation at 3000 rpm, 0.2 ml of supernatant was used to analyze protein, in which 2% Na_2CO_3 in 0.1 N NaOH, 1% Na-K tartrate in H_2O, 0.5% $CuSO_4$ in H_2O and Folin-Phenol were used as reagents. An optical density of the sample was recorded at 660 nm while the amount of protein was calculated by using the standard curve of bovine serum albumin (BSA).

A method by Hodge and Hofreiter (1962) was used to quantify the content of total carbohydrates in a sample wherein 500 mg of leaf was used homogenate with 10 ml of 80% ethanol and the mixture was heated at 80°C for 30 minutes. It was centrifuged at 3000 rpm for 10 minutes and an aliquot was collected. Subsequently, 1 ml of the aliquot and 1 ml of phenol were transferred to a test tube, followed by the addition of 5 ml of concentrated H_2SO_4. To enable the complete reaction, the sample was allowed to stand for up to 30 minutes at room temperature. Thereafter, optical density was measured at 490 mm using a visible spectrophotometer.

The amount of bond phenol present in the leaves of black gram was estimated following the method of Chattopadhyay and Samaddar (1980). A 0.5 g sample was homogenized with the sodium lauryl sulphate (3%) and centrifuged at 2000 rpm. To purify the substances, 5 ml of each of the SDS solution, water and ethanol, along with 10 ml of diethyl ether, were used. This was followed by evaporation after adding 3 ml of 0.5M NaOH. Folin ciocalteau reagent (FCR) was used as a coloring agent and the absorbance of the sample was recorded at 650 nm after heating the sample for 1 minute.

To estimate the flavanol content in the black gram leaf, A method by Akkol *et al.* (2008) was used wherein the known amount of the sample was homogenized with vanillin reagent prepared in 4% (w/v) in methanol. Subsequently, 1.5 ml of H_2SO_4 was introduced to catalyze the reaction. The resulting mixture was incubated in the dark at room temperature for the development of the chromophore. This was followed by the measurement of OD at 500 nm using a UV-Vis spectrophotometer, while a standard calibration curve made with catechin was used to calculate the content of flavanol.

Data regarding the grain yield and biological yield were recorded after the harvest of the crop and transformed into the qt ha^{-1}, while the harvest index (%) was derived as per the formula given (Donald and Hamblin, 1976).

The significance of data collected from the present piece of work was analyzed as per the analysis of variance at a p=0.05 level of significance described by Gomez and Gomez (1984), while the Duncan's Multiple Range Test was carried out using online software OPSTAT developed by Sheoran, CCS HAU, Hisar.

RESULTS AND DISCUSSION

Amino acid content

The effect of polyamines was assessed at 50 DAS and at harvest in the leaves of black gram (Table 1). It was observed that the application of putrescine 1.5 mM combined with cadaverine 1 mM (T_3) marked as a statistically significant at (p=0.05) and observed highest value of the total amino acid at 50 and harvest by 133.47 mg g^{-1} and 115.47 mg g^{-1}, respectively, as compared to control (T_0) 119.27 and 102.07 mg g^{-1} which was followed by T_5> T_6>T_4 and T_7. This result was supported by the similar findings (Talaat *et al.,* 2023; Aboohanah *et al.,* 2021; Haghshenas *et al.,* 2020). Putrescine and cadaverine increase total amino acid content by improving nitrogen uptake and regulating amino acid synthesis. These polyamines enhance nitrogen absorption, a key element for amino acid production, by activating critical enzymes such as glutamine synthetase and nitrate reductase (Jahanbakhsh *et al.,* 2022).

Total protein content

Data presented (Table 1) also revealed the improvement in protein content, which was statistically significant at (p=0.05). The maximum protein contents were obtained by the application of putrescine (1.5 mM) + cadaverine (1 mM) (T_3), which yielded 19.18% and 10.50%, respectively, as compared to the control 12.22% and 7.08% (Table 1). These results are consistent with (Singh *et al.,* 2002; Tomar *et al.,* 2013; Yang *et al.,* 2013) highlight the importance of protein in developing plant growth and its development and physiology. Proteins are essential catalysts for basic metabolic events such as respiration, photosynthesis, nitrogen fixation and other crucial biochemical reactions underlying plant resilience and productivity.

The increase in protein content achieved with putrescine and cadaverine application can be ascribed to higher nitrogen uptake and stimulation of protein synthesis. Principal enzymes, related to protein metabolism, found in this process are activated by polyamines, including nitrate reductase and glutamine synthetase. In addition, they increase nitrogen availability to stimulate amino acid production and thus support protein synthesis. Additionally, putrescine and cadaverine can contribute to the stabilization of ribosomal structures, therefore enhancing translational efficiency and facilitating protein accumulation in leaves, especially in stress or nutrient-limited conditions. These roles of polyamines in mediating plant growth regulators have been supported by studies that show they impact protein metabolism, resulting in improved overall plant health (Collado *et al.,* 2021; Suchak *et al.,* 2020; Islam *et al.,* 2022).

Total carbohydrate content

A significant increase in total carbohydrate content at 50 and harvest in T_3 (putrescine 1.5 mM combined with cadaverine 1 mM) by 54.81% and 34.28%, respectively, as compared to control 48.08% and 26.28%. This corresponds to a net gain of 12.27% at day 50 and 23.24% at harvest due to the treatment impact (Table 1), which was followed

Table 1: Effect of polyamines on amino acid, protein and carbohydrate.

Treatments	Amino acid (mg g^{-1})		Protein (%)		Total carbohydrate (%)	
	50 DAS	At harvest	50 DAS	At harvest	50 DAS	At harvest
T_0	119.27±4.64	102.07±2.40	12.22±0.29	7.08±0.10	48.08±1.63	26.28±9.43
T_1	123.07±0.61	105.13±3.18	15.43±0.54	8.07±0.07	49.83±0.71	30.29±11.69
	(3.09%)	(2.92%)	(20.83%)	(12.24%)	(3.51%)	(13.25%)
T_2	121.07±3.45	103.80±2.42	15.19±0.50	7.73±0.28	49.22±0.94	29.36±12.26
	(1.49%)	(1.67%)	(19.58%)	(8.40%)	(2.31%)	(10.49%)
T_3	133.47±13.5	115.47±0.99	19.18±2.47	10.50±0.70	54.81±1.63	34.28±14.47
	(10.6%)	(11.61%)	(36.29%)	(32.58%)	(12.27%)	(23.34%)
T_4	125.59±1.52	109.53±0.99	16.14±0.44	9.30±0.61	51.28±0.50	30.23±12.11
	(5.04%)	(6.82%)	(24.28%)	(23.85%)	(6.23%)	(13.06%)
T_5	128.79±0.69	114.13±1.33	17.35±0.25	9.70±0.47	53.36±1.18	31.69±13.58
	(7.39%)	(10.57%)	(29.60%)	(27.18%)	(9.89%)	(17.08%)
T_6	127.33±2.70	113.00±2.16	16.88±1.51	9.46±0.31	52.56±0.64	31.28±13.06
	(6.33%)	(9.68%)	(27.62%)	(25.18%)	(8.51%)	(15.99%)
T_7	124.93±5.25	108.60±0.20	15.89±0.52	8.88±0.98	50.89±1.67	30.35±9.09
	(4.54%)	(6.02%)	(23.10%)	(20.25%)	(5.51%)	(13.42%)
C.D. at (p=0.05%)	5.35	2.854	1.962	0.905	2.098	3.988

Note:

1-T_0= Control, T_1= Putrescine [1500 µl L^{-1}], T_2= Cdaverine [1000 µl L^{-1}], T_3= Putrescine + Cdaverine [1500 and 1000 µl L^{-1}], T_4= Putrescine + Cdaverine [3000 and 500 µl L^{-1}], T_5= Putrescine + Cdaverine [750 and 2000 µl L^{-1}], T_6= Putrescine + Cdaverine [750 and 500 µl L^{-1}] and T_7= Putrescine + Cdaverine [3000 and 2000 µl L^{-1}].

2-Data presented in parentheses represent the % increase over control.

by T_5> T_6>T_4 and T_7. Carbohydrates are stored as starch, which can be used for seed germination or when there is low photosynthetic activity (Haghshenas *et al.,* 2020; Seleem *et al.,* 2021). Polyamines play a crucial role in plant metabolism, particularly in the production and allocation of carbohydrates. They regulate key enzymes like sucrose synthase and starch synthase, which leads to an increased accumulation of sugars and starches within the plant (Marcińska *et al.,* 2020; ElSayed *et al.,* 2022; Rady *et al.,* 2021).

Bound phenol

The amount of bound phenol was significantly influenced by the treatment at harvest (p=0.05), whereas a nonsignificant difference was noticed at 50 DAS. According to the data shown in Table 2, the maximum phenol content was recorded by the application of putrescine (1.5 mM) combined with cadaverine (1 mM) (T_3), *i.e*., 0.323 and 0.246 mg g^{-1}, respectively. A similar result was also noticed by Chishti *et al.* (2024); Kumari *et al.* (2025). In plants, bond phenols are essential for defense, development and stress reactions. They serve as antioxidants, shield cells from oxidative stress and aid in forming lignin, which provides structural support by applying polyamines that influence plant growth, development and defense by affecting the levels of bound phenols. Polyamines like putrescine, spermidine and spermine interact with phenolic compounds and can stimulate their synthesis. This interaction is essential for processes such as lignin production, which strengthens cell walls and for enhancing the plant's defense mechanisms against pathogens (Mohammadrezakhani *et al.,* 2021; Kucuker *et al.,* 2023).

Total flavanol content

The data in Table 2 indicate that applying putrescine 1.5 mM combined with cadaverine 1 mM (T_3) significantly increased the total flavanol content at 50 and harvest by 0.618 mg g^{-1} and 0.502 mg g^{-1}, respectively, while a total of 19.75 and 10.94% increase was recorded over control. This result was supported by (Zeynali *et al.,* 2023; Kibar *et al.,* 2024). By promoting important metabolic processes and enhancing stress tolerance, putrescine and cadaverine raise the overall flavanol content in plant leaves. By promoting enzymes such as phenylalanine ammonia-lyase (PAL), these polyamines stimulate the phenylpropanoid pathway, essential for flavonoid production. As a result, flavanol synthesis rises (Sheteiwy *et al.,* 2023; Khalil *et al.,* 2016).

Biological yield, grain yield and HI%

The yield parameter indicates the final growth and productivity of the crop. With this parameter, it can easily distinguish between the effectiveness of treatments. As shown in Fig 1, T_3 (putrescine 1.5 mM combined with cadaverine 1 mM) showed effective results, as the maximum biological yield was 36 qt ha^{-1}. Similar results were observed in the economic yield of 10.9 qt ha^{-1} with putrescine 1.5 mM combined with cadaverine 1 mM (T_3). Additionally, data presented in Fig 2 revealed the highest % increase over control of 10.5, 17.7 qt ha^{-1}. These results are supported by (Hussein *et al.,* 2023; Bassiony *et al.,* 2018).

Table 2: Effect of polyamines on bound phenol and total flavanol content.

Treatments	Bound phenol (mg g^{-1})		Total flavanol content (mg g^{-1})	
	50 DAS	At harvest	50 DAS	At harvest
T_0	0.275^{a}±0.03	0.201^{b}±0.009	0.496^{d}±0.029	0.447^{a}±0.016
T_1	0.293^{a}±0.02	0.221^{ab}±0.006	0.578^{abc}±0.035	0.477^{a}±0.012
	(6.36%)	(9.15%)	(14.23%)	(6.21%)
T_2	0.287^{a}±0.03	0.216^{ab}±0.002	0.554^{bcd}±0.002	0.474^{a}±0.004
	(4.26%)	(6.97%)	(10.46%)	(5.72%)
T_3	0.323^{a}±0.04	0.275^{a}±0.025	0.618^{a}±0.033	0.502^{a}±0.008
	(15.04%)	(26.95%)	(19.75%)	(10.94%)
T_4	0.302^{a}±0.009	0.246^{ab}±0.009	0.558^{abcd}±0.026	0.474^{a}±0.006
	(9.11%)	(18.23%)	(11.08%)	(5.76%)
T_5	0.311^{a}±0.01	0.255^{ab}±0.008	0.604^{ab}±0.032	0.480^{a}±0.009
	(11.78%)	(21.23%)	(17.87%)	(6.89%)
T_6	0.306^{a}±0.005	0.253^{ab}±0.004	0.580^{abc}±0.012	0.479^{a}±0.007
	(10.29%)	(20.47%)	(14.58%)	(6.72%)
T_7	0.297^{a}±0.01	0.243^{ab}±0.004	0.540^{cd}±0.011	0.456^{a}±0.025
	(7.33%)	(17.49%)	(8.14%)	(1.94)
C.D. at (p=0.05%)	NS	0.015	0.047	0.023

Note:

1-T_0= Control, T_1= Putrescine [1500 µl L^{-1}], T_2= Cdaverine [1000 µl L^{-1}], T_3= Putrescine + Cdaverine [1500 and 1000 µl L^{-1}], T_4= Putrescine + Cdaverine [3000 and 500 µl L^{-1}], T_5= Putrescine + Cdaverine [750 and 2000 µl L^{-1}], T_6= Putrescine + Cdaverine [750 and 500 µl L^{-1}] and T_7= Putrescine + Cdaverine [3000 and 2000 µl L^{-1}].

2-Data presented in parentheses represent the % increase over control.

Putrescine and cadaverine, two key polyamines, are crucial for boosting legume crop production by regulating various physiological and biochemical processes. These compounds promote plant growth by facilitating cell division, elongation and differentiation. They also reduce oxidative stress and stabilize cell membranes by neutralizing reactive oxygen species (ROS), shielding plants from drought, salinity and extreme temperatures. Moreover, putrescine and cadaverine enhance nutrient uptake, particularly nitrogen, which is vital for legumes as nitrogen-fixing plants. By improving nodulation and nitrogen metabolism, they increase nitrogen fixation efficiency. This combination of effects strengthens plant health, increases biomass production and ultimately leads to higher legume crop yields.

The harvest index (HI) reflects the efficiency with which crop plants transfer assimilated food from the source to the sink or grains. Based on the harvest index values in Fig 1 and 2, the harvest index was significantly influenced by the T_3, recording the maximum harvest index of 30.3% and the per cent increase over control was 8%. This result was supported by (Hussein *et al.,* 2023; Gholipur *et al.,* 2022; Bassiony *et al.,* 2018). Zhou *et al.* (2024) also explained that by optimizing growth and effective distribution of resources towards seed development, putrescine raises the harvest index (HI) of legume crops. As a result, a more significant percentage of dry matter is allocated to the harvestable yield, raising seed output about the overall biomass of the plant and enhancing the harvest index (Ozmen *et al.,* 2023). The use of optimum concentrations of putrescine and cadaverine acts as growth regulators, significantly enhancing both physiological and morphological activities. These polyamines facilitate key processes such as cell division, elongation and differentiation, thereby promoting crop growth, development and yield of black gram. Conversely, de*via*tion from the optimal concentrations can diminish their positive effect due to due to inhibitory actions at higher or lower levels.

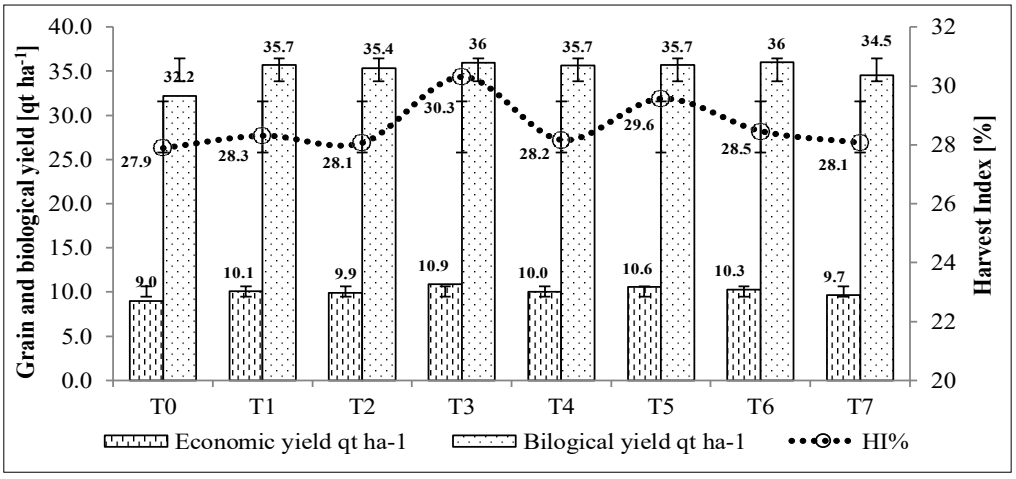


Fig 1: Effect of polyamines on grain yield, biological yield (qt ha^{-1}) and HI% of black gram.

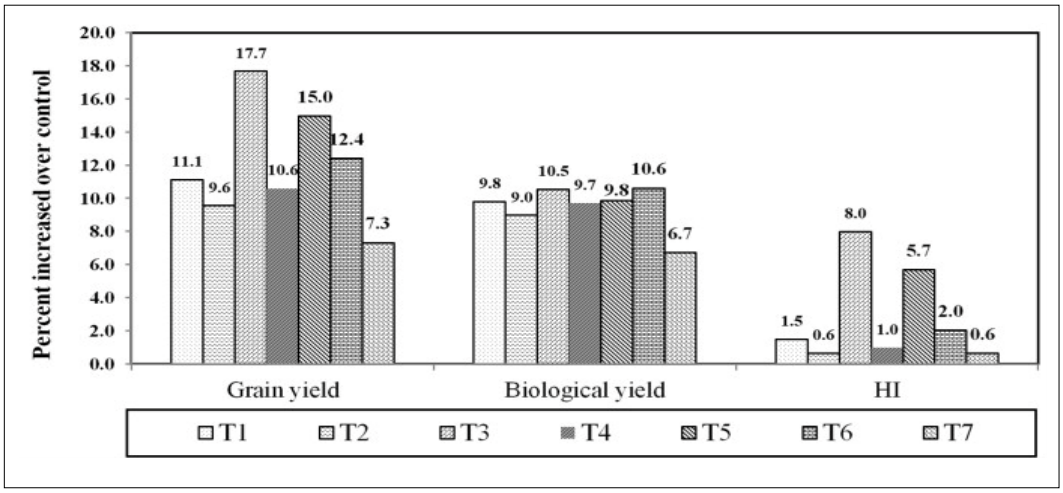


Fig 2: Effect of polyamines on per cent increase over control in grain yield, biological yield and HI.

CONCLUSION

The present study was undertaken to evaluate the significance of individual and combined applications of putrescine (0.75-3 mM) and cadaverine (0.5-2 mM) on the biochemical and yield attributes of black gram. Among all treatment combinations, (T_3) comprising a combined foliar application of putrescine (1.5 mM) and cadaverine (1 mM) proved most effective, significantly enhancing key biochemical traits and yield parameters which is clearly appearing in percent increase over control in biological yield grain yield and harvest index (17.7%, 10.5% and 8.0% respectively). These findings support the effectiveness of polyamine-based foliar treatments as a strategy for improving crop quality and productivity in black gram.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from using this content.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

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