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ORIGINAL RESEARCH

Effect of Zinc and Phosphorus Applications in Soil and Temperature Variation on Growth and Production of Black Gram (*Vigna mungo* L.)

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ABSTRACT

A clay pot experiment was conducted in glass top wire house to study the interactive effects of zinc and phosphorus application in soil on growth (length and dry weight), some biochemical constituents (pigments and protein contents and the activity of catalase and peroxidase) and production of fruits and seeds. Black gram (*Vigna mungo* L., Var. Haramoti) seeds were sown in clay pots field with alluvial soil (Texture- sandy loam, pH moderately alkaline and low organic matter content). Biochemical parameters were determined at day 65 of the growth in leaves of black gram. Before sowing treatments were made such as I- nil (control), II- $ZnSO_4$ @25 mg/kg soil, III- Single Super Phosphate (SSP) @50 mg/kg soil, IV- $ZnSO_4$ + SSP and V- $ZnSO_4$ + SSP + low temperature (20 ^{0}C) exposure for 4 hrs. up to the 25 days. Single application of zinc sulphate promoted all the parameters observed. Phosphorus application @50 mg/kg soil supported maximum plant growth such as dry weight by +54% but showed low value of pigments and protein content. Overall study showed the antagonistic effect of zinc and phosphorus fertilization on biochemical constituents and reproductive yield of black gram.

KEY WORDS: Zinc, Phosphorus fertilization, Pigments, Reproductive yield, Black gram

INTRODUCTION

The application of nutrient fertilizers is major practice to the management of nutrient deficient soils. Sometimes two or more nutrients sources are applied in the soil to improve the productivity of crops Zinc is a micronutrient very essential to plants and animals. Most of the agricultural land in India is zinc deficient (Khokhar *et al*., 2024). As zinc plays significant role in growth, production and protection of crops, its deficiency causes reduced crops production. It involves in the biosynthetic pathway of tryptophan involves in the synthesis of auxin. In C_4 plants, zinc is a constituent of carbonic anhydrase enzyme important to stabilize CO_2 during photosynthesis. After the production of reactive oxygen species during oxidative stresses, superoxide dismutase (SOD) activated by zinc to protect the cellular damage (Pandey, 2020; Fathi *et al.,* 2025). Due to the involvement of zinc in growth hormone (auxins) and chlorophyll production promotes growth of plants (Madaan *et al.,* 2025; Ammara *et al.,* 2026). Phosphorus is an essential macronutrient of plants and important constituent of nucleic acids, ATP and phospholipids of plasma membrane (Hu *et al*., 2025). About 30% of arable soils are phosphorus deficient, globally (MacDonald *et al.,* 2011) It is very important in energy transfer and stress resistance to plants (Khan *et al.,* 2023), comparatively, a higher concentration of phosphorus content has been reported in reproductive parts of plants (Poirier *et al.,* 2022). For the removal of phosphorus disorders in soil- plant systems, organic (rock phosphate, bone meal) and synthetic (Single Super Phosphate, Diammonium Phosphate etc.) sources are applied in the soil, oftenly but, when phosphorus applied in the soil with zinc in soil, both interact antagonistically (Nath *et al.,* 2024).

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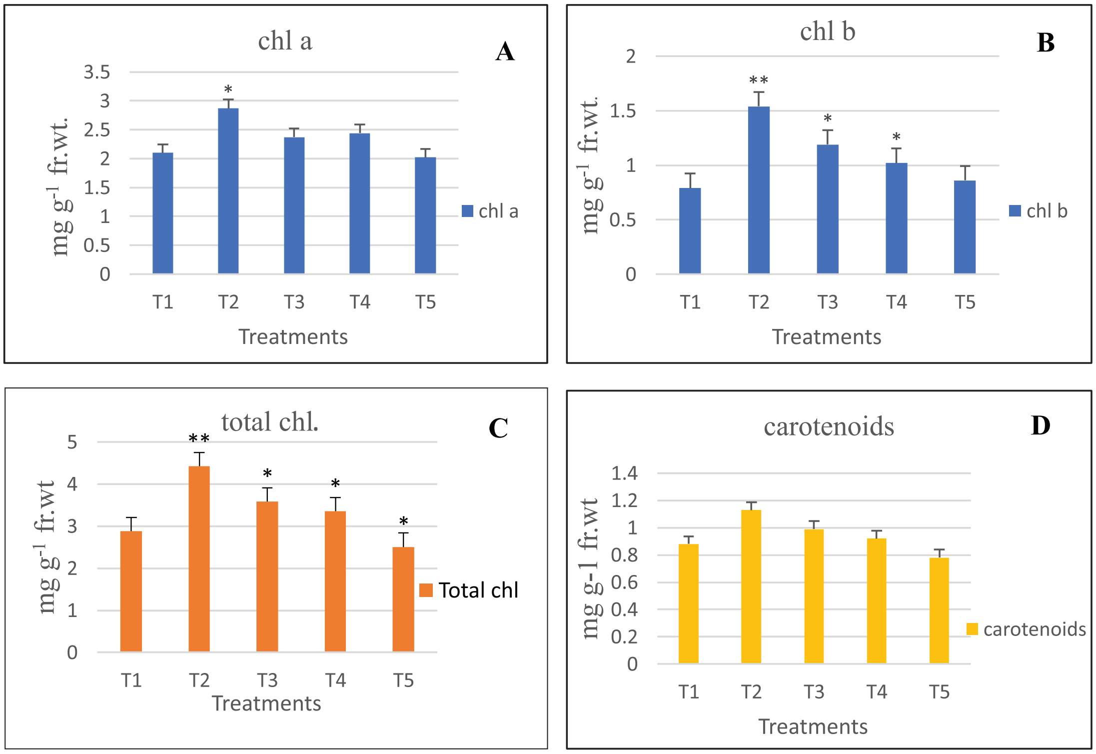

Figure 1: Effect of zinc and phosphorus treatments I- nil (control), II- $ZnSO_4$ @25 mg kg^{-1} soil, III- P_2O_5 @50 mg kg^{-1} soil, IV- $ZnSO_4$ @25 mg + P_2O_5 @50 mg kg^{-1} soil and V- $ZnSO_4$ @25 mg + P_2O_5 @50 mg kg^{-1} soil +low (20^{0}C) Temperature variations on (A) chlorophyll a (B) chlorophyll b (C) Total chlorophyll and (D) Carotenoids contents in black gram. * - indicate values significant at $P < 0.05$ and ** - value significant at $P < 0.01$ levels.

Zinc reacts with phosphoric acid to form an insoluble precipitate in soil, thus reduces their uptake and availability to plants (Nath *et al.,* 2024). The antagonistic effects to each other are concentration dependent (Imran *et al.,* 2016; Bhardwaj *et al.,* 2024). Black gram (*Vigna mungo* L.) is a fabaceous crop with high nutrition value sowing mostly in subtropical areas (25-35 ^{0}C) of India (Benergee *et al.,* 2021) in sandy to clayey soils. The production of black gram in India is about 2 to 2.45 million tonnes/ annually (Joshi *et al.,* 2023).

Least information is available to fertilization and interactive effects of zinc and phosphorus in soil on growth and production of black gram. Therefore, experiment was conducted to explore the effect of zinc and phosphorus fertilization in combination or single applications in soil on growth, some biochemical constituents and reproductive yield of black gram (*Vigna mungo* L. Hepper, Var. Haramoti).

Table 1: Physico- chemical properties of soil used in the experiment.

	Parameters					
	Soil texture Density (g/cm^3)	pH	O.M. (%)	$CaCO_3$ (%)	Available Zn	Fe (ppm)
Sandy loam	1.50	7.8	0.31	1.2	0.52	3.8

MATERIALS AND METHODS

A clay pot culture experiment was conducted in the Botany Department, University of Lucknow. Bulk composite soil sample was collected from the Gomti upland area of Lucknow district and analysed for their some important physico – chemical properties (Table 1). Then soil was filled in clay pots (10 kg size) and applied with zinc ($ZnSO_4$ @ 25 mg/kg soil) and phosphorus (SSP @ 50 mg/kg soil) and compared between Treatments viz. I- nil, II- $ZnSO_4$, III- SSP, IV- $ZnSO_4$ + SSP and V- $ZnSO_4$ + SSP + Temperature variation at 20^{0}C (4 hours/day) for 30 days. (After 3 weeks growth stage)

Experiment was conducted in three replicates. Ten seeds of black gram (*Vigna mungo* L. Hepper, Var. Haramoti) were sown in each pot of treatments and thinned to two plants in each pot after three weeks of the sowing. Plants were irrigated with distilled water at it's per the requirement, growth and visible symptoms appearance was observed regularly.

Soil Analysis

Soil sample was collected from the Gomti upland area of Lucknow district and analysed for their some important physico-chemical properties (Table 1).

Table 2: Effect of zinc and phosphorus application in soil and temperature variation on growth and production of black gram (*Vigna mungo* L.).

	Treatments				
Parameters	**I**	**II**	**III**	**IV**	**V**
Dr. wt./Pt. (g)	3.11 ± 0.01 (0.00)	3.63 ± 0.01 (+16.7)	4.78 ± 0.02* (+53.7)	4.80 ± 0.02** (+54.3)	2.70 ± 0.02 (-13.2)
Length (cm)	74.6 (0.0)	81.3 (+9.0)	100.2* (+34.4)	125.8 (+68.7)	60.9* (-18.3)
Protein ($\mu g\ g^{-1}$ fr. wt.)	88.8 (0.0)	94.0 (+5.89)	92.5 (+4.19)	111.6 (+25.69)	84.7 (-4.63)
Fruits /Pt (g)	17 (0.0)	21 (+23.53)	28 (+64.71)	23 (+35.29)	20 (+17.65)
Seeds/Pt (g)	54 (0.0)	95 (+75.93)	107 (+98.15)	88 (+62.96)	76 (+40.74)
Fruits wt./Pt (g)	4.71 (0.0)	6.81 (+44.58)	7.99 (+69.64)	5.74 (+21.87)	4.49 (-4.67)
Tissue zinc (mg g^{-1} dr. wt.)	12.5 (0.0)	23.8 (+90.4)	11.6 (-7.2)	26.2 (+109.6)	19.8 (+58.4)
Phosphorus (ppm)	880 (0.0)	910 (+3.4)	2200 (+150)	2110 (+139.8)	2010 (+128.4)

Treatments: I- Nil (Control), II- $ZnSO_4$ @25 mg kg^{-1} soil, III- P_2O_5 @50 mg kg^{-1} soil, IV- $ZnSO_4$ @25 mg + P_2O_5 @50 mg kg^{-1} soil and V- $ZnSO_4$ @25 mg + P_2O_5 @50 mg kg^{-1} soil +low (20^0C) Temperature variations.

Parenthesis indicate percentage increase (+) or (-) over control. ± S. E., * - indicate values significance at $P < 0.05$ ** - $P < 0.01$

Soil analysis was carried out for texture, density, pH, and organic matter content by the method described by Piper (1962). Available DTPA extractable zinc and iron content in soil were determined by the method of Lindsay and Norvell (1978).

Growth

Length and dry weight and visible symptoms appeared on plants were observed in each Treatment, regularly. In black gram plant (*Vigna mungo* L.), the Length and dry weight were observed at the time of harvesting.

Biochemical Constituents

The biochemical parameters were determined by the prescribed standard methods for the determination of Pigments (Licthenthaler & Welbern, 1983), Protein (Lowry *et al.,* 1951). Catalase activity (Euler & Josephson, 1927) and Peroxidase activity (Luck, 1936) in black gram leaves were used. Tissue phosphorus was determined in black gram by the method of Murphy & Riley (1962).

Statistical Analysis

Data were statistically analyzed with standard error (n=3) for their significance with the student t-test method. Data are presented in ± S.E., the tables and graphs are mean value of three replicates.

RESULTS AND DISCUSSION

Soil collected from the Gomti upland of Lucknow district was sandy loam in texture and slightly alkaline in reaction ranged between (pH 7.5-7.9). Organic matter content was very poor in soil (< 0.4%) (Pandey, 2018). Available zinc and iron content was very poor (< 0.55 Zn and < 5.56 ppm Fe) showed less amount of available essential element in soil (Agarwala & Sharma, 1979). Reduced growth of black gram can be correlated with a low level of zinc and iron as described earlier (Dey *et al.,* 2022; Tripathi *et al.,* 2023). The increase in shoot length by +68.7 and dry weight by +54.3% was noticed as compared to control might be due to the sufficient essential elements in plant tissue, specifically zinc and iron content (Kiruthika *et al.,* 2024). Maximum enhancement in pigment content was observed in plants grown at the soil applied with zinc fertilizer ($ZnSO_4$) @ 25 mg $ZnSO_4$/ kg soil. The increase in chlorophyll a (+36.7%), chlorophyll b (+94.4%), Total chlorophyll (+53.5%) and carotenoids content (+28.4%) was measured at the soil treated with $ZnSO_4$. This increase in pigments content may be attributed by sufficient zinc in soil, its uptake in plant and role of zinc in biosynthetic system of pigments (Shehzadi *et al.*, 2024). Among all pigments, a maximum increase was observed in chlorophyll b content (+95%) at the single application of fertilizer. Single application of phosphorus fertilizer or in combination with $ZnSO_4$ could not effective much more in enhancement of pigments content. These results indicated role

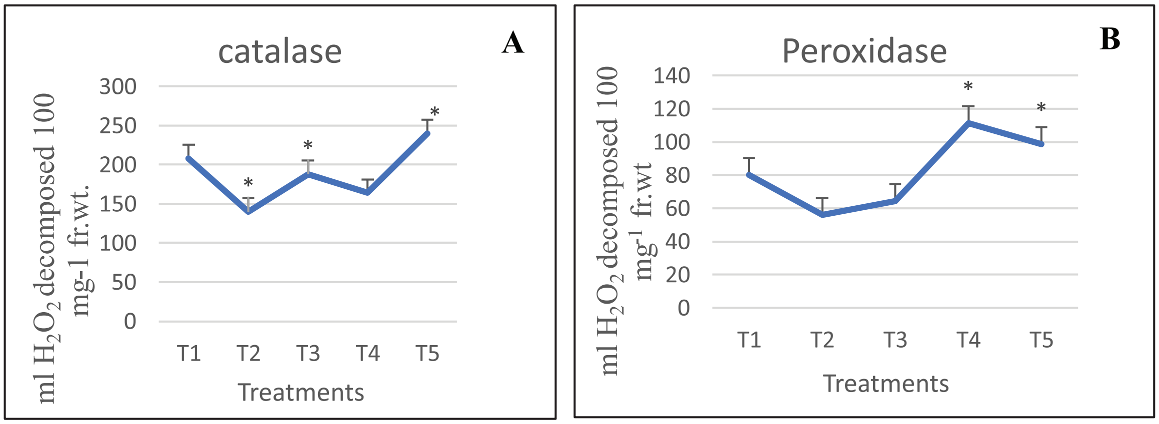

Figure 2: Effect of zinc and phosphorus treatments I- nil (control), II- $ZnSO_4$ @25 mg kg^{-1} soil, III- P_2O_5 @50 mg kg^{-1} soil, IV- $ZnSO_4$ @25 mg + P_2O_5 @50 mg kg^{-1} soil and V- $ZnSO_4$ @25 mg + P_2O_5 @50 mg kg^{-1} soil +low (20^0C) Temperature variations on (A) Catalase activity and (B) Peroxidase activity in black gram.

* - indicate values significant at $P < 0.05$ and ** - value significant at $P < 0.01$ levels.

of Zn in biosynthetic pathway of pigments as it is activator of enzymes involve in the synthesis of 5-aminolevolinic acid precursor of chlorophyll (Ammara *et al.,* 2026). Zinc deficiency in plants regulated by the soil factors may produce reactive oxygen species (ROS), leaching to break of pigment and reduce photosynthesis and consequently overall growth of plants have reported (Sudhalakshmi *et al.,* 2014; Dang *et al.,* 2024). The effectiveness of phosphorus application was less on pigments content could be due to their no direct effect in chlorophyll synthesis (Khan *et al.,* 2023). The applications of phosphorus singly or in combination with zinc showed a decrease in activities of antioxidative enzymes catalase and peroxidase (Table 2). Activity of these enzymes was observed to be high in plants at native soil. These results could be attributed due to the deficiency of nutrients zinc and phosphorus in the soil (Pandey, 2020; Dang *et al.,* 2024). The applications of zinc fertilizer significantly reduced the activity of catalase (-32.7%), whereas applied in combination with phosphorus it was reduced by -21%. The activity of peroxidase was reduced by -30% by $ZnSO_4$ fertilization singly, whereas enhanced peroxidase activity by +99% in combination with phosphorus fertilization in soil. Also, an increase in the activity of antioxidative enzymes and significant decrease in growth, pigments and protein contents was recorded in black gram exposed to low temperature (20^0C), even with the applications of zinc and phosphorus fertilizers. These results could be attributed due to the abiotic stresses of nutrients (Zn, P) and temperature (Pandey, 2018; Sharma, 2006). Protein content in black gram was found maximum at the combined application of zinc and phosphorus fertilization in soil (+25.7%). Reproductive production (Fruiting and seeds) of black gram was increased by the applications of zinc and phosphorus fertilizations in soil. The enhancement in fruits and seeds production was most supported by phosphorus fertilizer. The enhancement in the reproductive production of black gram could be due to the adequate zinc and phosphorus content in plants, and they played their role in biosynthetic pathways of biomolecules and energy metabolism of plants (Dang *et al.,* 2024).

CONCLUSION

Therefore, study concluded that, single fertilization of $ZnSO_4$ increased the growth pigments and reproductive yield of black gram grown in Gomti upland alluvial soil, whereas single phosphorus fertilization increased growth (length and dry weight), significantly. The combined application of zinc and phosphorus fertilizers reduced the effectiveness of enhancement of the above parameters determined. Thus, showed antagonistic relationships to each other. The exposure of low temperature (20^0C) for 4 hrs./day of 25 days reduced growth, biochemical constituents, and reproductive yield of black gram.

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