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Evaluation of the Response of Vinca Catharanthus Roseus (L.) G. Don to Biofertilization and Foliar Application of 24-Epibrassinolide in Selected Vegetative, Floral, And Chemical Traits

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ABSTRACT: To evaluate the expected response of vinca to biofertilization and foliar application of the plant growth regulator EBL, the study employed a 3×3 factorial experiment in a completely randomized design with three replicates, giving a total of 27 experimental units. The biofertilization factor included an uninoculated treatment B0, a combined bacterial BioNPK inoculant containing nitrogen-fixing and phosphorus- and potassium-solubilizing bacteria B1, and an arbuscular mycorrhizal fungus (AMF) inoculant B_2. The foliar application factor included water with solvent E_0, 0.01 µM E_1, and 0.10 µM E_2. The studied traits were plant height, shoot dry weight, number of flowers, flower diameter, total chlorophyll, and total soluble sugars. The experimental results showed significant effects of both factors and their interaction on all six traits, with B2E2 being superior; compared with B_0E_0, it increased the respective traits by 70.8, 88.5, 116.7, 34.8, 72.0, and 63.0%. These results indicate the potential integration of mycorrhizal inoculation with foliar application at 0.10 µM.

KEYWORDS: vinca, biofertilization, BioNPK, arbuscular mycorrhizal fungi, brassinosteroids.

INTRODUCTION

Vinca is a long flowering ornamental plant and it is also an important plant system for the study of terpenoid indole alkaloids of pharmaceutical value. Light levels, nutrition and biostimulants affect the efficiency of biomass and secondary-metabolite formation, thus the combination of vegetative growth and flowering with chemical indicator is helpful for assessing the overall plant response, rather than one of these alone (Quadri et al., 2026).

The beneficial microorganisms-based biofertilizers act in rhizosphere or plant tissues. These microorganisms can improve nutrient availability, generate growth stimuli and affect the signalling of plants. In addition to nutrient provision, the effect of microbial inoculation may also extend to the plant metabolic response, as suggested by a recent study in vinca which found that inoculation with beneficial bacteria of the genus Sphingomonas significantly impacted the MAPK signaling pathway that is linked to secondary metabolism (Gao et al., 2025).

A practical approach for the selection of the biofertilization rates in this model was obtained by a direct study on Vinca where bacterial inoculants with the ability to nitrogen fixation and to solubilize phosphorus and potassium were applied along with a consortium of arbuscular mycorrhizal fungi. This leads to improvement in growth, photosynthetic parameters, dry biomass and mycorrhiza had significant superiority in some parameters and is a justifiable comparison between no inoculation, BioNPK and AMF (Sharma et al., 2023).

A microbial consortium consisting of a cocktail of several bacterial isolates that produce biofilm and exopolysaccharide showed better results with regard to plant length, fresh weight, dry weight and total chlorophyll of vinca than a single isolate and it was also correlated to earlier flowering and delayed senescence. This response reflects the indirect effect of the improvement in nutrient and photosynthetic status of the plant on the floral responses (Thakur & Yadav, 2025).

EBL is a physiologically active brassinosteroid and varying concentrations have been directly applied to vinca. The concentrations 0, 0.01 and 0.10 µM were chosen as the best response for several growth traits, dry weight and chlorophyll in comparison with the control treatment and higher concentrations. Therefore, this study aimed to evaluate the response of Vinca (*Catharanthus roseus*) to the combined application of biofertilizers (BioNPK and AMF) and foliar spraying of 24-epibrassinolide (EBL) across selected vegetative, floral, and chemical attributes

MATERIALS AND METHODS

Nature of the Experiment

The experiment was set up under a standard screenhouse. One plant per pot was used as an experimental unit. Growing medium was made up of a uniform mix of loamy soil, sand, and peat moss at a 2:1:1 ratio and moisture was kept close to 70% of the field capacity in pots. To limit the variation attributable to the two factors studied to minimize experimental error, the units were standardized with respect to irrigation, cultural practices and when measurements were taken.

Experimental Factors and Their Levels

•Factor B for biofertilization: B0 without inoculation, B1 a combined bacterial BioNPK inoculant, and B2 an arbuscular mycorrhizal fungi (AMF) consortium.

1. Factor E for foliar application of EBL: E0 at a concentration of 0, E1 at 0.01 µM, and E2 at 0.10 µM.
2. Treatment combinations: B0E0, B0E1, B0E2, B1E0, B1E1, B1E2, B2E0, B2E1, and B2E2.

Preparation of Biofertilization Treatments

The B_1 bacterial inoculant was an equal blend of *Azotobacter chroococcum* (nitrogen-fixing), Bacillus amyloliquefaciens (phosphorus-solubilizing), and Enterobacter asburiae (potassium-solubilizing) on a charcoal carrier at a density of approximately 1×10^8 colony-forming units (cfu g^{-1}). A dose of 10 g of the formulation was applied directly to the root zone area during transplanting. The B_2 treatment consisted of an arbuscular mycorrhizal fungal (AMF) consortium comprising Funneliformis mosseae, Rhizophagus intraradices, and a Gigaspora species, applied at a dose of 50 g per pot (containing approximately 90–100 propagules g^{-1}) placed beneath the root system at planting.

Preparation of EBL Solutions and Foliar Application

To prepare a 1 mM stock solution, 48.07 mg of EBL (MW = 480.68 g mol^{-1}) was dissolved in 100 mL of ethanol. Then, 10 µL and 100 µL of this stock solution were diluted in 1 L of distilled water to prepare E1 (0.01 µM) and E2 (0.10 µM), respectively. The plants were sprayed to ensure complete wetting at 30 and 45 days after transplanting, whereas the E0 plants were sprayed only with the solvent and surfactant solution. This concentration range aligns with previous findings establishing 0.10 µM as the optimal dose for vinca (Alam et al., 2016).

Experimental Design

The treatments were laid out in a completely randomized design (CRD) in a 3×3 factorial arrangement with three replications (27 experimental units). Treatment was randomly applied and weekly interchange of pots was carried out to minimize the impact of light gradients inside screenhouse. The B and E levels were not considered variable and were treated as fixed factors.

Studied Traits

1. Plant height (cm): measured from the surface of growing medium to the highest growing point 90 days after transplanting (DAT) with a graduated ruler.
2. Dry weight of shoots (g plant-1): Shoots were detached and oven dried at 70ºC to constant weight and then weighed on a sensitive balance.
3. Number of flowers (flowers $plant^{-1}$): The total number of open flowers on each plant was counted from 60 to 90 days after transplanting.
4. Flower diameter (cm): Five fully opened flowers were taken each plant and the maximum diameter was measured using Vernier caliper and then mean calculated.
5. Total chlorophyll (mg g-1 fresh weight): Absorbance reading was done at 663 and 646 nm and chlorophyll a and b calculated using the suggested extraction equations from the recent study in the neighboring area (Sharma et al., 2023) and total chlorophyll calculated as the sum of chlorophyll a and b.
6. Total soluble sugars (% of dry weight): leaf powder was extracted with 80% ethanol, the filtrate was reacted with 5% phenol and concentrated sulfuric acid, and absorbance was read at 490 nm against a glucose standard curve; the result was then converted to a glucose-equivalent percentage (Doran & De Souza, 2021).

Statistical Analysis

Data were presented as treatment means of three replications. A 3 × 3 factorial analysis of variance (ANOVA) was conducted based on a Completely Randomized Design (CRD). The sources of variation included biofertilization (B), EBL application (E), and their interaction (B × E), with degrees of freedom of 2, 2, 4, and 18 for experimental error (26 total). Treatment means for main effects (B and E) as well as their interaction (B × E) were compared using the Least Significant Difference (LSD) test at a significance level of $P \leq 0.05$.

RESULTS

Plant Height

Mean plant height increased from 27.33 cm in B0 to 35.00 cm in B2, representing an increase of 28.0%. E2 also increased the mean from 27.17 to 36.67 cm compared with E0. The highest mean, 41.00 cm, was recorded in B2E2, compared with 24.00 cm in B0E0, representing a increase of 70.8%.

Table 1. Effect of biofertilization, EBL foliar application, and their interaction on vinca plant height (cm)

Biofertilization	E0	E1	E2	Mean B
B0	24.00	27.00	31.00	27.33
B1	28.00	32.50	38.00	32.83
B2	29.50	34.50	41.00	35.00
Mean E	27.17	31.33	36.67	31.72
LSD (0.05)	B	E	B×E	
	0.56	0.56	0.97	

Shoot Dry Weight

Mean dry weight increased from 6.00 g $plant^{-1}$ in B0 to 8.10 g $plant^{-1}$ in B2, an increase of 35.0%. The E2 mean also increased by 42.8% relative to E0. B2E2 reached 9.80 g $plant^{-1}$, exceeding B0E0 at 5.20 g $plant^{-1}$ by 88.5%.

Table 2. Effect of biofertilization, EBL foliar application, and their interaction on shoot dry weight (g plant^{-1})

Biofertilization	E0	E1	E2	Mean B
B0	5.20	5.90	6.90	6.00
B1	6.20	7.40	9.00	7.53
B2	6.60	7.90	9.80	8.10
Mean E	6.00	7.07	8.57	7.21
LSD (0.05)	B	E	B×E	
	0.14	0.14	0.25	

Number of Flowers

The mean number of flowers was 21.33 flowers plant^{-1} in B0 and increased to 31.67 flowers plant^{-1} in B2, an increase of 48.4%. The E2 mean was 33.00 flowers plant^{-1} compared with 22.00 in E0. B2E2 recorded the highest number, 39.00 flowers plant^{-1}, which was more than double B0E0 at 18.00 flowers plant^{-1}.

Table 3. Effect of biofertilization, EBL foliar application, and their interaction on number of flowers (flowers plant^{-1})

Biofertilization	E0	E1	E2	Mean B
B0	18.00	21.00	25.00	21.33
B1	23.00	28.00	35.00	28.67
B2	25.00	31.00	39.00	31.67
Mean E	22.00	26.67	33.00	27.22
LSD (0.05)	B	E	B×E	
	1.28	1.28	2.21	

Flower Diameter

B2 improved flower diameter by 14.8% compared with B0, while E2 increased the mean by 18.4% compared with E0. Flower diameter reached 4.38 cm in B2E2 compared with 3.25 cm in B0E0, giving a increase of 34.8%.

Table 4. Effect of biofertilization, EBL foliar application, and their interaction on flower diameter (cm)

Biofertilization	E0	E1	E2	Mean B
B0	3.25	3.45	3.72	3.47
B1	3.50	3.82	4.18	3.83
B2	3.62	3.96	4.38	3.99
Mean E	3.46	3.74	4.09	3.76
LSD (0.05)	B	E	B×E	
	0.04	0.04	0.07	

Total Chlorophyll

Mean total chlorophyll increased by approximately 31.0% from B0 to B2 and by 33.2% from E0 to E2. The highest value was 2.15 mg g^{-1} fresh weight in B2E2 compared with 1.25 mg g^{-1} in B0E0, representing an increase of 72.0%.

Table 5. Effect of biofertilization, EBL foliar application, and their interaction on total chlorophyll (mg g^{-1} fresh weight)

Biofertilization	E0	E1	E2	Mean B
B0	1.25	1.39	1.58	1.41
B1	1.47	1.68	1.97	1.71
B2	1.56	1.82	2.15	1.84
Mean E	1.43	1.63	1.90	1.65
LSD (0.05)	B	E	B×E	
	0.03	0.03	0.06	

Total Soluble Sugars

B2 increased mean total soluble sugars by 27.3% compared with B0, while E2 increased the mean by 31.1% compared with E0. B2E2 achieved the highest value, 17.60%, compared with 10.80% in B0E0, representing a increase of 63.0%.

Table 6. Effect of biofertilization, EBL foliar application, and their interaction on total soluble sugars (%)

Biofertilization	E0	E1	E2	Mean B
B0	10.80	11.70	13.00	11.83
B1	12.10	13.80	16.20	14.03
B2	12.80	14.80	17.60	15.07
Mean E	11.90	13.43	15.60	13.64
LSD (0.05)	B	E	B×E	
	0.27	0.27	0.46	

DISCUSSION

Discussion of Plant Height

B2 is superior in plant height due to mycorrhizal hyphae that can expand the volume of soil explored and its role in enhancing uptake of phosphorus and water and potentially improve gas exchange and water status of the plants. The maximum shoot length was observed in the mycorrhizal treatment followed by some bacterial inoculants (Sharma et al., 2023).

The effects of height response to foliar application can be attributed to the stimulation of cell division and elongation and improvement of photosynthesis within the concentration range. The direct study on vinca resulted in the selection of 0.10 µM which gave the best response in growth traits as compared to a wider range of concentrations as compared to E1 and E0 (Alam et al., 2016).

Discussion of Shoot Dry Weight

Dry weight is a more integrative measure than linear measurements only, as it is produced by the loss of water. The fact that its B1 and B2 were increased is in line with an earlier study on vinca, which found that a bacterial consortium that produced biofilm and extracellular polysaccharides led to an increase in both dry weight and the rate of photosynthesis, indicating enhanced efficiency in root functioning and continued photosynthesis (Thakur & Yadav, 2025).

The ability of B2E2 to outperform EBL suggests that the plant may be better primed to respond to hormonal signal of EBL if it has more nutrients and water available from mycorrhiza, and this would help enhance carbon fixation and allocation to new tissues. The study on medicinal peppermint indicated that foliar spraying with the compound caused branch elongation, improved biomass, chlorophyll content and photosynthesis, the optimum concentration was different for species and cultivar, and the dose should be verified for vinca (Parrey et al., 2023).

Discussion of Number of Flowers

The number of flowers increased under the microbial treatments; this can be related to better vegetative growth and creation of more flowering points as well as to the prolongation of the functional period of leaves. The earlier flowering of plants of Vinca inoculated with a consortium of bacteria (Thakur & Yadav, 2025) supports this interpretation.

Discussion of Flower Diameter

Mycorrhizal plants might be able to use more phosphorus, micronutrients and water for flower production and growth, which could increase the available resources. The AMF consortia isolated from medicinal plants varied in their ability to enhance leaf area and dry biomass of vinca (De la Rosa-Mera et al., 2012).

Brassinosteroid might be involved in the regulation of the elongation of cells in the petal and the synthesis of cell-walls, which in turn may account for the observed increase in diameter of the flowers, but the evidence for this mechanism is based on gerbera and not vinca. Brassinosteroids were found to influence the expression of genes involved in petal growth and cell expansion pathways in gerbera (Huang et al., 2017).

Discussion of Total Chlorophyll

The superiority of B2 in chlorophyll was explained by the higher availability of nitrogen, magnesium and phosphorus, which are essential for the formation of chlorophyll and are important for the activity of the photosynthetic apparatus. Increased pigments and photosynthetic traits were recorded under bacterial and mycorrhizal inoculants, the order being B2, B1 and B0 (Sharma et al., 2023), similar to the above-mentioned study in vinca.

EBL effect could be related to the maintenance of plastid structure, stimulation of chlorophyll synthesis, and delay in its degradation. In the case of Vinca, the concentration of 0.10 µM showed an increase in total chlorophyll of around 27.1% at 6 months and 35.7% at 9 months in direct comparison to the control, which aids in the construction of a positive response under E2 in Vinca (Alam et al., 2016).

Discussion of Total Soluble Sugars

The rise in soluble sugars is caused by a balance between the production of soluble sugars (due to photosynthesis) and consumption of soluble sugars (due to growth and flowering). In a direct study of vinca, native fungi increased growth, nutrition, chlorophyll, and soluble and reducing sugars which had a biological explanation for the higher B2 mean (Srinivasan & Govindasamy, 2014).

Brassinosteroid can do this by improving the photosynthetic efficiency and by regulating the allocation of carbon into starch and the abundance of soluble sugars. In the tomato plant a physiological study revealed that brassinosteroids promoted starch synthesis and enhanced soluble sugars, especially in low light. Consequently, the rise in E2 is in line with this reaction (Liu et al., 2022).

CONCLUSIONS

1. All the data reflected a uniform superiority of biofertilization compared to BioNPK and uninoculated treatment, and in all six traits the mean of AMF was higher.
2. EBL was at the highest concentration of 0.10 µM compared to 0.01 µM and control treatments in the experimental range.
3. Plant height, dry weight, number of flowers, flower diameter, chlorophyll and total soluble sugars was significantly affected by the B2E2 treatment and the interaction effects.
4. Combined application of AMF inoculation (B2) and 0.10 µM EBL foliar spray (E2) effectively enhances growth, flowering, and physiological characteristics in *Catharanthus roseus*.

RECOMMENDATIONS

1. Evaluate the synergistic effects of AMF and EBL under field conditions and different soil types to confirm commercial application viability.
2. Bacterial density and percentage root colonization by mycorrhiza (30 and 60 days) should be measured before application, not just based on the weight of commercial formulation.
3. Use the same surfactant, spray volume and solvent throughout EBL treatments to ensure the same dose is applied; test at 0.01, 0.05 and 0.10 µM to fine tune the dose to the optimum level.

4. Include additional characteristics like leaf area, photosynthetic rate, phosphorus and nitrogen content in leaves, and the proportion of early flowering plants.

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