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Research Article

Assessment of Growth and Productivity of Zinnia (Zinnia Elegans) Against Exogenous Application of NPK and Boron

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ABSTRACT

Study was conducted to evaluate the effects of varying concentrations of macronutrients (NPK) and micronutrients (boron) on the growth and flowering of "*Zinnia elegans*" cv. *Zinnia Binary's Giant* under the subtropical climate of Lahore. The study was comprised ten treatments with four replications, including a control with different combination of boron and NPK applications. Seeds of "*Zinnia Benary's Giant*" were sown in germination tray having substrate of leaf manure, sand, and silt, and transplanted into 12-inch earthen pots at the age of 20 days. Foliar applications of nutrients were started 25 days after transplanting. Data regarding following parameters were recorded such as days to flowering, fresh and dry weight of plant and flower, plant height, root length, flower diameter, leaf area and chlorophyll content, proline content, superoxide dismutase, catalase, peroxidase and total phenolics using standardized measurement techniques. After statistical analysis, results indicated that 50 ppm boron significantly reduced the time to flower opening to 55.75 days, while NPK applications generally delayed flowering. The optimal combination of 50 ppm boron and 2g NPK resulted in maximum, plant fresh weight (32.4 g), and dry weight (7.575 g), root length (33.325 cm), shoot length (57 cm) than control showed smallest shoot length (22.125cm), root length (10.125cm), pant fresh weight (13.175g) and dry weight (2.7g). Flower diameter peaked at 8.5225 cm with this treatment. Notably, chlorophyll content reached 5.002 mg/g FW, while peroxidase (POD) and superoxide dismutase (SOD) and activities, indicators of plant stress response, were highest with the combined nutrient application. In contrast, higher boron concentrations (100 ppm) suppressed growth and enzyme activity. Generally, the outcomes suggest that the synergistic application of 50 ppm boron and 2g NPK significantly enhances the growth and flowering of "*Zinnia elegans*", emphasizing importance of optimal nutrient management in improving floral crop production in Pakistan's burgeoning floriculture sector.

Keywords: Flowers, Zinnia, Yield, Boron, Floriculture, Pakistan.

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INTRODUCTION

Flowers represent love, affection, peace, beauty, and economic importance and are widely used in many different kinds of daily activities and events. There may not be any social events or festivals where flowers are not used (Oyi et al., 2012). Floriculture is a rising sector in agriculture that deals with the development, maintenance, and marketing of ornamental plants and cut flowers. It contributes significantly to the market and net return to producers (van Uffelen and de Groot, 2005). Fresh-cut flower cultivation contributes considerably to global agriculture, with a trade value of around US $ 5.5 billion (Kumar and Vazhacharickal, 2020).

One of the most economical things that may be produced in a greenhouse or field is cut flowers (Gast, 2000). *Zinnia elegans*, well-known as common zinnia, youth and age or elegant zinnia, is a member of the Asteraceae family (Slathia et al., 2019). The Zinnia genus contains roughly 20 species, with *Z. elegans* being the most prominent. The Asteraceae family, originally known as Compositae have around 32000 flowering plant spread among 1900 genera. Compositae, also known as the sunflower, composite, daisy or aster family, was initially mentioned in 1740. Zinnias are true American indigenous, growing throughout the southern United States, Mexico, and Central America (Grissell, 2020). The 20–30 species, *Zinnia elegans* and *Zinnia haageana* are the two most promising (Javid et al., 2005). They have various cultivars and are frequently planted for their lovely blooms. However, most attractive cultivars are derived from *Zinnia elegans*. Zinnia flowers are colorful and attractive plants that are commonly seen in Indonesia (Triatmi & Akbar, 2021). Zinnia is a dicot herbaceous plant, grown from seed (Tugbaeva et al., 2023). Their petals can take the form of a solitary row (single flowered zinnia), several rows with a hidden midpoint (double flowered), or several rows of petals with noticeable centers (semi-double zinnia) and have Dahlia-like flower too (Pasha et al., 2015). It is among the top five most commonly grown specialty cut flowers (Loyola et al., 2019). They are highly valued in the cut flower market due to their adaptability, variety of colours, and ease of care. Zinnias attract butterflies, hummingbirds, and other birds to gardens (Ullah et al., 2019). In 1999, the 'Benary's Giant' zinnia series won award "Cut Flower of the year". Since then, “Benary's Giant" line has set the industry's benchmark for cut flower zinnia (Gast, 2000).

Production of high-quality blooming plants requires specific care (A. K. Singh, 2006). In nurseries and specialist cut flower farms, planting annuals is a critical task (Bachmann, 2006). Plant development and productivity are significantly enhanced by both macro and micronutrients (S. Kumar et al., 2021; Mushtaq et al., 2023). Climate and fertilizer management have significant effect on flower quality as well as growth and development of plant. Foliar fertilizer may be adapted to satisfy plants' requirement for one or more micro or macronutrients, including trace minerals. This approach can fix deficiencies, strengthen crops, speed up development, cultivate healthier and better-looking plants, and promote overall plant health (Singh et al., 2013). Plants require micronutrients from soil or by foliar application for optimal crop development and production, as well as efficient utilization of provided nitrogen, phosphorous and potash. Along with taking part in majority of the enzyme reaction, microelements also indirectly contribute to the production of several growth regulators (Khalifa et al., 2009). The appropriate combination of chemical fertilizers is essential for producing more leaves, which has a positive influence on flower quality and quantity, as well as extending the blooming time (Arab et al., 2015). Nitrogen, phosphorus, and potassium are the most vital nutrients for plant development and producing high-quality blooms. Additionally, Nitrogen, phosphorus, and potassium play important roles in producing high-quality seed yields (Saputra et al., 2019). Without these nutrients, plants have physiological abnormalities, leading to uneven development and poor yield.

Phosphorus has vital role in integrity of cell walls and plant (Li et al., 2024). Phosphorus is an significant component of plant structures and act as catalyst in biological functioning (Kolodiazhnyi, 2021) and is also essential for many metabolic processes, such as respiration and photosynthesis. It is a part of nucleotides found in DNA and RNA, and plays a role in structure of phospholipids in cell membranes. Phosphorus is also important for energy metabolism because of its presence in ATP, ADP, AMP, and pyrophosphate molecules (Shaukat et al., 2012).

Potassium is also key components of plant nourishment; it is a fundamental element and the structural support system for plants. K promotes root development and enhances drought adaptation and also promotes glucose synthesis and transport (Shen et al., 2017). Potassium (K) acts as a key enzyme in plant activities (Hasanuzzaman et al., 2018).

Boron is a vital micronutrient required for the growth, development, yield, and quality of crops. It serves a variety of tasks in plants, primarily engaged in cell wall production and structural integration (Bariya et al., 2014). Crop quality is improved by applying fertilizers that contain NPK, boron, and other macro and micronutrients. However, a deficiency of these elements results in a decline in plant photosynthesis and RNA eradication, as well as a reduction in the amount of protein and carbohydrate synthesis and a subsequent decline in crop performance and quality. In light of the previously mentioned facts, the current study was designed to investigate the combined or separate nutrient application effect of macronutrients like NPK and micronutrients like boron on the growth and flower quality of *Zinnia elegans* cv. *Zinnia Binary's Giant* and to withstand zinnia flower during the subtropical weather of Lahore in May & June.

MATERIALS AND METHODS

Experimental site

This study was conducted at Floriculture (Training and Research Institute), Agriculture Department Punjab, Lahore, (GPS Latitude 31.55829, GPS longitude 74.34839), Pakistan, in 2024, to investigate the influence of varying concentrations of NPK and Boron to enhance the flower quality, vase life as well as vegetative growth of *Zinnia elegans* (Zinnia Benary's Giant).

Planting material and experimental design

Seeds of Zinnia were imported from Benary, Germany. Seeds were sown in germination trays containing 25% leaf manure, 10% sand and 75% silt as a substrate and at age of 20 days, seedlings transplanted to 12-inch earthen pots (1 plant per pot) and plants were irrigated according to the need of the plants. After 25 days of transplanting the plants, NPK and Boron were applied by foliar application. At 2-4 leaf stage, seedlings were transplanted in a 12-inch earthen pot. All cultural activities, such as irrigation, pruning, removing offshoots, weeding, and pest management, were performed using standardized procedures that were uniformly applied to each plant. Treatments includes T_0 Control, T_1 Distilled Water, T_2 50 ppm Boron, T_3 100 ppm Boron, T_4 2g/L NPK, T_5 4g/L NPK, T_6 50 ppm Boron+2g/L NPK, T_7 50 ppm Boron + 4g/L NPK, T_8 100 ppm Boron + 2g/L NPK, T_9 100 ppm Boron +4g/L NPK.

Measurement of morphological parameters

Data was recorded about the morphological traits. Number of days until the flowering started was calculated by counting from the planting date to appearance of the first flower. Plant height (cm) of each plant was taken from the highest point of meristem to base of the plant by using measuring tape (Heady, 1957). Measurement of root length (cm) was taken by using a measuring tape (Abbas et al., 2024). After measuring the root length, the average root length for each replication was calculated. For the measurement of Leaf area (cm²), two healthy mature leaves were selected, and areas were determined by measuring the maximum length and maximum width of the leaf blade. Then these measurements multiplied by a constant factor of 0.68 (Birch et al., 1998). Plant fresh and dry weight (g) was recorded using a digital balance firstly fresh then placed in an open and sunny area to dry completely. Once fully dried, the samples were collected and weighed using a digital balance. To measure the flower's diameter (mm), choose fully opened flowers that were representative of the species' normal size. Using a Vernier caliper, measured the broadest part of the flower horizontally across its center, ensuring the measurements were straight and perpendicular to the stem. For the measurement of Flower fresh and dry weight (g) fresh flowers were picked in the morning, weighed using an analytical balance and mean was measured than fresh flowers were placed in an oven at 65ºC for 24 hours. After drying, weight was measured using analytical balance and expressed in grams. After harvest, the diameter of the stem was measured using a digital Vernier caliper in millimeters (mm).

Measurements of physiological parameters

Proline composition (μmol g^{-1} F.W)

To determine proline content, a sample was prepared using 2.0 ml of filtered leaf homogenate, 2.0 ml of ninhydrin solution (made by dissolving 1.25 g of ninhydrin in 30.0 ml of glacial acetic acid and 20.0 ml of 6 molar orthophosphoric acid), and 2.0 ml of glacial acetic acid. Then heated at 100°C for 60 minutes. After heating, the reaction was put to an end by transferring the test tubes to an ice bath. The mixture was then extracted with 10.0 ml of toluene while stirring strongly by passing a constant stream of air through it for 1-2 minutes. Chromophore toluene layer was removed, and the remaining aqueous phase was separated and allowed to warm to room temperature. Absorbance at 520 nm was measured using a double beam spectrophotometer with toluene as the blank. Proline content was measured based on fresh weight data and compared against a standard curve. The measurements were determined using a Hitachi-120 spectrophotometer from Japan.

Mole proline gm^{-1} fresh weight = [gm proline ml^{-1} × ml of toluene/115.5) / (gm of sample/5)]

Superoxide dismutase (Unit's/mg protein)

To determine superoxide dismutase (SOD), reaction mixture (3.0ml) was prepared, which contained 20–50 ml of enzyme extract, 50.0 mM nitroblue tetrazolium (NBT), 1.3 mM riboflavin, 13 mM methionine, 75.0 mM EDTA, and 50.0 mM phosphate buffer having pH 7.8. Then, test tubes were exposed to light from 15 fluorescent lamps (at an intensity of 78 μmol m-2 s-1) for 15 minutes. After exposure, the absorbance of the radioactive solution was measured at 560 nm using a spectrophotometer (Giannopolitis & Ries, 1977). One unit of SOD activity was defined as the amount of enzyme that was saved for 50% of NBT photo reduction. Measurements were taken with a Hitachi-650 spectrophotometer from Japan.

Peroxidase (Unit's/mg protein)

To measure peroxidase (POD) activities using the following procedures were followed with minor modifications by (Chance & Maehly, 1955) method. POD activity was reported per unit of protein quantity and one unit defined as an absorbance change of 0.01 units per minute (Bradford, 1976).

Chlorophyll content

Chlorophyll pigments were determined by the method of (Makeen et al., 2007). The chlorophyll content was determined using the formula given below:

Chla (mg g FW) = [12.7*(663)-2.69(645)] *(v/1000) *w

Chlb (mg gFW) = [22.9*(645) - 4.68(663)] *(v/1000) *w

Statistical analysis:

Data was analyzed using Statistix (version 8.1) software for analysis of variance (ANOVA). Treatment means were compared using LSD at a 5% probability level.

RESULTS

Days taken to open of first flower

The plants fertilized with Boron at 50 ppm and 100 ppm concentrations showed a notable reduction in the time taken to flower, with the flowers opening in 55.75 and 57.25 days, respectively (Table 1). When NPK was applied at 2g/L, the plants took 59.5 days to flower, while those treated with 4g/L of NPK took 61 days. Results further indicated that combined application of Boron and NPK 50 ppm Boron with either 2g/L or 4g/L NPK both resulted in flowers opening in 60.5 days. Results depicted that increasing the Boron concentration to 100 ppm, along with 2g/L or 4g/L NPK, slightly delayed flowering to 62.75 and 63.75 days, respectively. However, control plants took the longest time, requiring 70 days to open the first flower. While plants treated with distilled water opened their first flowers slightly earlier taking 68 days.

Table 1. Effects of various concentrations of NPK and Boron on Days to First flower, Shoot length (cm), Root length (cm), Plant fresh weight (g), Plant dry weight (g), Flower Diameter (cm), Stem Diameter (cm), Leaf Area (cm²), Flower fresh weight (g) and Flower dry weight (g) of Zinnia elegans plants. Data represent Means ± SD, followed by different letters indicate significant differences as per Tukey's LSD test (P ≤ 0.05).

Effects on shoot length (cm)

The control plants without application of Boron and NPK demonstrated shoot length of 22.125 cm, while plants treated with distilled water exhibited an increase in shoot length to 25 cm (Table 1).

Treatment	First flower	Shoot length	Root length	Plant fresh weight	Plant dry weight	Flower Diameter	Stem Diameter	Leaf Area	Flower fresh weight	Flower dry weight
T0	70±0.01a	22.12±0.02h	10.12±0.03i	13.17±0.04h	2.7±0.04i	5.09±0.03g	4.05±0.02i	22.38±0.06i	2.52±0.02g	0.59±0.04h
T1	68±1.00a	25±0.10g	11.90±0.04h	14.02±0.06h	2.54±0.02i	5.25±0.06g	4.37±0.03h	24.07±0.03h	2.72±0.04g	0.73±0.02g
T2	55.75±0.15f	48.12±0.02d	17.1±0.05f	19.07±0.05f	3.73±0.07g	6.69±0.01cd	5.80±0.02e	35.92±0.05f	6.37±0.01d	1.48±0.04de
T3	57.25±0.25ef	47.37±0.05d	14.05±0.02g	17.47±0.03g	3.55±0.06h	6.43±0.02e	5.70±0.03f	34.15±0.01g	5.62±0.06e	1.46±0.05ef
T4	59.5±0.05de	53.37±0.01b	26.62±0.03c	25.1±0.06c	5.45±0.03c	6.82±1.02c	6.24±0.05c	46.72±0.03c	6.67±0.05c	1.52±0.06bc
T5	61±1.00cd	49.87±0.03c	24.62±0.05c	23.17±0.06d	5.26±0.02d	6.50±1.03de	6.15±0.02d	45.74±0.03c	6.42±0.04d	1.50±0.07cd
T6	50.5±0.5cd	57.00±0.05a	33.32±0.02a	32.4±0.05a	7.57±0.01a	8.52±0.07a	6.55±0.04a	53.35±0.05a	7.52±0.06a	1.56±0.01a
T7	50.5±0.05cd	53.63±0.04b	30.07±0.05b	29.95±0.05b	7.31±0.03b	7.25±0.03b	6.33±0.06b	49.88±0.01b	7.20±0.08b	1.54±0.06ab
T8	62.75±1.25bc	36.00±0.04e	23.02±0.07d	22.8±0.07d	4.43±0.02e	5.86±0.02f	5.68±0.03f	42.42±0.02d	5.52±0.02ef	1.45±0.01ef
T9	63.75±1.25b	33.25±0.02f	22.05±0.02e	20.1±0.06e	4.21±0.03f	5.65±0.03f	5.58±0.04g	39.72±0.03e	5.38±0.03f	1.43±0.01f

Boron application at 50 ppm resulted in a notable increase in shoot length to 48.125 cm. However, increasing the boron concentration to 100 ppm slightly reduced shoot length to 47.375 cm, suggesting a potential negative effect at higher levels. NPK treatment produced increase in shoot length, with 2 g/L NPK achieving 53.375 cm and 4 g/L NPK resulting in 49.875 cm. Results further depicted that combined application of boron and NPK with 50 ppm boron along with 2 g/L NPK led to the maximum shoot length of 57 cm. The combination of 50 ppm boron with 4 g/L NPK also resulted in increased shoot length (53.625 cm). In comparison, combining 100 ppm boron with NPK resulted in reduced shoot lengths to 36 cm and 33.25 cm for 2 g/L and 4 g/L NPK, respectively.

Effect on root length *Zinnia elegans* (cm)

The root length of control plants of *Zinnia elegans* exhibited the shortest root length at 10.125 cm, while plants treated with distilled water showed a slight increase in root length to 11.9 cm (Table 1). The application of Boron alone at 50 ppm led to a significant increase of 17.1 cm in root length. However, increasing the Boron concentration to 100 ppm resulted in a moderate reduction in root length to 14.05 cm. The plants treated with 2g/L NPK exhibited a root length of 26.625 cm, while those treated with 4g/L NPK had a slightly lower root length of 24.625 cm. However, the treatment combining 50 ppm Boron with 2g/L NPK resulted in the longest root length of 33.325 cm, while combining 50 ppm Boron with 4g/L NPK led to a root length of 30.075 cm. Alternatively, increasing the Boron concentration to 100 ppm combined with 2g/L or 4g/L NPK reduced the root lengths to 23.025 cm and 22.05 cm, respectively.

Effect on Plant Fresh weight (g)

The control demonstrated the lowest fresh weight at 13.175 g, while plants treated with distilled water showed a slight increase in fresh weight to 14.025 g (Table 1). Boron showed an increased in plant fresh weight, with the 50 ppm Boron treatment achieving a fresh weight of 19.075 g, while the 100 ppm Boron treatment led to a slightly lower fresh weight of 17.475 g. The plants fertilized with NPK resulted an increase in fresh weight with 2g/L NPK exhibited a fresh weight of 25.1 g, while those treated with 4g/L NPK showed a fresh weight of 23.125 g. Furthermore, combination of Boron and NPK with 50 ppm Boron and 2g/L NPK resulted in a maximum fresh weight of 32.4 g. The combination of 50 ppm Boron with 4g/L NPK also produced a high fresh weight of 29.95 g. However, increasing the Boron concentration to 100 ppm combined with 2g/L and 4g/L NPK reduced the fresh weights to 22.8 g and 20.1 g, respectively.

Effect on Plant Dry Weight (g)

The control plants recorded the lowest dry weight of 2.7 g. Similarly, the plants treated with distilled water demonstrated a marginally lower dry weight of 2.545g (Table 1). Foliar application of Boron with 50 ppm Boron resulted in a dry weight of 3.735 g, while 100 ppm Boron led to a slightly lower dry weight of 3.55 g. Moreover, the application of 2g/L NPK resulted in a dry weight of 5.455 g, whereas the application of 4g/L NPK resulted in a slightly lower dry weight of 5.265 g. The treatment combining 50 ppm Boron and 2g/L NPK produced the highest dry weight of 7.575 g, followed by the combination of 50 ppm Boron and 4g/L NPK, which resulted in a dry weight of 7.315 g. However, when the Boron concentration was increased to 100 ppm, in combination with 2g/L and 4g/L NPK, the dry weights decreased to 4.4375 g and 4.2 g, respectively.

Effect on Flower Diameter (cm)

The flowers of *Zinnia elegans* in control group represented the smallest diameter of 5.0925 cm. A slight increase was observed in case of distilled water treatment, with diameter of 5.25 cm (Table 1). The 50 ppm Boron significantly increased the flower diameter (6.6975 cm), while a 100 ppm Boron slightly decrease the diameter (6.4325 cm). Likewise, the treatment with 2.0 g/L NPK produced a flower diameter (6.82 cm), whereas, 4.0 g/L NPK showed a small diameter (6.505 cm). However, the combination of 50 ppm Boron and 2.0 g/L NPK revealed in the highest flower diameter (8.5225 cm), whereas the Boron concentration combined with 4.0 g/L NPK, reduced the diameter (7.255 cm). Moreover, increasing the Boron dosage to 100 ppm in combination with 2.0 g/L and 4.0 g/L NPK led to a further decline in diameter (5.8625 cm) and (5.6575 cm), respectively.

Effect on Stem Diameter (mm)

The control showed a minimum stem diameter (4.0575 mm), whereas a nominal improvement was seen in distilled water application (4.375 mm) (Table 1). When treated with 50 ppm Boron, the stem diameter increased notably to 5.805 mm, while a higher concentration of 100 ppm Boron slightly reduced the stem diameter to 5.7 mm. Moreover, the application of NPK with 2g/L NPK resulted in a diameter of 6.2425 mm, while concentration of 4g/L NPK slightly decreased the diameter to 6.15 mm. Furthermore, the combined application of 50 ppm Boron with 2g/L NPK resulted in a maximum stem diameter of 6.5575 mm, while combining 50 ppm Boron with 4g/L NPK resulted in a slightly reduced diameter of 6.3375 mm. However, increasing the Boron concentration to 100 ppm in combination with 2g/L and 4g/L NPK led to a further reduction in stem diameter to 5.6825 mm and 5.585 mm, respectively.

Effect on Leaf Area (cm²)

The leaf area of *Zinnia elegans* plants showed the smallest leaf area of 22.3875 cm² in control group plants (Table 1). A slight increase in leaf area was observed with the application of distilled water, resulting in a leaf area of 24.075 cm². The treatment with 50 ppm Boron significantly enhanced the leaf area to 35.925 cm², while a higher concentration of 100 ppm Boron slightly reduced the leaf area to 34.15 cm². The NPK foliar spray with 2g/L concentration of NPK increased the leaf area to 46.725 cm², followed by 4g/L NPK, which resulted in a leaf area of 45.74 cm². Moreover, the combination of 50 ppm Boron combined with 2g/L NPK producing the maximum leaf area of 53.35 cm². However, the combination of 50 ppm Boron with 4g/L NPK slightly reduced the leaf area to 49.885 cm². When the Boron concentration was increased to 100 ppm, the combination with 2g/L and 4g/L NPK led to a further reduction of 42.425 cm² and 39.725 cm², respectively in leaf area.

Effect on Flower fresh weight (g)

The control plants recorded the lowest flower fresh weight of 2.525 g, while a slight increase of 2.725 g was observed with the application of distilled water. The treatment with 50 ppm Boron increased the flower fresh weight to 6.375 g (Table 1). However, increasing the Boron concentration to 100 ppm reduced the flower fresh weight to 5.625 g. The highest weight of 6.675 g was recorded with 2g/L NPK, while 4g/L NPK resulted in a slightly lower fresh weight of 6.425 g. Moreover, the maximum flower weight of 7.525g observed in 2g/L NPK and 50 ppm Boron combination. While concentration of 50 ppm Boron with 4g/L NPK resulted in a slightly lower fresh weight of 7.2 g. However, increasing Boron to 100 ppm in combination with both 2g/L and 4g/L NPK led to a notable reduction in flower fresh weight of 5.525 g and 5.3825 g, respectively.

Effect on Flower Dry weight (g)

The control plants exhibited the lowest flower dry weight at 0.595 g, whereas application of distilled water slightly increased the flower dry weight to 0.735 g (Table 1). The addition of 50 ppm Boron significantly enhanced the dry weight to 1.4825 g. However, increasing the Boron concentration to 100 ppm reduced the flower dry weight to 1.46 g. The concentration of NPK further enhanced the flower dry weight, with the highest dry weight of 1.525 g detected in plant treated with 2g/L NPK. Concentration of 4g/L NPK resulted in a slight reduction of 1.5075 g in weight. Moreover, the combination of 50 ppm Boron and 2g/L NPK produces the maximum dry weight of 1.5675 g. While the combination of 50 ppm Boron with 4g/L NPK also led to a high dry weight of 1.5475 g. alternatively, the high concentration of boron 100 ppm, with both 2g/L and 4g/L NPK combinations resulted in reduced dry weights of 1.4525 g and 1.4375 g, respectively.

Effect on superoxide dismutase mg g^{-1}

Lowest SOD activity observed in control plant, while distilled water treatment resulted in a slight increase activity (Table 2). Foliar application of 50 ppm Boron led to a notable increase in SOD activity, indicating its role in enhancing plant defense mechanisms. A slight reduction in SOD activity was observed with 100 ppm Boron compared to 50 ppm, suggesting that higher Boron concentrations may not further improve SOD activity. Results showed that NPK application exhibited a increase in SOD activity, with 2g/L NPK whereas 4g/L NPK presented a little response. The both 50 ppm Boron with 2g/L NPK led to the highest SOD activity. However, at Boron levels (100 ppm) combined with NPK, the SOD activity remained minimum.

Effect on peroxidase mg g^{-1} FWT

The Boron and NPK application significantly improved the peroxidase (POD) activity, while control treatment exhibited the minimum POD activity (Table 2). Foliar application of 50 ppm Boron showed an improvement in POD activity, indicating its role in boosting antioxidant enzymes, but 100 ppm Boron showed a slight decrease in POD activity. Similarly, foliar application of 2g/L NPK indicated a significant improved POD activity, with 4g/L NPK recorded a lowest impact as compared to 2g/L. In combination of 50 ppm Boron and 2g/L NPK presented the highest POD activity.

Effect on proline content

The interaction between boron and NPK treatments had a significant effect on proline accumulation in *Zinnia elegans* compared with the control, which showed the lowest proline content (12.34 μmol g^{-1} fwt) (Table 2). Foliar application of boron significantly enhanced proline levels, with 50 ppm boron increasing proline content to 17.33 μmol g^{-1} fwt and 100 ppm resulting in a slightly higher value of 17.69 μmol g^{-1} fwt. Similarly, NPK application promoted proline accumulation, with 2 g/L producing the highest increase (20.09 μmol g^{-1} fwt), while 4 g/L resulted in a moderate rise (18.52 μmol g^{-1} fwt). The combined application of 50 ppm boron and 2 g/L NPK produced the highest proline content (21.19 μmol g^{-1} fwt). A notable increase was also observed with 50 ppm boron plus 4 g/L NPK (20.57 μmol g^{-1} fwt). In

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contrast, combinations involving the higher boron concentration (100 ppm) with NPK showed comparatively lower proline accumulation.

Table 2: Effects of various concentrations of NPK and Boron on Super oxide dismutase (SOD) (mg/g), peroxidase (POD) (mg/g) and Proline (μmol g^{-1}) of Zinnia elegans plants. Data represent Means ± SD, followed by different letters indicate significant differences as per Tukey's LSD test (P ≤ 0.05)

Effect on Chlorophyll A Contents

Treatment	Super Oxide Dismutase	Peroxidase	Proline
T0	27±0.01e	5.63±0.2e	12.34±0.01e
T1	27±0.03e	5.94±0.03e	12.61±0.01e
T2	28±0.03cd	7.60±0.01d	17.32±0.052bc
T3	29±0.04d	7.28±0.01d	17.68±0.02b
T4	30±0.03bc	8.61±0.03bv	20.08±0.04a
T5	31±0.05c	7.85±0.06cd	18.52±0.05b
T6	35±0.04a	10.65±0.05a	21.18±0.07a
T7	33±0.03b	9.19±0.04b	20.56±0.09a
T8	29±0.02d	7.54±0.03d	16.50±0.02cd
T9	29±0.01d	7.31±0.07d	15.65±0.02d

The control treatment showed a minimum chlorophyll A content (1.0115 mg/g FW), the 50 ppm boron led to a considerable improvement in chlorophyll A content (1.3405 mg/g FW), and there was little bit decline at 100 ppm boron level (1.33175 mg/g FW). NPK application i.e., 2g/L NPK enhanced the chlorophyll A content to 1.46475 mg/g FW and 4g/L NPK increased it to 1.4915 mg/g FW showed the role of NPK in chlorophyll synthesis (Figure 1). Both of 50 ppm boron with 2g/L NPK together presented the highest chlorophyll A content (1.60125 mg/g FW), while the 50-ppm boron and 4g/L NPK combination significantly enhanced the chlorophyll content (1.56425 mg/g FW).

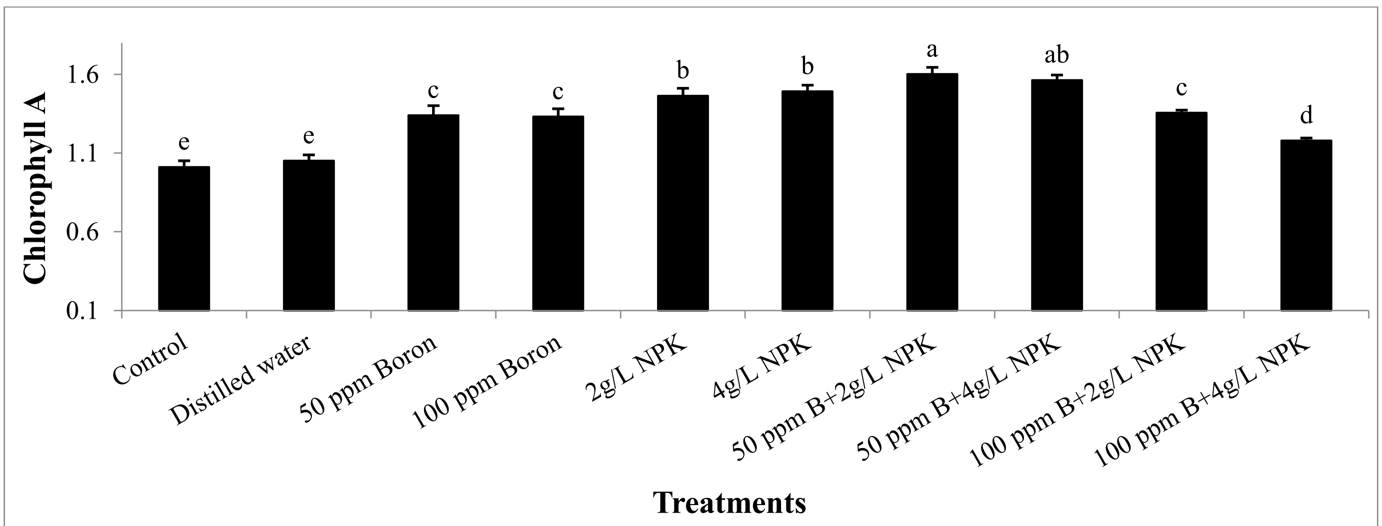

Figure 1. Effects of various concentrations of NPK and Boron on chlorophyll A contents of *Zinnia elegans* plants. Data represent Means ± SE, followed by different letters on the top of bars indicate significant differences as per Tukey's LSD test (P ≤ 0.05)

Effect on Chlorophyll B Contents

The control treatment showed the minimum chlorophyll B content, indicating lowest chlorophyll synthesis in the absence of nutrient supplementation (Figure 2). Boron application 50 ppm significantly increased the chlorophyll B content, whereas a little decline was noted at the higher level of 100 ppm. Similarly, NPK treatments improved chlorophyll B levels, with 2 g/L increasing the content and 4 g/L further raising it, highlighting the important role of NPK in chlorophyll formation. The combined application of 50 ppm boron and 2 g/L NPK resulted in the highest chlorophyll B content.

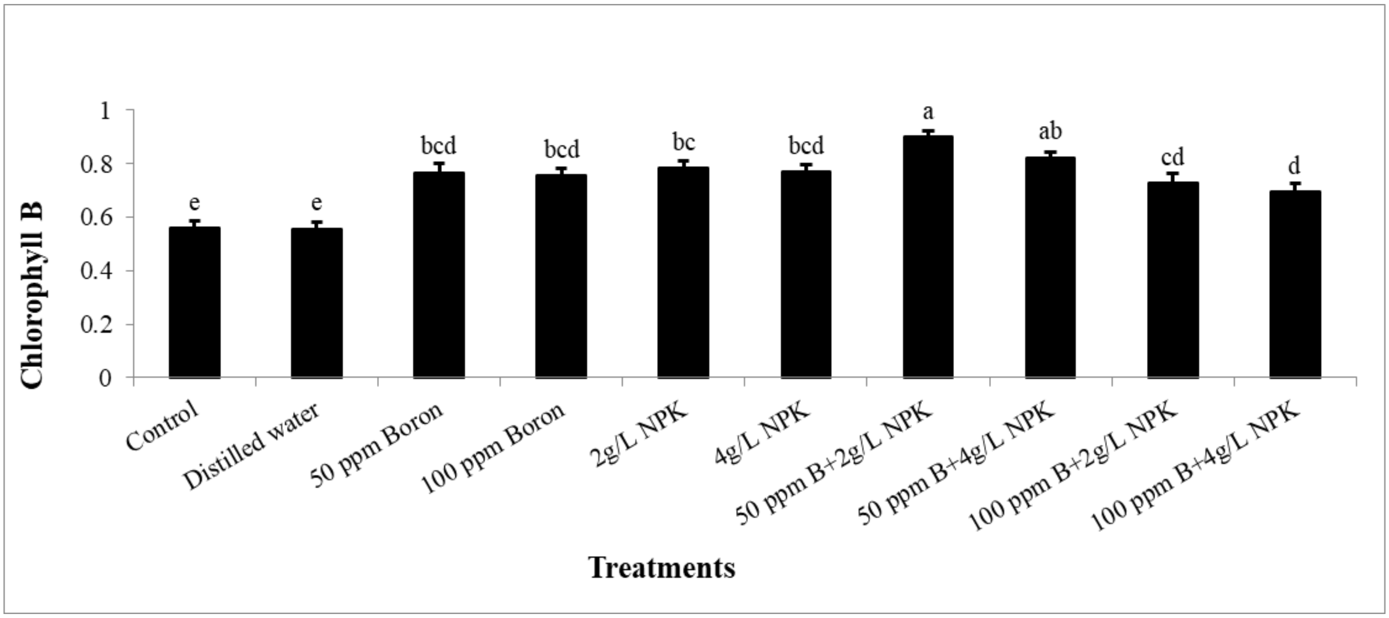

Figure 2. Effects of various concentrations of NPK and Boron on chlorophyll B contents of *Zinnia elegans* plants. Data represent Means ± SE, followed by different letters on the top of bars indicate significant differences as per Tukey's LSD test (P ≤ 0.05).

Effect Total Chlorophyll contents

Control plants depicted the lowest total chlorophyll content (3.1445 mg/g FW), while plants treated with distilled water demonstrated a slight increase (3.216 mg/g FW) (Figure 3). However, the foliar application of boron had a pronounced effect, with 50 ppm boron increasing total chlorophyll content to 4.2145 mg/g FW, while 100 ppm boron produced a similar value of 4.1735 mg/g FW. The 2g/L NPK treatment indicated the value of 4.4945 mg/g FW, and the 4g/L NPK treatment slightly improved chlorophyll content to 4.523 mg/g FW. While the combination of 50 ppm boron with 2g/L NPK produced the highest total chlorophyll content at 5.002 mg/g FW. The combination of 50 ppm boron with 4g/L NPK also resulted in a high chlorophyll level (4.776 mg/g FW). However, when 100 ppm boron was combined with NPK, there was a decline in total chlorophyll content to 4.1705 mg/g FW for 2g/L NPK and 3.751 mg/g FW for 4g/L NPK.

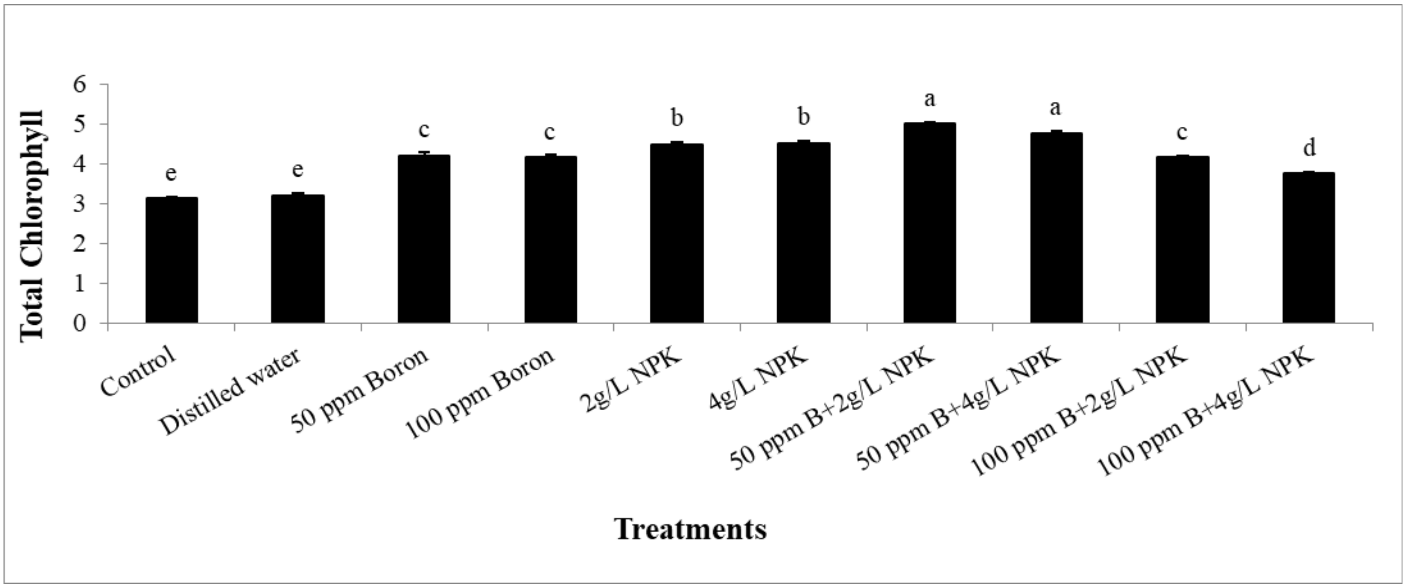

Figure 3. Effects of various concentrations of NPK and Boron on Total chlorophyll contents of Zinnia elegans plants. Data represent Means ± SE, followed by different letters on the top of bars indicate significant differences as per Tukey's LSD test (P ≤ 0.05)

DISCUSSION

This study was conducted to find out how NPK and boron have impact on vegetative and reproductive growth of *Zinnia elegans*. Stusy was comprised with two levels of boron i.e., (50 and 100 ppm) and two levels NPK (2.0 and 4.0 g/L) were foliarly applied and evaluate their impact on morphological, physiological, and biochemical attributes of *Zinnia elegans* cv. Giant Dahlia. The results clearly showed that proper nutrient management plays a vital role in improving plant growth and floral quality, which are essential for ornamental crops. Boron and NPK significantly influenced the vegetative and reproductive growth. Plants sprayed at 50 ppm boron level (T2) presented a significant improvement in fresh and dry biomass and root, shoot length as compared to control plants. Though, increasing the boron level to 100 ppm (T3) led to a slight decline in growth, particularly in root and shoot development, suggesting that higher levels may have inhibitory effects. This reduction may be associated with boron toxicity, which can interfere with cell division and elongation (Ali, 2018). Likewise, NPK application alone promoted plant growth, with 2 g/L (T4) producing considerable increases in biomass and plant height. The significant improvement in growth was noted under the combined application of 50 ppm boron and 2 g/L NPK (T6). In contrast, treatments involving 100 ppm boron combined with NPK (T8 and T9) exhibited a less growth, indicating that excessive nutrient supply may disturb optimal physiological functioning as reported by Khalid et al. (2024). Zinnia plants sprayed with 50 ppm boron and 2 g/L NPK recorded the highest fresh and dry weights, reflecting increased biomass production. Moderate boron levels likely improved photosynthetic activity and nutrient absorption, while higher concentrations may have limited growth by disrupting enzymatic processes and nutrient transport (M. Singh et al., 2019). Time to start flowering was also influenced by nutrient treatments, 50 ppm boron alone (T2) and in combination with NPK (T6) flowered earlier than the control. Boron is known to play an important role in cell wall formation and pollen tube growth, which supports reproductive development under optimal conditions (Mones Sardrodi et al., 2022).

In contrast, boron levels (100 ppm) showed delayed flowering, possibly due to toxic effects on reproductive tissues, similar results have been reported in sunflower, where excessive boron interfered with floral development and delayed blooming (Rerkasem et al., 2020). Flower size, and largest flower diameter was noted where 50 ppm boron and 2 g/L NPK was applied. Increasing the boron level 100 ppm resulted in smaller flowers, it is indicated that excess boron can negatively affect floral growth. This output is consistent with previous findings on ornamental plants, which have shown that balanced nutrient supply promotes flower development, whereas over-application leads to physiological stress and reduced flower quality. Physiological responses were also markedly influenced by boron and NPK application, chlorophyll content increased significantly with nutrient application, with the highest contents was recorded at 50 ppm boron and 2 g/L NPK (T6). These results suggest that boron supports chlorophyll formation, while NPK, particularly nitrogen, contributes to chloroplast development as per earlier findings (Khalid et al., 2024).

On the other hand, higher boron levels (100 ppm) decreased chlorophyll content, representing a possible interference with chlorophyll biosynthesis pathways (Mones Sardrodi et al., 2022). Antioxidant enzyme activities, especially superoxide dismutase (SOD) and peroxidase (POD), were also improved by moderate boron and NPK application. The maximum enzyme activities were noted under the 50 ppm boron and 2 g/L NPK treatment. These enzymes are essential components of the plant defense system and help neutralize reactive oxygen species produced under stress conditions (Zulfiqar et al., 2024). The increase in SOD and POD activity indicates improved stress tolerance in sprayed plants. Similar responses have been reported in *Brassica* species, where moderate boron levels stimulated antioxidant activity, while excessive level reduced enzyme efficiency (Masum et al., 2019). This suggests that balanced nutrient application strengthens plant defense mechanisms, whereas excessive levels may trigger oxidative damage. Proline production was maximum in plants where 50 ppm boron and 2 g/L NPK was sprayed. Proline is a stress-related amino acid that plays a protective role under unfavorable environments such as nutrient imbalance, salinity, and drought (Shafi et al., 2019). The increased proline content observed under moderate nutrient treatments indicates enhanced stress adaptation and metabolic stability in *Zinnia*.

CONCLUSIONS

The final conclusion is that 50 ppm boron and 2.0 g/L NPK foliar application showed maximum results in term of morphological, physiological and biochemical responses and these levels were suggested for better zinnia crop production under sub-tropical environment.

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AUTHOR CONTRIBUTIONS

All the authors contributed equally to this research.

COMPETING OF INTEREST

No conflicts of interest have been disclosed by the authors.

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