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Original Research

Foliar Application of Micronutrients Enhances Growth, Flowering, Minerals Absorption and Postharvest Life of Tuberose (*Polianthes tuberosa* L.) in Calcareous Soil

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ABSTRACT

Micronutrients applications are effective for better crop production in calcareous soils because these soils are usually deficit in iron (Fe), zinc (Zn) and boron (B). In Pakistan, most of soils are calcareous in nature. When tuberose is grown in such soils as a cut flower, its production is negatively affected. Therefore, a study was aimed to evaluate the effects of micronutrients as foliar sprays on cut tuberose production in calcareous soil. Micronutrients (Fe, Zn and B) alone and in combinations were sprayed on the plants after 60, 90 and 120 days of planting. The mixture of all these three micronutrients increased the plant height (95.77 cm), chlorophyll content (38.13 SPAD), number of leaves (79.63), leaf length (44.73 cm), fresh (111.64 g) and dry (16.16 g) plant weights, root length (15.13 cm), number of stalks (3.73), stalk length (79.03 cm), spike length (22 cm), number of florets (51.67), floret fresh weight (11.85 g), leaf Zn concentration (53.6 mg/g) and vase life (8.4 days). Fe + Zn enhanced the number of leaves (76.60), leaf length (45.83 cm), root length (15.05 cm), spike length (22.33 cm), and leaf Fe (128.18 mg/g) and Zn concentrations (55.02 mg/g). The Fe spray increased the leaf length (44.10 cm), days to flower initiation (142.47 days) and leaf Fe concentration (130.75 mg/g) in tuberose plants. Application of Zn improved the leaf length (45.87 cm) and diameter (1.32 cm), root (15.03 cm), spike (21.77 cm) and floret lengths (4.74 cm), floret dry weight (1.49 g) and leaf Zn concentration (57.5 mg/g). Foliar spray of B increased the B concentration (21.1 mg/g) in tuberose leaves. It is concluded that foliar application of micronutrients alone and in combinations improved the plant growth, flowering, leaf minerals concentrations and vase life of tuberose spikes. However, mixture of Fe, Zn and B was more effective as compared to other treatments. Moreover, current study encourages the foliar application of micronutrients in tuberose when grown in calcareous soils.

Keywords: *Leaf mineral content, nutrition management, spikes length, vase life.*

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INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is a famous bulbous perennial grown throughout the world as bedding plant and cut flower. The temperature requirement for optimum plant growth is 30 °C and plant does not require any shade and support (Muriithi et al., 2011). Sandy loam and loamy soils with pH ranging from 6.5 to 7.5 are needed for its optimal growth. It is grown commercially in USA, China, Taiwan, New Zealand, Netherlands, Rwanda, Japan, South Africa, Mexico, France, Egypt, Italy, and India (Barba-Gonzalez et al., 2012). It is used as cut flower and when production is in excess then essential oil can be extracted for export (Muriithi et al., 2011). In Pakistan, it is mostly grown around the big cities. Peri-urban areas of Multan, Lahore, Pattoki, Sheikhupura, Faisalabad, Quetta, Islamabad, Rawalpindi, Hyderabad, and Karachi are famous sites for tuberose cultivation in Pakistan (Usman and Ashfaq, 2013; Shahzad et al., 2021). Tuberose flower yield and spike quality are still low in Pakistan because of poor soils and inappropriate fertilization of micronutrients.

Most of the soils in developing countries are deficit in Zn and B (Sharma and Khare, 2014). Flowering crops are much responsive to micronutrients (Jatav et al., 2020), therefore their application is necessary for proper plant growth and flowering when roots are not going to uptake these nutrients. The appropriate amounts of suitable micronutrients, their time and method of application are vital for enhanced growth and quality flowering of tuberose plants. Foliar spray by direct application on aerial plant parts is effective method than root fertilization to rectify deficiency symptoms, maintain nutritional status and improve growth and produce quality in marginal soils (Otálora et al., 2018). The trend of foliar application is increasing to overcome the nutritional deficiency more quickly than root application (Niu et al., 2020). Soil pH may affect the availability

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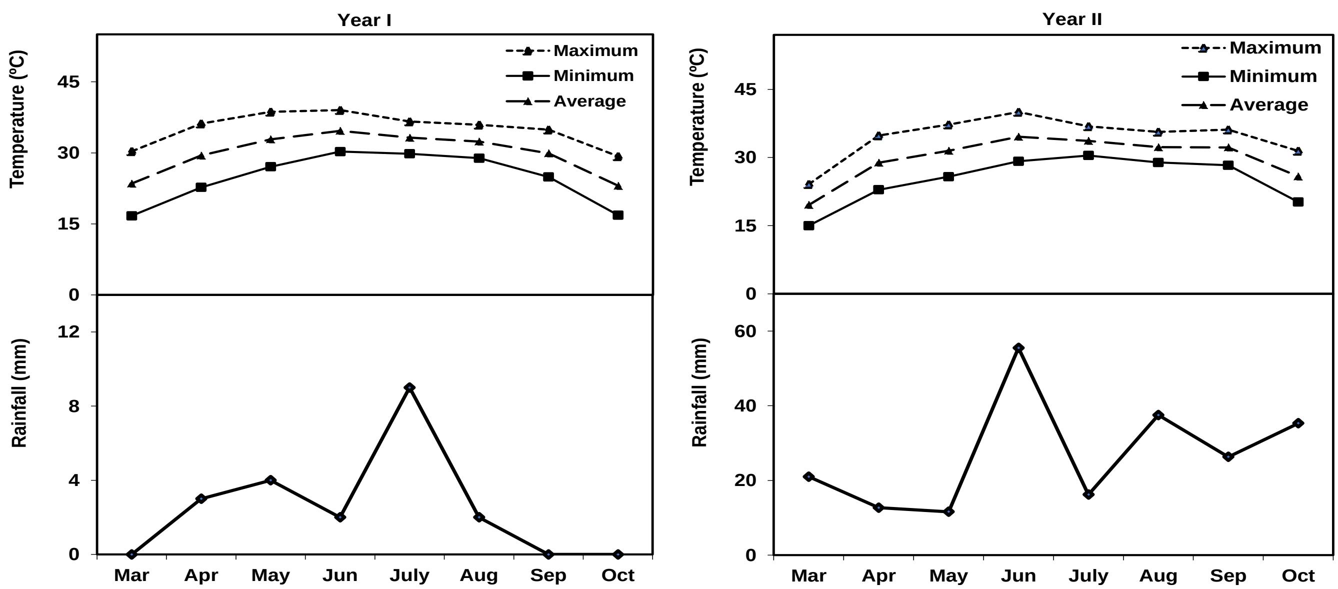

Figure 1: Monthly temperature and rainfall data collected from sowing to harvesting during 2018 and 2019.

and uptake of micronutrients. Higher soil pH increases the deficiency of micronutrients in crop plants and causes different physiological problems, *i.e.,* chlorosis, scorching and rosetting in plants which limit plant growth and flowering (Singh et al., 2012). In calcareous soils, application of micronutrients, viz. B, Zn, Fe, Cu and Mn is more effective through spray on leaves as compared to root application (Kaya et al., 2005) because liquid fertilizers spray rapidly recovers the deficiency of these micronutrients. Moreover, nutrients penetrate leaf cuticle and then transported into plant parts by plasmodesmata (Kannan, 2010) as in strawberry (Pestana et al., 2012).

Micronutrients usually improve chlorophyll pigments, synthesis of protein and nucleic acid, which further improves growth and flowering in floral crops *i.e.,* gladiolus (Sharma and Khare, 2014), chrysanthemum (Vanlalruati et al., 2019) and marigold (Yadegari, 2013). Fe is necessary for protein, chlorophyll and thylakoid synthesis, respiratory enzymes, energy transportation and cofactor to activate the enzymes (Marschner, 1995; George and Manuel, 2013). Fe deficiency reduces photosynthetic pigments and leaves show yellow colour. However, young leaves and new flushes are firstly affected than older leaves (Havlin, 2014). Zn is important for proper functioning of photosynthetic processes, nitrogen metabolism and uptake, carbon anhydrase activity, metabolism and synthesis of carbohydrate, tryptophan synthesis, protein quality, precursor of IAA and increase tolerance against oxidative stress, and enhances vase life of flowers (Sofy et al., 2020; Toor et al., 2020). Leaf size is reduced, and internodes are shortened due to deficiency of Zn. Moreover, susceptibility to light and temperature, and chances of fungal diseases increase in harvested produce (Cakmak, 2000). B is required for photosynthesis, enzymes activation, carbohydrate chemistry, cell wall strengthening, hormones production, respiration, stomatal regulation, IAA and RNA metabolism, sugars translocation, cell division and membranes integrity in plants (Yermiyahu et al., 2008). So, foliar application of Fe, Zn and B results in increased growth and yield of floral crops.

Nutritional deficiency is major problem for farmers having calcareous soils. In Pakistan, most of the soils are calcareous in nature, especially in arid and semi-areas of South Punjab. These soils have higher pH level range of 7.5 to 8.5, rich in calcium carbonate and low in organic matter (Anjum et al., 2019). Higher pH and calcium carbonate depress the availability and absorption of different nutrients, viz. P, K, Zn, Fe and B. Therefore, foliar application is effective strategy to improve the nutritional status of plants grown in such soils. Huge work has been conducted on application of macronutrients to tuberose plants; however, application of micronutrients still is neglected. A balanced fertilization is necessary for excellent growth and good quality spikes of tuberose. Hence, current work was conducted to evaluate the effects of foliar applications of micronutrients, *i.e.,* Fe, Zn and B alone and in various combinations on growth, flowering, leaf minerals and vase life of tuberose plants grown in calcareous soil.

MATERIALS AND METHODS

Experimental site

Current experiment was performed at the Horticultural Research Area, Bahauddin Zakariya University, Multan, Pakistan during 2018 and 2019. Monthly temperature and rainfall data collected from sowing to harvesting of tuberose crop during 2018 and 2019 is listed in Fig. 1.

Soil analysis

For soil analysis of experimental area, soil samples were taken from different field locations to a depth of 0-25 cm during both years of study. These samples were air dried to determine the soil physico-chemical properties as listed in Table 1.

Treatment applications

The detailed description of different micronutrients sprayed on

Table 1: Soil analysis before planting of tuberose.

Characteristics	2018	2019
Texture	Loam	Loam
pH	8.1	8.2
EC (mS/cm)	1.79	1.91
Saturation (%)	30	31
Organic matter (%)	0.69	0.77
Available phosphorous (mg/kg)	7.90	8.10
Available potassium (mg/kg)	220	231
Calcium carbonate (%)	8.7	8.9

tuberose plants are presented in Table 2. Experimental field was prepared with thorough ploughing followed by planking to obtain better soil tilth. Tuberose cv. Pearl Double was propagated through bulbs, obtained from a nursery (Sadabahar Nursery) at Pattoki, Kasur, Pakistan. The bulbs of 3-5 cm diameter were planted on ridges at 30 cm apart with 20 cm interplant distance on March 10, 2018. Sixty bulbs were planted in each treatment. First irrigation was applied immediately after planting and then at weekly intervals. The bulbs' sprouting was observed after 20-25 days of planting. During the second year (2019), the bulbs were planted on the same date (March 10). All the agronomic practices followed were same during both years.

Experimental Layout

Micronutrients (Fe, Zn and B) alone and in combinations were sprayed on the plants after 60, 90 and 120 days of planting. Per plant, 20 mL of solution was sprayed. The experiment was arranged in a randomized complete block design (RCBD) with three replications. Ten plants were randomly selected in each treatment from each replication for data collection.

Growth traits

Chlorophyll content was recorded by SPAD meter (MINOLTA-502). Plant height, leaf and root lengths were measured through a measuring scale. Leaf diameter was estimated through a Vernier calliper (IKKEGOL). Number of leaves was counted from each randomly selected plant. Fresh and dry weights of plant were recorded by using a digital weighing balance (HB-600). Fresh samples were dried in hotbox oven at 70 °C till constant dry weight.

Flowering traits

Flower- emergence, number of stalks and number of florets per spike were counted. Lengths of stalk, spike and floret were measured by using a measuring scale. Fresh and dry weights of stalk and floret were estimated through a digital weighing balance (HB-600). Vase life of flowers was recorded in days till the wilting of 50% florets as described by Shahzad et al. (2021). Half opened flowers were harvested and put in distilled water for estimation of vase life at room temperature.

Table 2: The detail of micronutrients treatments.

Treatments	Foliar applied chemicals
T_0	Control (Distilled water)
T_1	$FeSO_4.7H_2O$ (2%)
T_2	$ZnSO_4.7H_2O$ (2%)
T_3	H_3BO_3 (2%)
T_4	$FeSO_4.7H_2O$ (2%) + $ZnSO_4.7H_2O$ (2%)
T_5	$FeSO_4.7H_2O$ (2%) + H_3BO_3 (2%)
T_6	$ZnSO_4.7H_2O$ (2%) + H3BO_3 (2%)
T_7	$FeSO_4.7H_2O$ (2%) + $ZnSO_4.7H_2O$ (2%) + H_3BO_3 (2%)

Micronutrient estimation in leaves

Micronutrients *i.e.,* Fe and Zn in tuberose leaves were measured by a digestion procedure described by Rashid (1986). Dry-ashing method was adopted for determination of B in leaves as described by Chapman and Pratt (1961) and Bingham (1982).

Statistical analysis

A software, Statistix 8.1 (Tallahassee Florida, USA), was employed for evaluation of collected data using analysis of variance (ANOVA) technique. Two year's data were pooled, and averages were computed. Treatment means were separated through least significant difference (LSD) test at 5% probability level (Steel et al., 1997).

RESULTS

Growth traits

Tuberose plants had greatest plant height under application of Fe + Zn + B (95.77 cm), while lowest plant height was recorded in plants where micronutrients were not sprayed (control) (60 cm) (Table 3). Chlorophyll content were found to be highest when plants were supplied with foliar application of Fe + Zn + B (38.13 SPAD), followed by Zn, while lowest chlorophyll content was recorded in plants without any foliar spray (23.79 SPAD) and Fe only (26.27 SPAD) (Table 3). The maximum number of leaves per plant was counted under application of Fe + Zn + B (79.63) and Fe + Zn (76.6) than control (45.17) (Table 3). Larger leaf length was measured in Fe + Zn + B, Fe + Zn (44.73 cm), Zn (45.83 cm) and Fe (44.1 cm), followed by Fe + B (43.13 cm), while smaller leaf length was measured in control (35.27 cm), followed by B (37.53 cm) (Table 3). Leaf diameter was found to be larger under spray of Zn (1.32 cm), Fe + B (1.32 cm), followed by Fe + Zn + B (1.27 cm), Zn + B (1.21 cm) and Fe + Zn (1.19 cm) (Table 3). The maximum fresh weight per plant was recorded in Fe + Zn + B (111.64 g), while the minimum fresh weight per plant was obtained in control (43.77 g) (Table 3). Dry weight per plant was found to be greater in Fe + Zn + B (16.16 g), followed by Fe + Zn (14. 87 g) and Zn (14.38 g), while lower dry weight per plant was recorded in control (7.71 g) (Table 3). Root length was longer in Fe + Zn + B (15.13 cm), Fe + Zn (15.05 cm) and Zn (15.03 cm), followed by Fe + B (13.68 cm), Zn + B (14.03 cm) and Fe (14.05 cm), while shorter root length was measured in control (11.67 cm) and B (12.11 cm) (Table 3).

Flowering traits

Fe application delayed flowering in tuberose with the maximum days to flower emergence (142.47 days), followed by control (140.87 days) and Fe + B application (141.07 days), while the minimum days to flower emergence was observed in Zn + B (135.70 days), B (135.03 days) and Fe + Zn (136.70 days), followed by Zn (138.47 days) and Fe + Zn + B (137.17 days)

Table 3: Effect of foliar application of micronutrients on growth traits of tuberose plants.

Treatment	Plant height (cm)	Chlorophyll content (SPAD)	Number of leaves	Leaf length (cm)	Leaf diameter (cm)	Fresh weight/ plant (g)	Dry weight/ plant (g)	Root length (cm)
Control	60.00f	23.79e	45.17d	35.27d	1.02cd	43.77g	7.71f	11.67b
Fe	78.77d	26.27e	52.83c	44.10a	1.14bc	73.32e	13.34b-d	14.05ab
Zn	89.33b	35.84ab	60.97b	45.87a	1.32a	86. 19c	14.38a-c	15.03a
B	65.00e	31.41cd	44.53d	37.53cd	0.99d	58.66f	10.89e	12.11b
Fe + Zn	88.70b	34.23bc	76.60a	45.83a	1.19ab	99.81b	14.87ab	15.05a
Fe + B	79.87d	30.60d	53.20c	43.13ab	1.32a	79.81d	12.29cde	13.68ab
Zn + B	83.63c	33.80b-d	55.20bc	40.57bc	1.21ab	86.07c	11.48de	14.03ab
Fe + Zn + B	95.77a	38.13a	79.63a	44.73a	1.27ab	111.64a	16.16a	15.13a

Means with different letter(s) in a column are statistically significant at p = 0.05 (LSD) test.

(Table 4). Number of stalks was greater in Fe + Zn + B (3.73), followed by Fe + Zn (3.33), while least number of stalks was obtained in control (1.87) (Table 4). Stalk length was greater in Fe + Zn + B (79.03 cm), while shorter stalk length was obtained in B (36.17 cm) and control (38.50 cm) (Table 4). Spike length was larger in Fe + Zn (22.33 cm), Zn (21.77 cm) and Fe + Zn + B (22 cm), while smaller spike length was found in B (11.60 cm) and control (12.67 cm) (Table 4). Greater floret length was observed in Zn (4.74 cm), followed by Fe + Zn + B (4.33 cm), Fe (3.70 cm), Fe + Zn (4 cm), Fe + B (3.75 cm) and Zn + B (3.71 cm), while smaller floret length was measured in control (2.45 cm), followed by B (3.52 cm) (Table 4). The maximum number of florets was counted in Fe + Zn + B (21.57), followed by Fe + Zn (19.93), while the minimum number of florets was counted in control (8.20) (Table 4). Fresh weight of florets per spike was found to be greater in Fe + Zn + B (11.85 g), while lesser fresh weight of florets per spike was observed in control (3.25 g), followed by B (4.43 g) (Table 4). The maximum dry weight of florets per spike was obtained in Zn (1.49 g), while the minimum dry weight of florets per spike was fund in control plants (0.34 g) of tuberose (Table 4).

Leaf Fe, Zn and B contents

The presence of Fe content in tuberose leaves was found to be higher in Fe + Zn (128.18 mg/g) and Fe (130.75 mg/g) treatments, while lower Fe content was present in plants sprayed with B (11.71 mg/g) and Zn + B (11.97 mg/g) (Fig. 2A). Zn content in leaves was greater in Zn (57.5 mg/g), Fe + Zn (55.02 mg/g) and Fe + Zn + B (53.6 mg/g) sprayed plants, while lesser Zn content was estimated in control (8.79 mg/g) (Fig. 2B). The maximum B content was found in leaves of B treated plants (21.1 mg/g), while the minimum B content were present in leaves of control (6.53 mg/g), Fe + Zn (7.07 mg/g), Zn (6.53 mg/g) and Fe (7.27 mg/g) sprayed plants (Fig. 2C).

Vase life of spikes

Under application of different micronutrients, vase life of spikes was found to higher in Fe + Zn + B (8.4 days), while the lower vase life of spike was recorded in control (5.4 days) and B (5.7 days) treated plants of tuberose as compared to other micronutrients treatments (Fig. 3).

DISCUSSION

Calcareous soils are deficit in Fe, Zn and B, which result in poor quality of spikes in tuberose plants. Therefore, spray of Fe, Zn and B on leaves is very effective and rapid way of nutrients absorption in plants to protect from nutritional deficit conditions (Niu et al., 2020). In the present results, the enhanced chlorophyll content was due to foliar application of Fe + Zn + B in tuberose plants which indicated the improved level of chlorophyll content in leaves by fulfilling the plant nutrition. The increase of leaf chlorophyll content was possibly due to increased availability of nutrients in appropriate quantity and at right time (Balakrishnan et al., 2007; Rabnawaz et al., 2020). In the current study, the enhanced growth and biomass of tuberose plants grown in calcareous soils were observed under application of mixture of Fe + Zn + B. Recently, similar increase in growth of a flowering crop "Ascocenda orchid" was observed under foliar application of micronutrients (Kumar and Singh, 2020). Fe sprays on leaves regulate the development of chloroplast, formation of chlorophyll, photosynthesis, and respiration activities of plants (Miller et al., 1995). Moreover, physiological, and biochemical mechanism of plants were also

Table 4: Effect of foliar application of micronutrients on flowering of tuberose plants.

Treatment	Flower emergence (days)	Number of stalks	Stalk length (cm)	Spike length (cm)	Number of florets	Floret length (cm)	Floret fresh weight/ spike (g)	Floret dry weight/ spike (g)
Control	140.87ab	1.87d	38.50e	12.67c	8.20e	2.45c	3.25e	0.34h
Fe	142.47a	2.93b	59.23c	18.27b	15.70c	3.70ab	6.95bc	0.83f
Zn	138.47bc	3.00b	69.33b	21.77a	17.60bc	4.74a	8.16bc	1.49a
B	135.03c	2.40c	36.17e	11.60c	11.67d	3.52bc	4.43de	0.81g
Fe + Zn	136.70c	3.33ab	70.73b	22.33a	19.93ab	4.00ab	9.28b	1.33b
Fe + B	141.07ab	2.93b	53.70d	16.90b	17.63bc	3.75ab	5.88cd	0.85e
Zn + B	135.70c	2.33c	58.27cd	16.63b	15.17c	3.71ab	5.91cd	0.90d
Fe + Zn + B	137.17bc	3.73a	79.03a	22.00a	21.57a	4.33ab	11.85a	1.01c

Means with different letter(s) in a column are statistically significant at p = 0.05 (LSD) test.

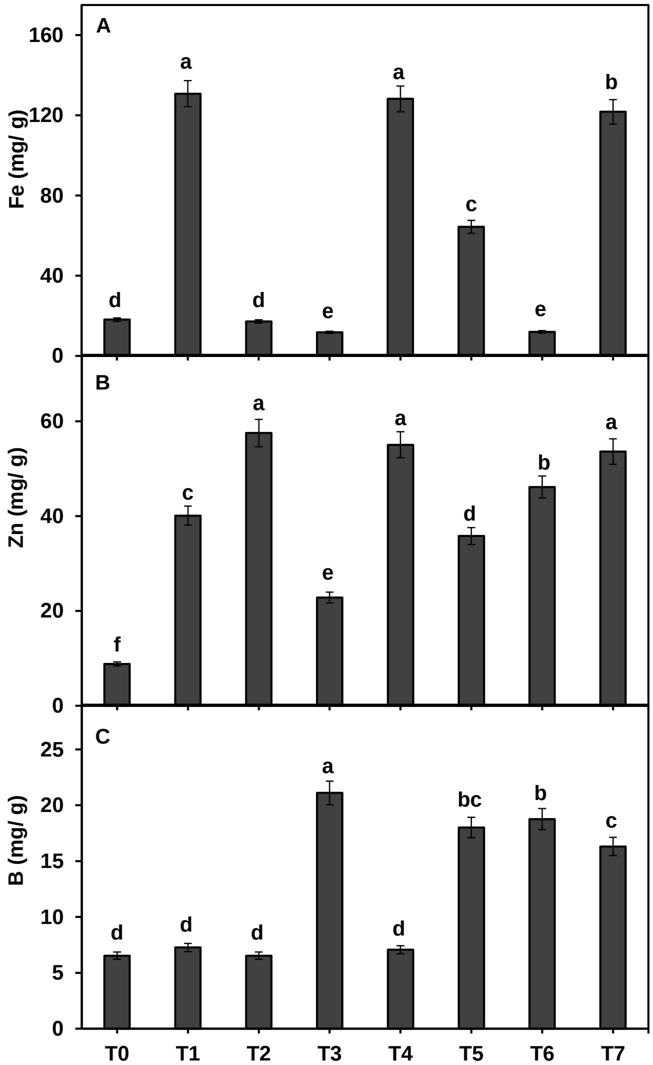

Figure 2: Effect of foliar application of micronutrients on minerals in leaves of tuberose plants. Vertical bars indicate SE of the means and data are mean of three biological replicates. Different letters indicate significant differences among the means according to LSD test $p \leq 0.05$. T_0 = control, T_1 = Fe, T_2 = Zn, T_3 = B, T_4 = Fe + Zn, T_5 = Fe + B, T_6 = Zn + B, T_7 = Fe + Zn + B.

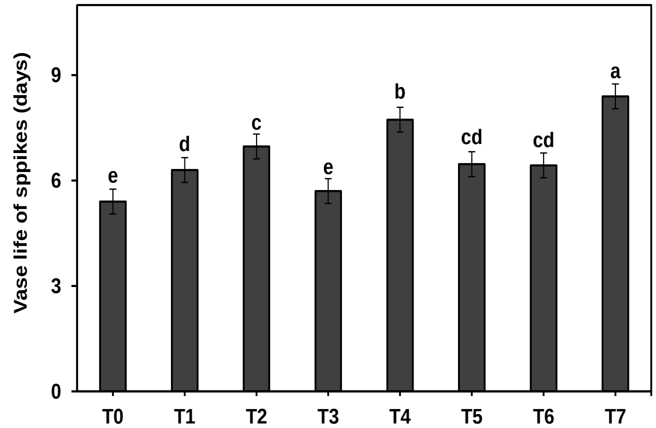

Figure 3: Effect of foliar application of micronutrients on vase life of tuberose flowers. Vertical bars indicate SE of the means and data are mean of three biological replicates. Different letters indicate significant differences among the means according to LSD test $p \leq 0.05$. T_0 = control, T_1 = Fe, T_2 = Zn, T_3 = B, T_4 = Fe + Zn, T_5 = Fe + B, T_6 = Zn + B, T_7 = Fe + Zn + B.

improved by Fe application. Zn acts as a catalyst in activation of enzymes and chlorophyll development (Patil et al., 2010). Foliar application of B, Zn and Fe alone and in mixture increased the plant growth possibly by improving auxin synthesis and chlorophyll content as obtained in present and earlier work (Ahmad et al., 2010). Plant height and biomass were found to be increased in numerous crops *i.e.,* tomato (Bhatt et al., 2004), brinjal (Kiran et al., 2010) and marigold (Yadegari, 2013) by the foliar application of micronutrients.

Application of micronutrients and their optimum concentrations greatly increased flowering traits and improve vase life of spikes (Ahmad et al., 2010). In the present study, enhanced flowering traits of tuberose were observed under foliar application of mixture of Fe, Zn and B. Current results are in line with earlier work because combination of different micronutrients may improve the flowering traits *i.e.,* weight, size, and vase life of floral crops e.g., gladiolus (Sharma and Khare, 2014), chrysanthemum (Vanlalruati et al., 2019) and marigold (Yadegari, 2013). B application improves cell elongation, IAA synthesis, cell maturation, sugar translocation and protein synthesis. All these compounds are necessary for increase of crop production (Kiran et al., 2010). Recently, Genaidy et al. (2020) also observed increase in pollen germination and improved elongation of pollen tube by application of B and its mixture with other micronutrients. Similarly, Fahad et al. (2014) applied mixture of Fe, Zn and B and obtained enhanced weight and size of stalks in gladiolus. Jat et al. (2007) and Chopde et al. (2015) observed the stimulation of RNA metabolism, DNA formation and carbohydrates synthesis which are necessary for increase of flowering traits in floral crops.

In the present study, foliar application of micronutrients like Fe, Zn and B increased their concentrations in tuberose leaves. Fe alone and in combination with Zn are found to be effective sprays for increase of Fe concentration in tuberose leaves. Absorption of minerals increases by application of different macro and micronutrient as confirmed by earlier work of Kolekar and Bhagyaresha (2018). However, enhanced concentration of Fe in tuberose leaves was due to foliar application of Fe. Applications of Zn alone and in combination with Fe and B are found to be effective for increase of Zn absorption in tuberose plants. B application also increased B concentration in tuberose leaves. So, current study is in agreement to earlier work of Ahmad et al. (2010) who found that the application of Zn and B increased the concentrations of Zn and B in leaves of roses. In another study, Khosa et al. (2011) also observed improved B concentration in Gerbera leaves under B fertilization.

Tuberose flower spikes are found to be highly perishable at higher temperature (Kumari et al., 2018). Flower quality is mainly based on vase life of flower spikes (Patel et al., 2017).

After harvesting, flower spikes lose their turgidity and internal carbohydrates deterioration is enhanced, therefore tuberose flowers have poor vase life (Vidhya and Bhattacharjee, 2002). Foliar applications of different chemicals are necessary to reduce the post-harvest losses of tuberose flower spikes. In the present study, pre-harvest application of mixture of Fe, Zn and B resulted in enhanced vase life of tuberose flowers. The current study is in line with earlier work as pre-harvest foliar application of mixture of Fe, Zn and B increased the vase life of gladiolus (Fahad et al., 2014).

CONCLUSION

From the present work, it is concluded that foliar application of mixture of Fe, Zn and B is effective for increased growth and flowering of tuberose plants, vase life of their spikes and leaf mineral composition when grown in calcareous soil. Thus, micronutrients foliar spray is effective for increased production of floral crops with better quality of flowers and longer vase life. In the light of current results, Fe, Zn and B mixture was more effective than other studied treatments.

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