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Effect of essential heavy metals on floral parameters and flower yield of tuberose *cv.* 'Prajwal'

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Abstract
Three essential heavy metals *viz.*, $MnSO_4$, $CuSO_4$, and $ZnSO_4$ at different graded levels were used in the present investigation with the main objective to study their influence on different floral parameters and fresh flower yield of tuberose *cv.* 'Prajwal'. The experiment was carried out in polybag culture method and conducted with a Completely Randomized Design with factorial concept using three replications. Data recorded on different floral parameters and fresh flower yield of tuberose *cv.* 'Prajwal' were analyzed statistically using OPSTAT software and least significant difference was used to differentiate the treatments. Statistical analysis of results indicated that application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded a significant improvement in many of the floral parameters *viz.*, early in spike emergence (66.73 days) and first floret opening (6.33 days), number of spikes produced per plant (1.53 and 1.16 respectively during 2019-20 and the pooled data analysis), number of florets per spike (23.83, 25.20 and 24.51 respectively during 2018-19, 2019-20 and the pooled data analysis), diameter of floret (18.00 mm, 22.63 mm and 20.31 mm respectively during 2018-19, 2019-20 and the pooled data analysis) and fresh weight of flowers per plant (42.10 g, 26.90 g and 34.50 g respectively during 2018-19, 2019-20 and the pooled data analysis) at 360 days after planting (DAP) interval.

Keywords: $CuSO_4$, flower yield, heavy metals, $MnSO_4$, spike emergence and $ZnSO_4$

Introduction
Presence of high concentration of essential and non-essential heavy metals in the soil are considered to affect the plant growth and development adversely and sometimes even lead to death under extreme conditions. Thus heavy metal toxicity has been considered as one of the major abiotic stresses leading to hazardous effects on plants as many of them are toxic even at very low level concentrations. Industrial revolution has accelerated the biosphere with heavy metals. A common response of heavy metal toxicity is the excessive accumulation of reactive oxygen species (ROS) which can cause peroxidation of lipids, oxidation of proteins, inactivation of enzymes, DNA damage and/or interact with other vital constituents of the plant cells (Bohra *et al*., 2015). Certain heavy metals are nutritionally essential for healthy growth of plant in very small quantities such as iron (Fe), copper (Cu), manganese (Mn), Magnesium (Mg) and Zinc (Zn). These metals are required in specific amounts and their deficiency or elevated concentration will result in deleterious effects on plant growth and development thus reduce plant productivity. Out of the several heavy metals of essential and non-essential, three essential heavy metals *viz.,* Mn, Cu and Zn were found required in trace amounts for better growth, development and metabolic activity of plants have been selected in the present investigation to identify their toxic effects on the metabolic activity of plant under heavily accumulated condition in the soil. Keeping all these things in view the present investigation was carried out to find out the influence of essential heavy metals on the floral parameters as well on the floret yield of tuberose *cv.* 'Prajwal'.

Materials and Methods
The present investigation was carried out during the period from *Rabi*-2018 to *Kharif*-2020 at College of Horticulture, Dr. Y. S. R. Horticultural University, Anantharajupeta, Kadapa district of Andhra Pradesh F, India. The experiment was laid out in a completely randomized design (CRD) with factorial concept and replicated thrice. The experiment has consisted of 10 treatments *viz.*, T_1 = RDF+$MnSO_4$ @ 1,000 mg kg^{-1} soil, T_2 = RDF+$MnSO_4$ @ 2,000 mg kg^{-1} soil, T_3 = RDF+$MnSO_4$ @ 3,000 mg kg^{-1} soil, T_4 = RDF+$CuSO_4$ @ 100 mg kg^{-1} soil, T_5 = RDF+$CuSO_4$ @ 200 mg kg^{-1} soil, T_6 = RDF+$CuSO_4$ @ 300 mg kg^{-1} soil, T_7 = RDF+$ZnSO_4$ @

200 mg kg^{-1} soil, T_8 = RDF+ $ZnSO_4$ @ 400 mg kg^{-1} soil, T_9 = RDF+ $ZnSO_4$ @ 600 mg kg^{-1} soil, T_{10} = Control (No RDF and no heavy metals). The main objective of the investigation was to find out the influence of graded levels of essential heavy metals (Mn, Cu, Zn) on the floral parameters and floret yield of tuberose *cv*. 'Prajwal'. All the reproductive parameters *viz.*, number of days to spike emergence, number of days to first floret opening, number of spikes per plant and floret yield per plant were recorded as per the standard procedures established by several research workers earlier. Number of days taken for emergence of flower spike from the date of planting was observed and recorded as mean number of days. Number of days taken for opening of basal floret was recorded as the number of days taken from the date of initiation of flower spike to opening of floret and expressed as mean number of days. Number of flower spikes harvested on five randomly selected plants in each replication was counted for all the pickings in a year and the mean was calculated as number of spikes per plant. Length of flower spike was measured from base to the tip of the spike with a meter scale and was expressed in centimeters. Numbers of florets (both opened and unopened) present on a mature flower spike were counted when 50 per cent of florets have fully opened and expressed as number of florets per spike. Maximum diameter of five florets from five randomly selected plants at fully opened stage was measured using a meter scale and the mean arriving was expressed as flower diameter in centimeters. Weight of five individual florets was recorded from five randomly selected plants at fully opened stage with the help of electronic weighing balance and expressed in grams as weight of single floret. All the opened florets per spike were weighed as and when harvested by using digital electronic weighing balance and the cumulative floret yield per plant was expressed as grams per plant. The data arrived at was analyzed using OPSTAT software and the treatments were differentiated by using the least significant difference.

Results and Discussion

The data pertaining to the number of days taken to spike emergence of tuberose *cv.* 'Prajwal' by soil application of graded levels of essential heavy metal concentrations was found significantly different among the treatments (Table 1.). Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly early (66.73 days) spike emergence in tuberose *cv.* 'Prajwal' followed by application of $ZnSO_4$ @ 200 mg kg^{-1} soil (76.40 days), whereas significantly highest number of days taken to spike emergence in tuberose *cv.* 'Prajwal' was recorded with the untreated control plants (361.06 days) followed by application of $MnSO_4$ @ 3000 mg kg^{-1} soil (325.33 days). The data pertaining to number of days taken to spike emergence in tuberose was found significantly increased with each increasing concentration of $MnSO_4$ from 1000 to 3000 mg kg^{-1} soil application. The data pertaining to number of days taken to spike emergence of tuberose has recorded a significant decrease at each successive increasing concentration of $CuSO_4$ from 100 to 300 mg kg^{-1} soil application. Based on the analysis of results, it may be concluded that a significant reduction noticed in the juvenile phase of tuberose *cv.* 'Prajwal' might be due to active role played by Zn in many of the physiological and biochemical reactions in the plant. Further, application of $ZnSO_4$ @ 400 mg kg^{-1} soil enhanced vegetative growth parameters like the number of leaves produced per plant, plant spread and leaf area per plant (data presented) and thus tuberose plants could complete their juvenile phase at a faster rate in comparison to the other essential heavy metal concentrations as well as the untreated control plants. The present result was found in close conformity with the earlier findings of Jadhav *et al.* (2005) [7] in gerbera, Kumar *et al.* (2012) [16] and Lahijie (2012) [17] in gladiolus. However, an inordinate delay noticed in the spike emergence of tuberose plants in the untreated control plants (361.06 days) as well as soil application of graded levels of $CuSO_4$ and $MnSO_4$ might be attributed to lack of Zn, an essential trace element to trigger the physiological and biochemical processes in the plant. Further, prolonged delay observed in the spike emergence of tuberose *cv.* 'Prajwal' plants could also be attributed to the sudden spurt of *Alternaria* leaf spot disease on many of the essential heavy metal treated plants excepting the $ZnSO_4$ applied plants which have crossed the vegetative phase in advance and reached to the reproductive stage earlier in comparison to plants lacking Zn in the soil. Based on the analysis of results at a broader sense, it may be concluded that application of $ZnSO_4$ encouraged the vegetative growth of plants by increased production of tryptophan, the precursor of IAA oxidase enzyme thus increased the plant defense mechanism in the $ZnSO_4$ applied tuberose plants especially at an optimum dose of 400 mg kg^{-1} soil. Tsui (1948) [30] reported that increased absorption of water in the plant was thought to be a function of auxin activity influenced by Zn in plants which was directly related to the growth of plants, thus the time taken for spike emergence was reduced. The present result was found in agreement with the earlier findings of Aruna *et al.* (2007) [3] in crossandra.

Significant differences were observed in the number of days to first floret opening by soil application of graded levels of essential heavy metal concentrations (Table 2.). Significantly early floret opening after emergence of flower spike was observed in the plants applied with $ZnSO_4$ @ 400 mg kg^{-1} soil (6.33 days) and was found at par with the application of $ZnSO_4$ @ 200 mg kg^{-1} soil (7.80 days), whereas significantly highest number of days taken to first floret opening in tuberose was recorded in the untreated control plants (19.26 days). The remaining all other heavy metal concentrations were found in between these treatments. Among the graded levels of $MnSO_4$, significantly increased number of days taken to first floret opening was noticed with an increase in the concentration of chemical. Among the graded levels of $CuSO_4$, significantly lowest number of days taken to first floret opening was noticed with the application of $CuSO_4$ @ 200 mg kg^{-1} soil (8.40) and was found at par with the application of $ZnSO_4$ @ 200 mg kg^{-1} soil. Based on the analysis of these results it may be concluded that plants treated with $ZnSO_4$ @ 400 mg kg^{-1} soil was found optimum and helped in the early opening of florets on the flower spike in tuberose *cv.* 'Prajwal'. This might be attributed to enhanced growth and development of the floret by application of $ZnSO_4$ @ 400 mg kg^{-1} soil. Activation of tryptophan production in the plant due to optimal availability of zinc element might have led to increased levels of auxin production in the plant which in turn promoted early flowering and development of florets on the spike thereby early opening of florets was observed in tuberose *cv.* 'Prajwal'. Presence of optimal levels of zinc concentrations in the plant tissue helped in proper regulation of auxin concentration in the plants, thus early spike emergence and early opening of florets was noticed in tuberose *cv.* 'Prajwal'. The present result was found in close agreement with the earlier findings of Manisha and Syamal (2002) [18] in rose, Singh and Singh (2004) [29], Jauhari *et al.* (2005) [8], Reddy and Chaturvedi (2009) [23] and Sharma *et al.* (2013) [27] in gladiolus.

Significant differences were noticed with respect to number of spikes produced per plant in tuberose *cv.* 'Prajwal' by soil application of graded levels of essential heavy metal concentrations (Table 3). Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest number of flower spikes produced per plant (0.46, 0.88 and 0.67 respectively during 2018-19, 2019-20 and the pooled data analysis) followed by application of $ZnSO_4$ @ 200 mg kg^{-1} soil. Among the concentrations of $ZnSO_4$, application of $ZnSO_4$ @ 600 mg kg^{-1} soil recorded significantly lowest number of flower spikes produced per plant in tuberose during both the years of study as well as in the pooled data analysis. Non-significant differences were noticed among the graded levels of $MnSO_4$ with respect to number of flower spikes produced per plant during 2018-19 and the pooled data analysis, however a significant reduction was noticed in the number of spikes produced per plant with an increase in the concentration of $MnSO_4$ from 1000 to 2000 mg kg^{-1} soil during 2019-20, whereas non-significant difference was noticed in between the concentrations of $MnSO_4$ @ 2000 and 3000 mg kg^{-1} soil application. Non-significant differences were noticed in the number of flower spikes produced per plant in tuberose *cv.* 'Prajwal' by soil application of graded levels of $CuSO_4$ during both the years of study as well as in the pooled data analysis. Among all the treatments, significantly lowest number of flower spikes produced per plant in tuberose *cv.* 'Prajwal' was noticed in control (0.08, 0.09 and 0.08 respectively during 2018-19, 2019-20 and the pooled data analysis). Based on the analysis of results, it may be concluded that soil application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest number of spikes produced per plant during both the years of study as well as in the pooled data analysis. Soil application of Zn might have exerted the beneficial effect in increasing the vegetative growth of tuberose by enhancing the production levels of tryptophan, the precursor of auxin in the plant system thus increasing the translocation of carbohydrates, minerals and amino acids from the site of synthesis to the floral tissue, thereby increased the number of flower spikes produced per plant. However, the influence of graded levels of $MnSO_4$ and $CuSO_4$ concentrations with respect to number of spikes per plant was found non-significant during most part of the period of experimentation as well as in the pooled data analysis. Further, it may concluded that manganese and copper were found non-reactive with the soil application which may be attributed to the immobile or slow mobile nature of the elements in the plant system.

Significant differences were observed among the intervals of observation recorded with respect to number of flower spikes produced per plant. Significantly highest number of flower spikes produced per plant (0.38, 0.68 and 0.53 respectively during 2018-19, 2019-20 and the pooled data analysis) in tuberose was recorded at 360 days after planting, whereas significantly lowest number of spikes produced per plant (0.13, 0.10 and 0.12 respectively during 2018-19, 2019-20 and the pooled data) was noticed at the initial 90 DAP interval during both the years of study as well as in the pooled data analysis. Based on the analysis of results it is quite obvious that non-significant differences were observed between any two consecutive intervals during the first year of study except between 270 and 360 DAP intervals, whereas significant differences were noticed in between any two consecutive intervals, except between the intervals 180 and 270 DAP during 2019-20 and the pooled data analysis.

The data pertaining to interaction effect between graded levels of soil applied essential heavy metal concentrations and the intervals with respect to number of flower spikes produced per plant was found non-significant during the 1st year of study *i.e.,* 2018-19, whereas significant differences were observed during the 2nd year of study *i.e.,* 2019-20 and the pooled data analysis. Among the combination treatments, significantly highest number of flower spikes produced per plant (1.53 and 1.16 respectively during 2019-20 and the pooled data analysis) was recorded by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 360 DAP interval followed by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 270 DAP interval (1.40 and 1.00 respectively during 2019-20 and the pooled data analysis), whereas significantly lowest number of flower spikes produced per plant (0.00 and 0.00 respectively during 2019-20 and the pooled data analysis) was recorded in control at 90 DAP interval. Based on the analysis of results, it may be concluded that an increase observed in the number of flower spikes produced per plant by soil application of $ZnSO_4$ might be attributed to the beneficial role of Zn in enhancing the vegetative growth of tuberose *cv.* 'Prajwal' by the enhanced production of tryptophan, the precursor of auxin, thus increased the translocation of carbohydrates, minerals and amino acids from the site of synthesis to the floral tissue, thereby enhanced early flowering and increased number of flower spikes per plant in tuberose. Kumar *et al.* (2003a) [12] reported similar kind of observation in gladiolus by application of Zn to the plants. The present results were found in consonance with the earlier findings of Kumar *et al.* (2003b) [13] in tuberose, Nag *et al.* (2003) [21] and Balakrishnan *et al.* (2007) [4] in marigold and Sharma *et al.* (2013) [27] in gladiolus.

Significant differences were noticed among the essential heavy metal concentrations with respect to spike length of tuberose *cv.* 'Prajwal' (Table 4). Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest spike length (22.59 cm, 35.15 cm and 28.87 cm respectively during 2018-19, 2019-20 and the pooled data analysis) and was found at par with the application of $ZnSO_4$ @ 200 mg kg^{-1} soil (19.83 cm, 30.41 cm and 25.12 cm respectively during 2018-19, 2019-20 and the pooled data analysis). Among the concentrations of $ZnSO_4$, application of $ZnSO_4$ @ 600 mg kg^{-1} soil recorded significantly lowest spike length during both the years of study as well as in the pooled data analysis. Soil application of graded levels of $MnSO_4$ and $CuSO_4$ recorded non-significant differences among their concentrations with respect to the spike length of tuberose *cv.* 'Prajwal' during 2018-19, whereas non-significant differences were noticed in between the concentrations of lowest and highest in the respective chemicals during 2019-20 and the pooled data analysis, but application of moderate concentration of $MnSO_4$ and $CuSO_4$ recorded significantly lowest spike length during 2019-20 and the pooled data analysis. Among all the treatments, untreated control recorded significantly lowest spike length (1.33 cm, 5.85 cm and 3.59 cm respectively during 2018-19, 2019-20 and the pooled data analysis). Based on the analysis of results, it may be concluded that significant differences noticed in the spike length of tuberose *cv.*

'Prajwal' might be attributed to the fact that heavy metal elements in general activate several enzymes in the plant system *viz.*, catalase, peroxidase, alcohol dehydrogenase and carbonic dehydrogenase. The role of zinc element in the synthesis of tryptophan was evidenced by many researchers in triggering the production of auxin in the plant to enhance cell division and cell enlargement thus increased the length of flower spike in tuberose *cv.* 'Prajwal'. Kumar and Arora (2000) [11] reported that plant growth and development were encouraged by involvement of Zn in various physiological activities of the plant as well as in the synthesis of chlorophyll. The present result was found in agreement with the earlier findings of Ahlawat *et al.* (2003) [1], Kumar *et al.* (2003c) [14] and Kumar *et al.* (2004) [15] in tuberose, Jauhari *et al.* (2005) [8] in the bulbous ornamental plants and Sharma *et al.* (2013) [27] in gladiolus.

Significant differences were noticed among the intervals with respect to spike length of tuberose *cv.* 'Prajwal'. Among the intervals of observation, significantly highest spike length (17.23 cm, 27.73 cm and 22.48 cm respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded at 360 days after planting, whereas significantly lowest spike length (5.93 cm, 5.80 cm and 5.87 cm respectively during 2018-19, 2019-20 and the pooled data analysis) was noticed at the initial 90 DAP interval. However, non-significant differences were noticed in the spike length between the successive intervals from 180 to 270 days after planting during 2018-19 and 2019-20, but significant increase in spike length was noticed at each successive interval in the pooled data analysis. Based on the analysis of results, it may be concluded that an increase observed in the length of flower spike in tuberose *cv.* 'Prajwal' might be attributed to the increased cell division and cell elongation (Kumar and Arora, 2000) [11].

The data pertaining to interaction effect between graded levels of soil applied essential heavy metal concentrations and the intervals with respect to spike length of tuberose *cv.* 'Prajwal' was found non-significant during 2018-19, 2019-20 and the pooled data analysis. However, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded highest spike length during both the years of study as well as in the pooled data analysis at 360 DAP interval.

Significant differences were noticed in the number of florets per spike in tuberose *cv.* 'Prajwal' by soil application of graded levels of essential heavy metal concentrations (Table 5). Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest number of florets per spike (13.68, 15.40 and 14.54 respectively during 2018-19, 2019-20 and the pooled data analysis) followed by application of $ZnSO_4$ @ 200 mg kg^{-1} soil and was found at par with the application of $ZnSO_4$ @ 600 mg kg^{-1} soil. Non-significant differences were noticed among the graded levels of $MnSO_4$ and $CuSO_4$ concentrations with respect to number of florets per spike during both the years of study as well as in the pooled data analysis. Significantly lowest number of florets per spike was recorded in the untreated control plants (0.95, 1.22 and 1.08 respectively during 2018-19, 2019-20 and the pooled data analysis). Based on the analysis of results, it may be concluded that application $ZnSO_4$ @ 400 mg kg^{-1} soil increased the number of florets per spike in tuberose *cv.* 'Prajwal'. In general, the number of florets present on the flower spike was determined by the genotype as well as proper nutrition of the plant. Increase in number of florets per spike observed in tuberose *cv.* 'Prajwal' by application $ZnSO_4$ @ 400 mg kg^{-1} soil could be attributed to the increased number of leaves as well as leaf area (data not presented) on the plant there by recorded an increase in the rate of photosynthesis (data not presented) in the plant thus resulted an increase in the number of florets spike.

Significant variation was noticed among the intervals of observation recorded with respect to the number of florets per spike in tuberose *cv.* 'Prajwal'. Significantly highest number of florets per spike (7.20, 9.84 and 8.52 respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded at 360 DAP interval, whereas significantly lowest number of florets per spike (2.46, 4.12 and 3.29 respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded at the initial 90 DAP interval during both the years of study as well as in the pooled data analysis. However, non-significant differences were noticed in between the successive intervals from 180 to 360 DAP during 1st year of study, whereas during the 2nd year of study non-significant differences were noticed in between 90 and 180 DAP intervals with respect to number of florets per spike. Significant differences were noticed in between the intervals of 180 and 270 DAP in the pooled data analysis. Based on the critical analysis of results, it may be concluded that an increase noticed in the number of florets per spike in tuberose *cv.* 'Prajwal' might be attributed to the increased production of tryptophan in the developing tissues which might have acted as a precursor of auxin and thus might have helped the plant to increase its vegetative growth at a faster rate there by suppressing the juvenile phase of plant. Series of these biological processes in the plant might have led to production of increased photo assimilates in the plant which in turn might have caused more number of florets produced per spike in tuberose *cv.* 'Prajwal'. The present investigation was found in close conformity with the earlier findings of Muthumanickam (1999) [20] in gerbera.

The data pertaining to interaction effects between graded levels of soil applied essential heavy metal concentrations and the intervals of observation recorded with respect to number of florets per spike was found significant during 2018-19, 2019-20 and the pooled data analysis. Significantly highest number of florets per spike (23.83, 25.20 and 24.51 respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 360 DAP interval followed by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 270 DAP interval (15.13, 19.26 and 17.19 respectively during 2018-19, 2019-20 and the pooled data analysis), whereas significantly lowest number of florets per spike (0.00, 0.00, 0.00) was recorded in the untreated control plants at 90 DAP interval during 2018-19, 2019-20 and the pooled data analysis. Based on the analysis of results, it may be concluded that application $ZnSO_4$ @ 400 mg kg^{-1} soil increased the number of florets per spike in tuberose *cv.* 'Prajwal' during both the years of study as well as in the pooled data analysis. Increase in number of florets per spike in tuberose *cv.* 'Prajwal' by soil application of graded levels of $ZnSO_4$ especially at the rate of 400 mg kg^{-1} soil in comparison with all other treatments might be attributed to an increase in the leaf area which in turn might be attributed to an increase in the synthesis of photo assimilates in the plant thus contributed to an increase in the number of florets per spike. Further, an increase observed in the number of florets per spike might be attributed to improved translocation of water, amino acids, minerals especially the Zn and photo assimilates from source to sink *i.e.*, to the flower spike as a result of which the number of florets per spike increased. The present finding was found in close agreement with the earlier findings of Nath and Biswas (2002) [22] and Yadav *et al.* (2002) [31] in tuberose as well as Kumar *et al.* (2012) [16], Sharma *et al.* (2013) [27] and Amin *et al.* (2014) [2] in gladiolus. Further, number of florets produced per spike in tuberose *cv.* 'Prajwal' might have also been controlled by its genetic architecture with proper nutrition.

Significant variation was noticed among the graded levels of essential heavy metal concentrations with respect to diameter of floret (Table 6). Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest diameter of floret (11.56 mm, 14.29 mm and 12.44 mm respectively during 2018-19, 2019-20 and the pooled data analysis). However, non-significant differences were noticed in the diameter of floret by application of $ZnSO_4$ @ 200 mg kg^{-1} soil (5.12 mm and 7.05 mm) and application of $ZnSO_4$ @ 600 mg kg^{-1} soil (5.99 mm and 5.78 mm) respectively during 2018-19 and the pooled data analysis. Non-significant differences were noticed among the graded levels of $MnSO_4$ and $CuSO_4$ with respect to diameter of floret in tuberose *cv.* 'Prajwal' during 2018-19, 2019-20 and the pooled data analysis. Significantly lowest diameter of floret was recorded in control (0.27 mm, 0.77 mm and 0.46 mm respectively during 2018-19, 2019-20 and the pooled data analysis). An increase observed in the diameter of floret in tuberose *cv.* 'Prajwal' by soil application of graded levels of essential heavy metal concentrations might be attributed to the increased cell division and cell elongation especially with the application of $ZnSO_4$ @ 400 mg kg^{-1} soil. Kumar and Arora (2000) [11] also reported similar kind of observation in gladiolus. Further, the present result was found in close in conformity with the earlier findings of Kumar *et al.* (2003b) [13] and Sharma *et al.* (2013) [27] in gladiolus and Khan (2000) [10] in chick pea genotypes. A drastic reduction noticed in the diameter of floret in the untreated control might be due to insufficiency in the availability of essential nutrients as well as malnutrition especially at the critical stages of plant growth and development.

Significant differences were noticed among the intervals with respect to diameter of floret in tuberose *cv.* 'Prajwal'. Among the intervals of observation recorded, significantly highest diameter of floret (6.89 mm, 9.04 mm and 7.97 mm respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded at 360 DAP interval, whereas significantly lowest diameter of floret (2.77 mm, 3.11 mm and 2.93 mm respectively during 2018-19, 2019-20 and the pooled data analysis) was noticed at the initial 90 DAP interval during both the years of study as well as in the pooled data analysis. However, the data recorded at 90 and 180 DAP were found at par with each other during 2018-19, 2019-20 and the pooled data analysis. Significant variation recorded in the diameter of floret, specially an increase, was noticed with the passage of time *i.e.*, 180 DAP during both the years of study as well as in the pooled data analysis.

The data pertaining to interaction effects between graded levels of essential heavy metal concentrations and the intervals with respect to diameter of floret was found significant during 2018-19, 2019-20 and the pooled data analysis. Significantly highest diameter of floret (18.00 mm, 22.63 mm and 20.31 mm respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 360 DAP interval followed by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 270 DAP interval (12.33 mm, 19.36 mm and 15.84 mm respectively during 2018-19, 2019-20 and the pooled data analysis), whereas significantly lowest diameter of floret (0.00, 0.00, 0.00) was recorded in control at 90 DAP during 2018-19, 2019-20 and the pooled data analysis which means no flowering was observed during the initial 90 DAP interval, even during the 2nd year of experimentation also. Based on the analysis of results, it may be concluded that an increase observed in the diameter of floret might be attributed to an increase in the cell division and cell elongation due to application of $ZnSO_4$ @ 400 mg kg^{-1} soil which helped in the synthesis of tryptophan, the precursor of auxin. Further, it may be reported that an increase observed in the diameter of floret in tuberose *cv.* 'Prajwal' might be due to soil application of optimum dose of $ZnSO_4$ which involved in the metabolism of RNA as well as might have increased the ribosomal content in the plant that led to stimulation of carbohydrate production as well as synthesis of protein and DNA content in the leaves. All these series of incidents might have helped in the synthesis of tryptophan which acted as growth promoting substance in the plants. The present result was found in close conformity with the earlier findings of Balakrishnan *et al.* (2007) [4] in marigold, Karuppaiah (2014) [9] and Saini *et al.* (2015) [24] in chrysanthemum.

Significant differences were observed in the fresh weight of flowers per plant of tuberose *cv.* 'Prajwal' by soil application of graded levels of essential heavy metal concentrations (Table 7). Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest fresh weight of flowers per plant (22.66 g, 18.31 g and 17.83 g respectively during 2018-19, 2019-20 and the pooled data analysis) followed by application of $ZnSO_4$ @ 200 mg kg^{-1} soil and was found at par with the application $ZnSO_4$ @ 600 mg kg^{-1} soil during 2018-19, but during 2019-20 and in the pooled data analysis significant differences were noticed between the application of $ZnSO_4$ @ 200 and $ZnSO_4$ @ 600 mg kg^{-1} soil. Soil application of graded levels of $MnSO_4$ and $CuSO_4$ recorded non-significant differences among their concentrations with respect to fresh weight of flowers per plant in tuberose *cv.* 'Prajwal' during 2018-19, 2019-20 and the pooled data analysis. Significantly lowest fresh weight of flowers per plant in tuberose *cv.* 'Prajwal' was recorded in the untreated control (0.57 g, 1.39 g and 0.98 g respectively during 2018-19, 2019-20 and the pooled data analysis). Based on the critical analysis of results, it may be concluded that an increase observed in the fresh weight of flowers per plant in tuberose *cv.* 'Prajwal' might be due to application of $ZnSO_4$ @ 400 mg kg^{-1} soil and it may be argued that Zn might have involved in the activation of several enzymes *viz.* catalase, peroxidase, tryptophan synthase, etc., as well as involved in the synthesis of chlorophylls and various other physiological activities of plant growth and development thereby accumulation of photo assimilates might have taken place in the plant parts, ultimately led to increased fresh flower yield per plant. The present result was found in agreement with the earlier findings of Sharma *et al.* (1990) [26] in maize and Marschner (1995) [19] in higher plants. The present study has clearly indicated that application of $ZnSO_4$ @ 400 mg kg^{-1} soil enhanced the vegetative growth parameters of tuberose *cv.* 'Prajwal' which in turn increased the accumulation of photo assimilates in the plant thus helped in increasing the fresh weight of flowers per plant in comparison with other treatments.

Table 1: Influence of applied essential heavy metals (Mn, Cu, Zn) on number of days to spike emergence of *Polianthes tuberosa cv.* 'Prajwal'

Treatment (mg of element kg^{-1} soil)	Number of days to spike emergence
$MnSO_4$ 1000	234.73
$MnSO_4$ 2000	256.13
$MnSO_4$ 3000	325.33
$CuSO_4$ 100	285.00
$CuSO_4$ 200	216.20
$CuSO_4$ 300	207.13
$ZnSO_4$ 200	76.40
$ZnSO_4$ 400	66.73
$ZnSO_4$ 600	157.53
Control	361.06
S.Em±	2.15
CD at 5%	6.40

Table 2: Influence of applied essential heavy metals (Mn, Cu, Zn) on number of days to first floret opening of *Polianthes tuberosa cv.* 'Prajwal'

Treatment (mg of element kg^{-1} soil)	Number of days to first floret opening
$MnSO_4$ 1000	9.46
$MnSO_4$ 2000	11.93
$MnSO_4$ 3000	14.33
$CuSO_4$ 100	10.33
$CuSO_4$ 200	8.40
$CuSO_4$ 300	11.33
$ZnSO_4$ 200	7.80
$ZnSO_4$ 400	6.33
$ZnSO_4$ 600	10.00
Control	19.26
S.Em±	0.63
CD at 5%	1.87

Table 3: Influence of soil applied essential heavy metals (Mn, Cu and Zn) on number of spikes $plant^{-1}$ of *Polianthes tuberosa cv.* 'Prajwal'

Treatment (mg kg^{-1})	Number of spikes $plant^{-1}$														
	2018 – 2019					2019 - 2020					Pooled (2018 - 2020)				
	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean
$MnSO_4$ 1000	0.06	0.13	0.13	0.40	0.18	0.06	0.33	0.40	0.86	0.41	0.06	0.23	0.26	0.63	0.29
$MnSO_4$ 2000	0.13	0.20	0.06	0.26	0.16	0.13	0.13	0.10	0.40	0.19	0.13	0.16	0.08	0.33	0.17
$MnSO_4$ 3000	0.06	0.13	0.33	0.26	0.19	0.13	0.20	0.33	0.60	0.31	0.09	0.16	0.33	0.43	0.25
$CuSO_4$ 100	0.06	0.20	0.26	0.40	0.23	0.13	0.33	0.26	0.66	0.34	0.09	0.26	0.26	0.53	0.28
$CuSO_4$ 200	0.13	0.13	0.20	0.53	0.24	0.13	0.06	0.13	0.53	0.21	0.13	0.09	0.16	0.53	0.23
$CuSO_4$ 300	0.10	0.13	0.26	0.33	0.20	0.06	0.66	0.13	0.26	0.27	0.08	0.39	0.19	0.29	0.24
$ZnSO_4$ 200	0.26	0.20	0.43	0.53	0.35	0.06	0.33	0.20	1.26	0.46	0.16	0.26	0.31	0.89	0.40
$ZnSO_4$ 400	0.33	0.13	0.60	0.80	0.46	0.26	0.33	1.40	1.53	0.88	0.29	0.23	1.00	1.16	0.67
$ZnSO_4$ 600	0.20	0.20	0.20	0.20	0.20	0.13	0.60	0.33	0.73	0.44	0.16	0.40	0.26	0.46	0.32
Control	0.00	0.13	0.10	0.10	0.08	0.00	0.26	0.06	0.06	0.09	0.00	0.19	0.08	0.08	0.08
Mean	0.13	0.15	0.25	0.38		0.10	0.32	0.33	0.68		0.12	0.24	0.29	0.53	

Factor	T	I	T × I	T	I	T × I	T	I	T × I
S.Em±	0.06	0.03	0.12	0.09	0.05	0.18	0.04	0.02	0.08
CD at 5%	0.17	0.11	NS	0.26	0.16	0.52	0.12	0.07	0.24

Table 4: Influence of applied essential heavy metals (Cu, Mn, Zn) on spike length of *Polianthes tuberosa cv.* Prajwal

Treatment (mg of element kg^{-1} soil)	Spike length (cm)														
	2018 - 2019					2019 - 2020					Pooled (2018-20)				
	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean
$MnSO_4$ 1000	3.06	11.26	3.60	15.60	8.38	2.66	19.90	26.33	28.60	19.37	2.86	15.58	14.96	22.10	13.87
$MnSO_4$ 2000	6.40	10.93	3.93	11.66	8.23	5.66	0.00	9.46	18.70	8.45	6.03	5.46	6.70	15.18	8.34
$MnSO_4$ 3000	2.40	7.46	8.53	11.80	7.54	6.66	18.06	19.06	26.26	17.51	4.53	12.76	13.80	19.03	12.53
$CuSO_4$ 100	2.53	13.06	7.00	18.13	10.18	7.13	26.03	29.93	41.76	26.21	4.83	19.55	18.46	29.95	18.19
$CuSO_4$ 200	4.33	6.60	13.00	23.13	11.76	7.40	7.03	5.93	18.63	9.74	5.86	6.81	9.46	20.88	10.75
$CuSO_4$ 300	0.00	6.26	17.63	17.53	10.35	3.73	14.43	41.46	27.43	21.76	1.86	10.35	29.55	22.48	16.06
$ZnSO_4$ 200	13.13	14.40	23.13	28.66	19.83	4.13	40.33	35.93	41.26	30.41	8.63	27.36	29.53	34.96	25.12
$ZnSO_4$ 400	18.20	7.30	28.96	35.92	22.59	13.26	41.50	42.70	43.16	35.15	15.73	24.40	35.83	39.54	28.87
$ZnSO_4$ 600	9.33	11.46	12.60	9.90	10.82	7.46	18.70	25.73	26.36	19.56	8.40	15.08	19.16	18.13	15.19
Control	0.00	5.33	0.00	0.00	1.33	0.00	6.63	11.60	5.20	5.85	0.00	5.98	5.80	2.60	3.59
Mean	5.93	9.40	11.83	17.23		5.80	19.26	24.81	27.73		5.87	14.33	18.32	22.48	

Factor	T	I	T × I	T	I	T × I	T	I	T × I
S.Em±	2.25	1.42	4.51	3.09	1.95	6.18	1.94	1.22	3.88
CD at 5%	6.36	4.02	NS	8.71	5.51	NS	5.47	3.46	NS

Table 5: Influence of applied essential heavy metals (Cu, Mn, Zn) on number of florets spike^{-1} of *Polianthes tuberosa cv.* Prajwal

Treatment (mg of element kg^{-1} soil)	Number of florets spike^{-1}														
	2018 - 2019					2019 - 2020					Pooled (2018-20)				
	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean
$MnSO_4$ 1000	1.40	3.80	6.40	8.60	5.05	1.93	2.00	5.20	10.86	4.99	1.66	2.90	5.8	9.73	5.02
$MnSO_4$ 2000	3.66	1.40	3.56	3.20	2.95	2.06	0.00	6.40	5.20	3.41	2.86	0.70	4.98	4.20	3.18
$MnSO_4$ 3000	1.20	4.16	4.33	3.90	3.39	4.60	7.33	6.23	5.06	5.80	2.90	5.74	5.28	4.48	4.60
$CuSO_4$ 100	0.73	5.43	6.33	5.06	4.38	5.20	3.56	8.06	5.40	5.55	2.96	4.49	7.19	5.23	4.97
$CuSO_4$ 200	2.60	2.73	4.03	8.13	4.37	5.33	3.53	7.96	2.46	4.82	3.96	3.13	5.99	5.29	4.59
$CuSO_4$ 300	0.00	1.93	4.26	8.56	3.68	1.66	6.40	3.50	14.26	6.45	0.83	4.16	3.88	11.41	5.07
$ZnSO_4$ 200	2.53	11.70	7.60	8.36	7.54	7.33	7.63	6.76	13.00	8.68	4.93	9.66	7.18	10.68	8.11
$ZnSO_4$ 400	9.53	6.26	15.13	23.83	13.68	8.60	8.56	19.26	25.20	15.40	9.06	7.41	17.19	24.51	14.54
$ZnSO_4$ 600	3.00	4.90	8.36	2.43	4.67	4.53	2.46	7.43	13.73	7.03	3.76	3.68	7.89	8.08	5.85
Control	0.00	1.70	2.13	0.00	0.95	0.00	0.00	1.63	3.26	1.22	0.00	0.85	1.88	1.63	1.08
Mean	2.46	4.40	6.21	7.20		4.12	4.14	7.24	9.84		3.29	4.27	6.72	8.52	
Factor	T		I		T × I	T		I		T × I	T		I		T × I
S.Em±	1.06		0.67		2.13	0.83		0.52		1.67	0.68		0.43		1.37
CD at 5%	3.01		1.90		6.02	2.35		1.48		4.70	1.94		1.22		3.87

Table 6: Influence of applied essential heavy metals (Cu, Mn, Zn) on diameter of floret of *Polianthes tuberosa cv.* Prajwal

Treatment (mg of element kg^{-1} soil)	Diameter of floret (cm)														
	2018 - 2019					2019 - 2020					Pooled (2018-20)				
	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean
$MnSO_4$ 1000	1.80	3.53	4.86	4.28	3.61	3.03	1.00	1.88	4.65	2.64	2.41	2.35	3.37	4.46	3.14
$MnSO_4$ 2000	2.70	0.00	2.80	2.42	1.98	3.26	2.36	1.16	4.79	2.89	2.98	2.26	1.98	3.60	2.70
$MnSO_4$ 3000	2.66	3.00	4.10	4.51	3.56	2.36	1.10	8.70	4.90	4.26	2.51	2.32	6.40	4.70	3.98
$CuSO_4$ 100	2.93	3.06	4.63	9.61	5.05	3.70	1.20	3.90	7.30	4.02	3.31	2.84	4.26	8.45	4.71
$CuSO_4$ 200	3.03	1.60	4.70	1.47	2.70	2.93	2.63	4.50	12.20	5.56	2.98	2.63	4.60	6.83	4.26
$CuSO_4$ 300	1.36	1.60	2.90	8.36	3.55	2.46	0.00	7.60	8.73	4.69	1.91	1.46	5.25	8.54	4.29
$ZnSO_4$ 200	1.70	5.30	5.10	8.38	5.12	4.70	10.46	8.76	17.63	10.38	3.20	5.07	6.93	13.00	7.05
$ZnSO_4$ 400	7.63	8.30	12.33	18.00	11.56	4.70	10.50	19.36	22.63	14.29	6.16	7.45	15.84	20.31	12.44
$ZnSO_4$ 600	3.90	3.80	5.40	10.86	5.99	3.96	5.03	7.80	6.10	5.72	3.93	4.12	6.60	8.48	5.78
Control	0.00	0.00	0.00	1.10	0.27	0.00	0.77	0.76	1.56	0.77	0.00	0.15	0.38	1.33	0.46
Mean	2.77	3.01	4.68	6.89		3.11	3.50	6.44	9.04		2.93	3.06	5.56	7.97	
Factor	T		I		T × I	T		I		T × I	T		I		T × I
S.Em±	1.06		0.67		2.12	0.70		0.44		1.41	0.67		0.42		1.34
CD at 5%	3.00		1.89		6.00	1.99		1.26		3.99	1.89		1.20		3.79

Table 7: Influence of applied essential heavy metals (Cu, Mn, Zn) on fresh flower yield plant^{-1} of *Polianthes tuberosa cv.* Prajwal

Treatment (mg of element kg^{-1} soil)	Fresh flower yield plant^{-1} (g)														
	2018 - 2019					2019 - 2020					Pooled (2018-20)				
	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean	I_{90}	I_{180}	I_{270}	I_{360}	Mean
$MnSO_4$ 1000	0.39	0.66	6.23	7.66	3.73	3.30	6.76	9.53	8.02	6.90	1.84	3.71	7.88	7.84	5.32
$MnSO_4$ 2000	0.40	2.20	8.46	10.20	5.31	2.20	5.66	5.83	5.86	4.88	1.30	3.93	7.14	8.03	5.10
$MnSO_4$ 3000	0.98	1.70	4.86	8.56	4.02	4.02	4.96	5.66	8.03	5.66	2.50	3.33	5.26	8.30	4.84
$CuSO_4$ 100	1.70	2.43	7.26	10.23	5.40	4.90	5.05	5.60	7.86	5.85	3.30	3.74	6.43	9.05	5.63
$CuSO_4$ 200	3.40	5.72	5.70	10.46	6.32	2.50	2.63	4.96	7.66	4.43	2.95	4.17	5.33	9.06	5.37
$CuSO_4$ 300	2.00	3.63	4.46	16.23	6.58	2.46	2.63	5.03	15.16	6.32	2.23	3.13	4.74	15.69	6.44
$ZnSO_4$ 200	1.30	4.46	4.96	19.20	7.48	3.66	10.46	10.73	19.03	10.97	2.48	7.46	7.84	19.11	9.22
$ZnSO_4$ 400	6.03	10.50	32.03	42.10	22.66	12.26	13.83	20.26	26.90	18.31	9.14	12.16	26.14	34.50	20.48
$ZnSO_4$ 600	2.83	3.80	6.63	9.86	5.78	5.03	5.16	7.80	14.16	8.03	3.93	4.48	7.21	12.01	6.91
Control	0.00	0.00	0.00	2.30	0.57	0.00	0.00	2.13	3.46	1.39	0.00	0.00	1.06	2.88	0.98
Mean	1.90	3.51	8.05	13.68		4.03	5.71	7.75	11.61		2.96	4.61	7.90	12.64	
Factor	T		I		T × I	T		I		T × I	T		I		T × I
S.Em±	1.25		0.79		2.49	1.03		0.65		2.06	0.84		0.53		1.68
CD at 5%	3.52		2.22		7.04	2.91		1.84		5.83	2.37		1.50		4.75

Significant differences were noticed among the intervals of observation recorded by application of graded levels of essential heavy metal concentrations with respect to fresh weight of flowers per plant in tuberose *cv.* 'Prajwal'. Among the intervals of observation recorded, significantly highest fresh weight of flowers per plant (13.68 g, 11.61 g and 12.64 g respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded at 360 DAP interval, whereas significantly lowest fresh weight of flowers per plant (1.90 g, 4.03 g and 2.96 g respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded at the initial 90 DAP interval in tuberose *cv.* 'Prajwal'. A gradual increase in the fresh weight of flowers per plant of tuberose *cv.* 'Prajwal' was observed with the passage of time during both the years of study as well as in the pooled data analysis. However, the differences recorded in the fresh weight of flowers per plant between the successive intervals of 90 and 180 DAP were found at par with each other during 2018-19 and 2019-20, whereas in the pooled data analysis fresh weight of flowers per plant recorded was found at par with each other in between the intervals 180 and 270 DAP intervals.

The data pertaining to interaction effect between graded levels of essential heavy metal concentrations and the intervals with respect to fresh weight of flowers per plant was found significant during 2018-19, 2019-20 and the pooled data analysis. Significantly highest fresh weight of flowers per plant (42.10 g, 26.90 g and 34.50 g respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 360 DAP interval followed by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 270 DAP interval (32.03 g, 20.26 g and 26.14 g respectively during 2018-19, 2019-20 and the pooled data analysis), whereas significantly lowest fresh weight of flowers per plant (0.00, 0.00, 0.00) was recorded in the untreated control plants at 90 DAP interval during 2018-19, 2019-20 and the pooled data analysis. However, non-significant differences were noticed among the different concentrations of $MnSO_4$ and $CuSO_4$ with respect to fresh weight of flowers per plant during both the years of study as well as in the pooled data analysis. Based on the analysis of results, it may be concluded that a significant increase recorded in the fresh weight of flowers per plant of tuberose *cv.* 'Prajwal' might be attributed to the influence of $ZnSO_4$ in increasing the nutrient and water uptake from soil thus increased the biosynthesis of auxin in the developing tissue of plant thereby enhanced the metabolism of plant (Cakmak *et al.*, 1999) [6]. The beneficial effects of soil applied micronutrients especially $ZnSO_4$ on flower yield of tuberose *cv.* 'Prajwal' might be attributed to the activation of various enzymes in the plant system as well as efficient utilization of soil applied nutrients thereby ultimately an in increase in the fresh weight of flowers per plant was recorded. Similar kind of observation was reported earlier by Shanker *et al.* (1999) [25] and Singaravel *et al.* (2001) [28] in sesamum.

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