Plant Archives Vol. 26, Supplement 2, 2026 pp. 2034-2041 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-2.254

RESPONSE OF ESSENTIAL HEAVY METALS ON LIPOXIGENASE ACTIVITY, PROLINE AND TOTAL SUGARS IN *Polianthus tuberosa* CV. 'PRAJWAL'

L. Gowthami[1*], V. Vijaya Bhaskar[2] and R.P. Premalatha[1]

[1]ICAR-Indian Institute of Oil Palm Research, Pedavegi mandal, Eluru district - 534450, Andhra Pradesh, India.

[2]College of Horticulture, Dr. Y.S.R. Horticultural University, Chinalataripi, Kandkur mandal, SPSR Nellore district - 523105, Andhra Pradesh, India

*Corresponding author E-mail: floriglori8@gmail.com

(Date of Receiving : 09-04-2026; Date of Revision : 28-05-2026; Date of Acceptance : 15-06-2026)

ABSTRACT

Presence of high concentration of both essential and non-essential heavy metals are considered to affect 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 in plants as many of them are toxic even at very low level concentrations. Out of the several heavy metals of essential and non-essential, three essential heavy metal containing chemicals *viz.*, $MnSO_4$, $CuSO_4$ and $ZnSO_4$ which are required in trace amounts for better growth, development and metabolic activity, lipoxigenase activity, proline and total sugars of plants have been selected in the present investigation to identify their toxic effects on the plant's metabolic activity under heavily accumulated condition in the soil. Statistical analysis of results indicated that application of $ZnSO_4$ @ 400 mg/kg at 360 DAP soil recorded a significant improvement in the lipoxigenase activity (0.65 n moles g^{-1}, 0.72 n moles g^{-1} and 0.68 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis), proline (12.70 µ moles g^{-1}, 16.90 µ moles g^{-1} and 14.80 µ moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis), total sugars (136.86%, 147.53% and 142.20% respectively during 2018-19, 2019-20 and the pooled data analysis).

Keywords **:** LOX, proline, total sugars, yield, $MnSO_4$, $CuSO_4$, $ZnSO_4$.

Introduction

Industrial revolution accelerated the biosphere with essential micronutrients. A common response of essential micronutrient 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). Manganese has been an essential micronutrient throughout all stages of plant growth and development. It plays a vital role in many functions of the plant system. Manganese acts as a cofactor of various enzymes such as Mn–superoxide dismutase, Mn–catalase, pyruvate carboxylase and phosphoenolpyruvate carboxy kinase. Therefore, incorporation of Mn by cells is essential, particularly in photosynthesis where Mn plays a critical role as an accumulator of positive charge equivalents in a reaction catalyzed in photosystem-II (Marschner, 1995). Manganese aids in the synthesis of chlorophyll and assimilation of nitrate and activates enzymes of fat biosynthesis. It's function in the formation of riboflavin, ascorbic acid and carotene is a well-known fact. Copper is required for plant nutrition only in trace amounts and at higher concentrations it can be toxic to cells. Critical deficiency levels are in the range of 1-5 mg Cu kg^{-1} plant dry mass and the threshold for toxicity was found above 20-30 mg Cu kg^{-1} dry mass (Marschner, 1995). Some hyper accumulators may accumulate up to 1000 mg of Cu kg^{-1} in leaves (Morrison *et al.*, 1981). Copper is a redox active metal with an electrochemical potential of -260mV. Thus, it is not surprising that copper is an essential component of many electron carriers involved in reactions occurring in the range from -420 mV to +800 mV. Zinc is an essential element in the plant system which is required in trace amounts, but at higher concentrations it is toxic. Since it is assimilated early by plants, it can be highly phytotoxic. Growth inhibition is a general phenomenon associated with zinc toxicity. Zinc is also a constituent of metallo-enzyme or a co-factor for several enzymes such as anhydrases, dehydrogenases, oxidases and peroxidases and plays an important role in regulating the nitrogen metabolism, cell multiplication, photosynthesis and auxin synthesis in plants. It also plays an important role in respiration and carbohydrate metabolism, synthesis of nucleic acid and proteins and helps in the utilization of phosphorous and nitrogen during seed formation. Hyper accumulation of zinc has been observed in many plant species and accumulation of 1% of the dry weight being common among plants grown on zinc mine wastes. Lipoxygenase (linoleate : oxygen oxidoreductase, EC 1.13.11.12), which catalyzes the oxidation of unsaturated fatty acids containing a cis,cis-1,4-pentadiene system, has been implicated in the senescence and wounding response of plants (Galliard, 1978; Galliard and Chan, 1980; Lesham *et al*., 1981). LOX activity results in the formation of lipid peroxides that accumulate during aging of animal and plant cells (Dhindsa *et al*., 1981; Pauls and Thompson, 1984). Lipid peroxide, levels have been correlated with senescence and increased membrane permeability (Kockritz *et al*., 1985; Peterman and Siedow, 1985). Essential micronutrients can significantly impact total sugar levels in plants, often leading to increased soluble sugars and decreased starch content in foliage. This shift in carbohydrate distribution is a common response to heavy metal stress, as plants attempt to cope with the toxic effects. In the recent times, it has been observed a wide spread contamination of soils and ground water with Mn, Cu and Zn mainly due to indiscriminate application of agricultural chemicals and human activities mainly mining which is posing a serious threat to the future generations. In order to maintain sustainability of soil and keeping the ground water free from contamination with heavy metals, continuous efforts have been made to develop technologies that are easy to use, sustainable and economically feasible. Physico-chemical approaches have been widely used for remedying the polluted soil and ground water, especially at small scale. However, they experienced more difficulties for a large scale of remediation because of involvement of high cost and side effects. The use of plant species for cleaning the polluted soils and ground waters named as phytoremediation, has gained increasing attention since last decade, as an emerging cheaper technology. A few research workers reported that *Acalypha indica*, *Abutilon indicum* and *Physalis minima* (Subhashini *et al*., 2017), chrysanthemum and pelargonium (Abdullah and Sarem, 2010) have the ability to accumulate heavy metals apart from tuberose which also possess the phytoremediation capability to remove heavy metals from contaminated soils (Ramana *et al*., 2012 and Ramana *et al*., 2013b). Thus, the present investigation has been planned to utilize the potential heavy metal accumulating capability of tuberose is cultivated on commercial scale in Andhra Pradesh Keeping all these things in view, the present investigation was planned.

Materials and Methods

The present investigation was carried out during rabi-2018 to kharif-2020 at College of Horticulture, Dr. Y.S.R. Horticultural University, Anantharajupeta, Kadapa district of Andhra Pradesh, India. The location is situated at an altitude of 162 meters (531 feet) above mean sea level. The geographical location was 13°59' North latitude and 79°19' East longitude. Poly bags of 12 kg capacity were used in the present investigation and bags were filled with 10 kg of virgin soil brought from uncultivated barren land. Soil was dried, grounded and sieved through 2 mm sieve and then 3 samples of soil were collected at random just before filling the poly bags. Further, regularly soil samples were collected from poly bags at every 90 days interval and the samples were used for analysis of essential heavy metals content present in the soil. The experimental material used for the present study was tuberose single type cultivar 'Prajwal'. 'Prajwal' also known as 'Arka Prajwal' is a cross between 'Shringar' x 'Mexican Single' varieties released by Indian Institute of Horticultural Research, Bangalore. It bears single type flowers on tall and stiff spikes. Pinkish red tinge is observed on the bud before opening. The variety has been reported as tolerant to high concentrations of heavy metals and has the ability to extract the heavy metals from contaminated soils. The experiment was laid out in a completely randomized design with three replications under open field conditions as pot culture experiment. The experiment consisted of 10 treatments, *viz*., T_1 = RDF+$MnSO_4$ @ 1,000 mg/ kg soil, T_2 = RDF+$MnSO_4$ @ 2,000 mg/kg soil, T_3 = RDF+$MnSO_4$ @ 3,000 mg/ kg soil, T_4 = RDF+$CuSO_4$ @ 100 mg/kg soil, T_5 = RDF+$CuSO_4$ @ 200 mg/kg soil, T_6 = RDF+$CuSO_4$ @ 300 mg/kg soil, T_7 = RDF+$ZnSO_4$ @ 200 mg/kg soil, T_8 = RDF+ $ZnSO_4$ @ 400 mg/kg soil, T_9 = RDF+ $ZnSO_4$ @ 600 mg/kg soil, T_{10} = Control (No RDF and no essential heavy metals). One bulb was planted in each poly bag and there were 300 plants in each replication. Poly bags of size 13" x 15" with 12 kg capacity were used in the present study. Poly bags were filled with 10 kg of growing medium containing red earth, sand and farmyard manure in the ratio of 2:1:1. Calculated quantities of heavy metals as per the concentration were incorporated into the growing medium just before filling the poly bags and all the poly bags were left over on polythene mulch sheet for ten days to get settle down the growing medium. Based on the calculated area of poly bag (12.56 m^2), NPK fertilizers were applied in the form of Urea @ 540 g, SSP @ 1570 g and MOP @ 410 g per annum of the crop growth period at every three months interval as per the recommended package of practice suggested by Dr. Y.S.R. Horticultural University, Venkataramannagudem, West Godavari district of Andhra Pradesh. A day before planting all the selected tuberose bulbs were soaked in carbendazim solution at a concentration of 0.1% for half an hour and the bulbs were dried in shade. Bulbs weighing about 40 g were planted at a depth of about 3-4 inches in the soil filled in poly bags and watered with rose can immediately after planting. Five plants were randomly selected and tagged with a label in each replication of the treatment to record the non-destructive observations which include vegetative growth, physiological, floral and flower yield parameters. In case of destructive sampling, one plant at each stage of observation recording was removed carefully by tearing the polythene bag and removed the soil adhered to the root system of the plant, washed thoroughly with clean water to remove the soil particles adhered to the plant and dried in shade for recording the observations on growth analysis and estimation of heavy metals in different parts of the plant at regular intervals of 90 days *i.e.*, at 90, 180, 270 and 360 days after transplanting (DAT). Lipoxigenase activity was measured by the amount of malondialdehyde (MDA), a product of lipid peroxidation in the sample by the thiobarbituric acid (TBA) reaction as described by Heath and Packer (1968). Homogenized 0.1 g of leaf tissue by adding 0.5 ml 0.1% Tri chloro acetic acid (TCA). Centrifuged the homogenate at 4^{o}C for 10 minutes at 15,000 rpm. 0.5 ml of supernatant solution collected and mixed with 1.5 ml 0.5% TBA diluted in 20% TCA. Incubated the samples in water bath at 95°C for 25 minutes and cooled.In case the solution is not clear, then centrifuged at 4^{o}C for further 5 minutes at 15,000 rpm. The absorbance of supernatant solution for lipid peroxidation activity was read at 532 nm and the value for the non-specific absorption at 600 nm was subtracted. The concentration of MDA was calculated using its extinction co-efficient of 155 mM cm^{-1}. Proline content in the fresh leaves of each replication of the treatment was determined by using rapid colorimetric method as suggested by Bates *et al.* (1973). Proline concentration in the sample was determined based on the standard curve using analytical grade proline (SRL, Mumbai) and calculated on fresh weight basis. 0.5 g of fresh leaf tissue was homogenized in 10 ml of 3% aqueous Sulphosalicylic acid and ground with pre-cooled mortar and pestle. The leaf extract was centrifuged at 18^{o}C for 30 minutes at 6,000 rpm. The supernatant solution thus collected was stored in a refrigerator for further use. Pipetted out 2 ml of filtrate, 2 ml of glacial acetic acid, 2 ml of acid ninhydrin in a test tube and heated the test tube in the boiling water bath at 80^{o}C for 1 hour. Cooled rapidly by placing on ice-bath to terminate the reaction. Added 4 ml of toluene to each sample. Stirred well for 30 seconds with glass rod and left for 5 minutes. Chromophore containing toluene layer was aspirated from aqueous phase then brought to room temperature. Read the absorbance at 520 nm using UV-VIS Spectrophotometer. Proline content in the leaves was expressed as μ mol/gram FW. Total sugars in the fresh leaves of each replication of the treatment were determined by the method explained by Yemm and Willis (1954). Anthrone reagent was prepared by dissolving 200 mg of anthrone in 100 ml of 95% sulphuric acid under chilled condition. 0.5 g leaf sample was ground with 10 ml of hot 80% ethanol using mortar and pestle. The homogenate was centrifuged at 20^{o}C for 20 minutes at 5000 rpm. The supernatant solution was collected in the conical flask. Washed the residue repeatedly for 3 times and pool the supernatant. Made the volume to 100 ml with distilled water and pipette out 0.5 ml of the above sample, 0.5 ml of distilled water, 4 ml of pre-cooled anthrone reagent in test tube. Heated the sample in boiling water bath for 8 minutes at 60^{o}C and cool it rapidly and read the absorbance at 630 nm. Blank or reference cuvette contained 1 ml of distilled water in place of the sample. Based on the standard graph, the amount of glucose present in the sample was calculated and expressed as per cent of total sugars.

Statistical analysis

The data arrived at pertaining to lipid peroxidation, proline and total sugars in leaf samples were subjected to statistical analysis by following standard methods developed by Panse and Sukhatme (1985). Statistical significance was tested by using ‘F’ value at 5 per cent and 1 per cent level of significance. Critical difference at 5 per cent level was worked out for the effects which were found significant. The results were depicted graphically wherever necessary.

Results and Discussion

The data pertaining to influence of graded levels of soil applied essential heavy metal concentrations and their interaction effects on lipoxygenase activity of tuberose *cv.* 'Prajwal' was presented in Table 1. Significant variation was observed in the lipoxygenase activity (LOX) of tuberose *cv.* 'Prajwal' by soil application of graded levels of essential heavy metal concentrations. Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest LOX activity (0.52 n moles g^{-1}, 0.56 n moles g^{-1} and 0.52 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) followed by application of $ZnSO_4$ @ 200 mg kg^{-1} soil (0.34 n moles g^{-1}, 0.46 n moles g^{-1} and 0.38 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis). Among the zinc sulphate concentrations, application of $ZnSO_4$ @ 600 mg kg^{-1} soil recorded significantly lowest LOX activity (0.10 n moles g^{-1}, 0.17 n moles g^{-1} and 0.14 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) during both the years of study as well as in the pooled data analysis. Soil application of graded levels of $MnSO_4$ recorded either non-significant or marginally significant differences in the lipoxygenase activity of tuberose during both the years of study as well as in the pooled data analysis. Soil application of graded levels of $CuSO_4$ recorded significant differences in their LOX activities, but the changes noticed in the LOX activity were found different with different concentrations of $CuSO_4$ without any specific rhythm to the graded levels of soil applied $CuSO_4$ during both the years of study as well as in the pooled data analysis. Among all the treatments, untreated control plants recorded significantly lowest LOX activity (0.02 n moles g^{-1}, 0.04 n moles g^{-1} and 0.03 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) in tuberose *cv.* 'Prajwal'.

Significant variation was found among the intervals of observation recorded with respect to the LOX activity of tuberose *cv.* 'Prajwal' during both the years of study as well as in the pooled data analysis. Among the intervals of observation recorded, significantly highest LOX activity (0.22 n moles g^{-1}, 0.33 n moles $g^{-1}$and 0.27 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded at 360 DAP, whereas significantly lowest LOX activity (0.10 n moles g^{-1}, 0.13 n moles g^{-1} and 0.12 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) was noticed at the initial 90 DAP interval. A gradual and significant increase in the LOX activity of tuberose *cv.* 'Prajwal' was noticed with the passage of time at each successive interval of observation during both the years of study as well as in the pooled data analysis.

The data pertaining to interaction effect between graded levels of essential heavy metal concentrations and the intervals of observation recorded with respect to lipoxygenase activity in tuberose *cv.* 'Prajwal' was found significant during 2018-19, 2019-20 and the pooled data analysis. Among the combination treatments, significantly highest LOX activity (0.65 n moles g^{-1}, 0.72 n moles g^{-1} and 0.68 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) was noticed by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 360 DAP followed by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 270 DAP (0.56 n moles g^{-1}, 0.64 n moles g^{-1} and 0.60 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis), whereas significantly lowest LOX activity (0.005 n moles g^{-1}, 0.02 n moles g^{-1} and 0.01 n moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) was observed in the untreated control plants at 90 DAP. The results of the present study have indicated that an increase in the oxidative stress was noticed by an increase in the H_2O_2 content with the age of the plant and thus recorded an increase in the lipid degradation or lipid peroxidation as reflected through an increase in the MDA content. Chakraborty *et al.* (2009) observed an increase in the lipid peroxidation of *Hemerocallis* leaves during the period of senescence which was confirmed by higher degree of membrane deterioration expressed as an increase in the electrolyte leakage which indicated a gradual loss in the cell membrane's permeability of selective ion leakage. Singh *et al.* (2008) and Auty (2011) also noticed similar kind of observation with regard to lipid peroxidation while working on gladiolus and orchid respectively. Already it has been reported that LOX activity might play a positive role in promoting the senescence through oxidative membrane damage explained by several research workers *viz.*, Peary and Prince, (1990) in gladiolus, Rubinstein (2000) in day lily and Hossain *et al.* (2006) in gladiolus.

The data pertaining to influence of graded levels of soil applied essential heavy metal concentrations and their interaction effects with intervals on proline content was presented in Table 2. Significant differences were noticed in the proline content of tuberose *cv.* 'Prajwal' treated with graded levels of essential heavy metal concentrations. Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest proline content (8.83 µ moles g^{-1}, 13.98 µ moles g^{-1} and 11.40 µ moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) followed by application of $ZnSO_4$ @ 600 mg kg^{-1} soil (5.86 µ moles g^{-1}, 8.34 µ moles g^{-1} and 7.09 µ moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis). Among the zinc sulphate concentrations, application of $ZnSO_4$ @ 200 mg kg^{-1} soil recorded significantly lowest proline content in the leaves during both the years of study as well as in the pooled data analysis. Soil application of graded levels of $MnSO_4$ recorded a decreasing trend in the proline content of tuberose *cv.* 'Prajwal' with an increase in the concentration of $MnSO_4$ during 2018-19, whereas decreasing trend in the proline content of tuberose *cv.* 'Prajwal' was observed with an increase in the concentration of $MnSO_4$ up to 2000 mg kg^{-1} soil and thereafter a sudden increase in the proline content was noticed with further application of $MnSO_4$ @ 3000 mg kg^{-1} soil which was found significantly superior among their concentrations during 2019-20 and the pooled data analysis. Soil application of graded levels of $CuSO_4$ recorded a gradual increase in the proline content of tuberose *cv.* 'Prajwal' during both the years of study as well as in the pooled data analysis. Among all the treatments, untreated control plants recorded significantly lowest proline content (0.60 µ moles g^{-1}, 2.19 µ moles g^{-1} and 1.31 µ moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis). Significant variation was noticed among the intervals of observation recorded with respect to the proline content of leaves in tuberose *cv.* 'Prajwal' by soil application of graded levels of essential heavy metal concentrations. Among the intervals of observation recorded, significantly highest proline content (4.03 µ moles g^{-1}, 8.07 µ moles g^{-1} and 6.04 µ moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) was observed at 360 DAP, whereas significantly lowest proline content (2.67 µ moles g^{-1}, 3.25 µ moles g^{-1} and 2.96 µ moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded at the initial 90 DAP interval. A significant and gradual increase in the proline content of leaves was observed with the passage of age of tuberose *cv.* 'Prajwal' during both the years of study as well as in the pooled data analysis.

The data pertaining to interaction effect between graded levels of essential heavy metal concentrations and the intervals of observation recorded with respect to proline content was found significant during 2018-19, 2019-20 and pooled data analysis. Among the combination treatments, significantly highest proline content (12.70 µ moles g^{-1}, 16.90 µ moles g^{-1} and 14.80 µ moles g^{-1} 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 followed by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 270 DAP (8.62 µ moles g^{-1}, 16.20 µ moles g^{-1} and 12.41 µ moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis), whereas significantly lowest proline content (0.40 µ moles g^{-1}, 0.16 µ moles g^{-1} and 0.28 µ moles g^{-1} respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded in the untreated control at 90 DAP. Based on the analysis of results, it may be concluded that increasing concentrations of heavy metals especially zinc accumulation in tuberose exerted increased amount of stress, which in turn increased the content of proline which is an osmoprotectant. Further, it was argued that accumulation of proline in plant system helps to conserve the nitrogenous compounds and protect the plant against heavy metal stress. Accumulation of proline in the cell acts as a membrane stabilizer under stress conditions. Similar kind of observation was reported earlier by Sayyari *et al.* (2013) in lettuce, Hayat *et al.* (2014) in *Cicer arietinum* and Ansari *et al.* (2017) in musk melon.

The data pertaining to influence of graded levels of soil applied essential heavy metal concentrations and their interaction effects with intervals on total sugars was presented in Table 3. Significant variation was noticed in the total sugars content of tuberose *cv.* 'Prajwal' by soil application of graded levels of essential heavy metal concentrations. Among the graded levels of essential heavy metal concentrations, application of $ZnSO_4$ @ 400 mg kg^{-1} soil recorded significantly highest total sugars (112.29%, 146.19 % and 129.24% respectively during 2018-19, 2019-20 and the pooled data) followed by application of $ZnSO_4$ @ 600 mg kg^{-1} soil. Among the concentrations of zinc sulphate, application of $ZnSO_4$ @ 200 mg kg^{-1} soil recorded significantly lowest total sugars content in leaves. Soil application of graded levels of $MnSO_4$ and $CuSO_4$ recorded gradual decrease in the total sugars content of tuberose *cv.* 'Prajwal' with an increase in the concentrations of $MnSO_4$ and $CuSO_4$ during 2019-20 and the pooled data analysis, whereas minor fluctuation was noticed in the total sugars content during 2018-19 where decreased up to the second level of concentration and then increased significantly with an increase in the concentration of chemicals. However, non-significant differences were noticed with the higher concentrations of both the chemicals in the pooled data analysis. Among all the treatments, untreated control recorded significantly lowest total sugars content (14.37%, 26.94% and 20.65% respectively during 2018-19, 2019-20 and the pooled data analysis).

Significant variation was noticed among the intervals of observation recorded with respect to the total sugars content in the leaves of tuberose cv.

'Prajwal' by soil application of graded levels of essential heavy metal concentrations. Among the intervals of observation recorded, significantly highest total sugars content (50.60%, 63.79% and 57.20% respectively during 2018-19, 2019-20 and the pooled data analysis) was observed at 360 DAP, whereas significantly lowest total sugars content (20.77%, 57.01% and 38.89% respectively during 2018-19, 2019-20 and the pooled data) was recorded at the initial 90 DAP interval. A gradual and significant increase in the total sugars content of tuberose *cv.* 'Prajwal' was noticed at each successful interval of observation recorded with the passage of time during both the years of study as well as in the pooled data analysis.

The data pertaining to interaction effect between graded levels of essential heavy metal concentrations and the intervals with respect to total sugars content was found significant during 2018-19, 2019-20 and the pooled data analysis. Among the combination treatments, significantly highest total sugars content (136.86%, 147.53% and 142.20% 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 followed by application of $ZnSO_4$ @ 400 mg kg^{-1} soil at 270 DAP (135.00%, 147.16% and 141.08% respectively during 2018-19, 2019-20 and the pooled data analysis), whereas significantly lowest total sugars content (7.80%, 26.06% and 16.93% respectively during 2018-19, 2019-20 and the pooled data analysis) was recorded in the untreated control plants at 90 DAP during 2018-19, 2019-20 and the pooled data analysis. From the study, it is evident that a significant increase in the total sugars content of tuberose leaves was observed with the application of $ZnSO_4$ @ 400 mg kg^{-1} soil. Lal and Maurya (1981) reported a significant increase in the total sugars content of onion leaves by application of $ZnSO_4$ which might be attributed to the increased content of chlorophylls in leaves which in turn increased the carbohydrates content by increased rate of photosynthesis. Jawaharlal and Veeraraghavathatham (1988) also reported similar kind of observation in onion. Marschner (1995) explained that zinc had been identified as an important element in many of the enzymes in plant system and plays as major functional, structural or regulatory co-factor in the photosynthesis of plants and participates in the synthesis of protein, cell division and maintenance of membrane structure. Marschner (1995) also reported that application of zinc to plant regulates the sexual fertilization in plants. Ballab *et al.* (2013) reported multifarious roles of zinc as an element in the physiological and biochemical processes of onion plant as well as Marschner (1995) explained the increased content of total sugars in the leaves of onion.

Conclusion

Lipoxigenase activity, proline and total sugars were found to be significantly higher during the 2nd year of study i.e., 2019-20 in comparison to the 1st year of study i.e., 2018-19 in tuberose cv. 'Prajwal' by soil application of graded levels of essential heavy metals viz., Mn, Cu and Zn.

Table 1 : Influence of applied essential heavy metals (Cu, Mn, Zn) on lipid peroxidation of *Polianthes tuberosa cv.* Prajwal.

Treatment (mg of element kg^{-1} soil)	Lipid peroxidation (n mol g^{-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	0.03	0.03	0.04	0.26	**0.09**	0.03	0.21	0.06	0.28	**0.14**	0.03	0.06	0.05	0.27	**0.10**
$MnSO_4$ 2000	0.05	0.15	0.12	0.07	**0.09**	0.05	0.17	0.17	0.31	**0.17**	0.05	0.09	0.14	0.19	**0.11**
$MnSO_4$ 3000	0.14	0.06	0.15	0.19	**0.13**	0.08	0.13	0.18	0.24	**0.15**	0.11	0.10	0.16	0.21	**0.14**
$CuSO_4$ 100	0.05	0.14	0.14	0.05	**0.09**	0.01	0.15	0.34	0.42	**0.23**	0.03	0.07	0.24	0.23	**0.14**
$CuSO_4$ 200	0.15	0.08	0.17	0.35	**0.18**	0.07	0.24	0.19	0.17	**0.16**	0.12	0.13	0.18	0.27	**0.17**
$CuSO_4$ 300	0.01	0.13	0.23	0.15	**0.13**	0.07	0.35	0.27	0.35	**0.26**	0.04	0.12	0.25	0.24	**0.16**
$ZnSO_4$ 200	0.26	0.45	0.31	0.34	**0.34**	0.36	0.42	0.43	0.63	**0.46**	0.31	0.36	0.37	0.48	**0.38**
$ZnSO_4$ 400	0.35	0.52	0.56	0.65	**0.52**	0.43	0.48	0.64	0.72	**0.56**	0.39	0.43	0.60	0.68	**0.52**
$ZnSO_4$ 600	0.02	0.12	0.15	0.13	**0.10**	0.24	0.18	0.14	0.15	**0.17**	0.13	0.13	0.14	0.14	**0.14**
Control	0.005	0.023	0.02	0.05	**0.02**	0.02	0.01	0.06	0.09	**0.04**	0.01	0.02	0.04	0.07	**0.03**
Mean	**0.10**	**0.17**	**0.18**	**0.22**		**0.13**	**0.23**	**0.24**	**0.33**		**0.12**	**0.15**	**0.22**	**0.27**	

Factor	T (2018-2019)	I (2018-2019)	T × I (2018-2019)	T (2019-2020)	I (2019-2020)	T × I (2019-2020)	T (Pooled)	I (Pooled)	T × I (Pooled)
SEm±	0.005	0.003	0.009	0.006	0.004	0.013	0.005	0.003	0.009
CD at 5%	0.013	0.008	0.027	0.018	0.011	0.036	0.013	0.008	0.026

Table 2 : Influence of applied essential heavy metals (Cu, Mn, Zn) on proline content of *Polianthes tuberosa cv.* Prajwal.

Treatment (mg of element kg^{-1} soil)	Proline content ($\mu g\ g^{-1}$ FW)														
	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	4.61	4.63	3.64	4.27	**4.29**	2.70	3.60	4.06	8.40	**4.69**	3.66	4.10	3.85	6.33	**4.49**
$MnSO_4$ 2000	1.61	1.90	3.18	3.53	**2.55**	5.14	3.26	3.66	3.53	**3.90**	3.37	2.58	3.42	3.53	**3.23**
$MnSO_4$ 3000	1.70	1.57	1.49	2.30	**1.76**	3.18	9.43	9.23	9.10	**7.73**	2.44	5.50	5.36	5.70	**4.75**
$CuSO_4$ 100	1.33	1.74	2.38	1.36	**1.70**	3.46	6.70	6.90	6.48	**5.88**	2.39	4.22	4.64	3.92	**3.79**
$CuSO_4$ 200	2.14	1.59	2.14	3.16	**2.25**	2.71	6.73	7.16	6.70	**5.82**	2.42	4.16	4.65	4.93	**4.04**
$CuSO_4$ 300	3.15	1.51	3.15	1.65	**2.36**	2.72	8.40	8.50	8.66	**7.07**	2.93	4.95	5.82	5.15	**4.71**
$ZnSO_4$ 200	1.80	1.66	1.68	3.30	**2.11**	3.16	7.50	7.80	6.93	**6.34**	2.48	4.58	4.74	5.11	**4.23**
$ZnSO_4$ 400	6.56	7.44	8.62	12.70	**8.83**	6.73	16.10	16.20	16.90	**13.98**	6.64	11.77	12.41	14.80	**11.40**
$ZnSO_4$ 600	3.43	6.85	5.70	7.45	**5.86**	2.59	9.63	9.63	11.50	**8.34**	3.01	8.24	7.66	9.47	**7.09**
Control	0.40	0.63	0.83	0.55	**0.60**	0.16	3.23	2.19	2.53	**2.19**	0.28	1.93	1.51	1.54	**1.31**
Mean	**2.67**	**2.95**	**3.28**	**4.03**		**3.25**	**7.42**	**7.64**	**8.07**		**2.96**	**5.20**	**5.40**	**6.04**	

Factor	**T**	**I**	**T × I**	**T**	**I**	**T × I**	**T**	**I**	**T × I**
SEm±	0.09	0.05	0.18	0.12	0.08	0.25	0.081	0.051	0.162
CD at 5%	0.26	0.16	0.52	0.36	0.22	0.72	0.229	0.145	0.458

Table 3 : Influence of applied essential heavy metals (Cu, Mn, Zn) on total sugars of *Polianthes tuberosa cv.* Prajwal.

Treatment (mg of element kg^{-1} soil)	Total sugars (%)														
	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	19.36	35.00	53.33	54.73	**40.60**	63.13	61.66	59.30	59.53	**60.90**	41.25	48.33	56.31	57.13	**50.75**
$MnSO_4$ 2000	16.76	16.00	22.33	34.60	**22.42**	45.46	51.00	53.33	53.80	**50.90**	31.11	33.50	37.83	44.20	**36.66**
$MnSO_4$ 3000	18.63	15.76	24.66	40.00	**24.76**	43.53	46.66	47.40	48.60	**46.55**	31.08	31.21	36.03	44.30	**35.65**
$CuSO_4$ 100	14.40	34.93	42.13	43.93	**33.85**	55.06	57.33	57.70	57.86	**56.99**	34.73	46.13	49.91	50.89	**45.41**
$CuSO_4$ 200	13.06	24.10	32.66	35.86	**26.42**	38.00	45.73	46.10	47.46	**44.32**	25.53	34.91	39.38	41.66	**35.37**
$CuSO_4$ 300	14.60	26.40	33.90	35.06	**27.49**	38.73	42.06	43.13	43.90	**41.95**	26.66	34.23	38.51	39.48	**34.72**
$ZnSO_4$ 200	15.16	33.56	38.70	54.26	**35.42**	62.66	66.13	66.73	66.93	**65.61**	38.91	49.85	52.71	60.60	**50.52**
$ZnSO_4$ 400	53.83	123.46	135.00	136.86	**112.29**	143.33	146.73	147.16	147.53	**146.19**	98.58	135.10	141.08	142.20	**129.24**
$ZnSO_4$ 600	34.16	36.20	44.00	50.33	**41.17**	54.13	83.86	84.33	84.60	**76.73**	44.14	60.03	64.16	67.46	**58.94**
Control	7.80	8.46	20.83	20.40	**14.37**	26.06	26.83	27.16	27.70	**26.94**	16.93	17.64	23.99	24.05	**20.65**
Mean	**20.77**	**35.38**	**44.75**	**50.60**		**57.01**	**62.80**	**63.23**	**63.79**		**38.89**	**49.09**	**53.99**	**57.20**	

Factor	**T**	**I**	**T × I**	**T**	**I**	**T × I**	**T**	**I**	**T × I**
SEm±	0.64	0.41	1.29	0.66	0.41	1.32	0.44	0.28	0.89
CD at 5%	1.82	1.15	3.65	1.86	1.17	3.72	1.26	0.79	2.52

References

Abdullah, W.S. and Sarem, S.M. (2010). The potential of chrysanthemum and pelargonium for phyto-extraction of lead contaminated soils. *Jordan Journal of Civil Engineering*. **4**(4), 409-16.

Ansari, W.A, Atri, N, Singh, B. and Pandey, S. (2017). Changes in antioxidant enzyme activities and gene expression in two muskmelon genotypes under progressive metal stress. *Biologia Plantarum*. **61**, 333-41.

Auty, Y.K. (2011). Standardization of harvesting stage and chemical treatments in Orchid (*Dendrobium sp.*) cv. Sonia Red. M.Sc. (Hort.) thesis submitted to Navsari Agricultural University, Navsari, Gujarat, India.

Ballabh, K, Rana, D.K. and Rawat, S.S. (2013). Effects of foliar application of micronutrients on growth, yield and quality of onion. *Indian Journal of Horticulture.* **70**(2), 260-65.

Bates, L.S, Waldren, R.P. and Teare, I.D. (1973). Rapid determination of free proline for water stress studies. *Plant and Soil*. **39**, 205-07.

Bohra, A, Dheera, S. and Chauhan, R. (2015). Heavy metal toxicity and tolerance in plants with special reference to cadmium: A Review. *Journal of Plant Science Research*. **31**(1), 51-74.

Chakraborty, D, Verma, A.K. and Datta, S.K. (2009). Oxidative stress and anti-oxidant activity on the basis of senescence in hemerocallis (daylily) flowers. *Journal of Horticulture and Forestry*. **1**(6), 113-19.

Dhindsa, R., Plumb-Dhindsa, P. and Thorpe, T.A. (1981). Leaf senes- cence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Expt. Bot*. **32**(126), 93-101.

Galliard, T. (1978). Lipolytik and lipoxygenase enzymes in plants and their action in wounded tissues, p. 155-201. In: G. Kahl (ed.). Biochemistry of wounded plant tissues. De Gruyter, New York.

Galliard, T. and Chan, H.W.S. (1980). Lipoxygenases, p. 131-161. In: P.K. Stumpf and E.E. Conn (eds.). The biochemistry of plants. vol.4. Academic, New York

Hayat, S., Hayat, Q., Alyemeni, M.N. and Ahmad, A. (2014). Cumulative effects of proline & salicylic acid on the cadmium-induced changes in *Cicer arietinum* L. *Fresenius Environmental Bulletin*. **23**(2), 330-40.

Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts-I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophys*ics, **125**, 189 -98.

Hossain, Z., Mandal, A.K.A., Datta, S.K. and Biswas, A.K. (2006). Decline in ascorbate peroxidase activity - a prerequisite factor for tepal senescence in gladiolus. *Journal of Plant Physiol*ogy, **163**, 186-94.

Jawaharlal, M. and Veeragavathatham, D. (1988). Effect of zinc and iron on the quality of onion (*Allium cepa* L.) bulbs. *South Indian Horticulture*. **36**(3), 114 -17.

Kockritz, A., Schewe, T., Hieke, B. and Hass, W. (1985). The effects of soybean lipoxygenase-1 on chloroplasts from wheat. *Phytochemistry*, **24**(3), 381-384

Lal, S. and Maurya, A.N. (1981). Effect of zinc on onion. *Haryana Journal of Horticultural Sciences*, **10** (3-4): 231-35.

Leshem, Y.Y., Liftman, Y., Grossman, S. and Frimer, A.A. (1981). Free radicals and pea foliage senescence: increase of lipoxigenase and ESR signals and cytokinin-induced changes, p. 676-678. In: M.A.J. Rodgers and E.L. Powers (eds.). Oxygen and oxy-radicals in chemistry and biology. Academic, New York.

Marschner, H. (1995). Mineral nutrition of higher plants. Academic Press, London.

Morrison, R.S, Brooks, R.D., Reeves, R.D., Malaise, F., Horowitz, P., Aronson, M. and Merriam, G.R. (1981). The diverse chemical form of heavy metals in tissue extracts of some metallophytes from Shaba province, Zaire. *Phytochemistry*, **20**, 455-58.

Panse, V.G. and Sukhatme, P.V. (1978). Statistical methods for agricultural workers. *Indian Council of Agricultural Research*, New Delhi. *pp*: 1-317.

Pauls, K.P. and Thompson, J.E. (1984). Evidence for the accumulation of peroxidized lipids in membranes of senescing cotyledons. *Plant Physiol*. **75**, 1152-1157.

Peary, J.S. and Prince, T.A. (1990). Floral lipoxygenase activity during senescence and inhibition by phenidone. *Jornal of American Society for Horticultural Sciences*, **115**, 455-57.

Peterman, T.K. and Siedow, J.N. (1985). Behavior of lipoxigenase during establishment, senescence, and rejuvenation of soybean cotyledons. *Plant Physiology*. **78**, 690-695.

Ramana, S., Biswas, A.K., Ajay, B., Singh, A.B., Ahirwar, N.K. and Rao, A.S. (2013b). Phytoremediation ability of some floricultural plant species. *Indian Journal of Plant Physiology*, **18**(2), 187-90.

Ramana, S., Biswas, A.K., Singh, A.B., Ajay, B., Kumar, N.P., Ahirwar, N.K., Behara, S.K. and Rao, A.S. (2012). Phytoremediation of cadmium contaminated soils by tuberose. *Indian Journal of Plant Physiology*. **17**(1), 61-64.

Rubinstein, B. (2000). Regulation of cell death in flower petals. *Plant Molecular Biology*. **44**, 303-18.

Sayyari, M., Ghavami, M., Ghanbari, F. and Kordi, S. (2013). Assessment of growth rate and some physiological parameters of lettuce plants grown under metal stress conditions. *International Journal of Agriculture and Crop Sciences*, **5**(17), 1951-57.

Singh, A., Kumar, J. and Kumar, P. (2008). Effects of plant growth regulators and sucrose on postharvest physiology, membrane stability and vase life of cut spikes of gladiolus. *Plant Growth Regulation*, **55**, 221-29.

Subhashini, V., Swamy, A.V.S., Harika, D. and Rao, K.V. (2017). Phytoremediation of heavy metals in the contaminated soils. *International Journal of Current Microbiology and Applied Science*, **5**, 19-30.

Yemm, E.W. and Willis, A.J. (1954). The estimation of carbohydrates in plant extracts by anthrone. *Biochemical Journal*, **57**, 508-14.