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EFFECT OF FOLIAR APPLICATION OF DIFFERENT CONCENTRATIONS OF NANO AND BULK MICRONUTRIENTS (Fe AND Zn) ON BIOCHEMICAL PARAMETERS OF GERBERA GROWN UNDER POLYHOUSE CONDITIONS

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ABSTRACT

A study was carried out to determine the effect of foliar application of different concentrations of nano scale zinc oxide and iron oxide on biochemical parameters of gerbera (*Gerbera jamesonii Bolus ex. Hooker*). The results revealed that foliar application of T_9- ZnO @ 200 ppm + FeO @ 300 ppm recorded maximum chlorophyll-a (1.17 mg g^{-1} and 1.28 mg g^{-1}), chlorophyll-b (0.39 mg g^{-1} FW and 0.42 mg g^{-1} FW) and total chlorophyll content in leaves (1.56 mg g^{-1} FW and 1.69 mg g^{-1} FW) at 180 and 360 DAP respectively. Similar trend was observed with respect to carotenoid content in flower petals. Maximum carotenoid content in flower petals was recorded in T_9- ZnO @ 200 ppm + FeO @ 300 ppm (6.73 mg 100 g^{-1} and 8.84 mg 100 g^{-1}) at 180 and 360 DAP respectively. The catalase and peroxidise activity was found to be highest in T_{12}- control.
***Keywords*:** Gerbera, Nano, Iron, Zinc and physiological parameters

Introduction

Gerbera (*Gerbera jamesoni bolus ex.hooker*) belongs to the family Asteraceae is one of the most important cut flower in the world and it currently ranks as the fifth most popular cut flower in the world after the roses, carnations, chrysanthemums and tulip. Gerbera can contribute largely to floriculture industry by virtue of its yield potential, colour variation and long vase life. Cultivation of Gerbera under polyhouse has emerged as a very important option to progressive farmers in a many parts of India. But farmers are facing some problems in gerbera cultivation such as environmental and technological changes, disease pest infestation and physiological disorders caused by micro nutrient deficiencies which reduces the quality and yield. So commercialization of gerbera by using modern technologies will improve the cultivation condition and also enhances the quality and yields. Recently, Nanotechnology is gaining a lot of importance and has been used in all stages of crop production. Physiological and biochemical processes in plants are significantly affected by the application of nano micronutrients. Application of nano scale nutrients inhibits or perturbs the biosynthesis of proteins, the endogenous hormone balance, gas-exchange in leaves, water exchange, and enzyme activities. Foliar application of different concentrations of nano micronutrients on plants influences the plant biochemical characteristics like amount of chlorophyll synthesised which regulates the photosynthesis and thus increasing the flower yield and quality. Nanoscale nutrients also influences the antioxidant enzyme activities which are involved in protecting themselves against the harmful effects of reactive oxygen species (ROS). Evidence has shown that increase in the biochemical activity of plants significantly affects the growth and yield of plants. The main aim of present study was to determine the effect of nano scale micronutrients on biochemical parameters of Gerbera.

Material and Methods

The experiment was carried out at Central Laboratory, College of Horticulture, Anantharajupeta, Dr. Y.S.R. Horticultural University, A.P. India. The leaves and flowers of gerbera were collected from plants grown under naturally ventilated polyhouse located at College of Horticulture, Anantharajupeta. The plants were treated with different concentrations of nano zinc oxide and nano iron oxide as foliar spray. The leaves were collected during morning hours and placed in ice cold box. The procedures used for estimation of chlorophyll, carotenoids, catalase and peroxidase are as below.

1. Chlorophyll content

500 mg of fresh leaf sample was chopped into fine bits and immersed in 10 ml of DMSO. Then, samples were incubated at 70°C for 4 hours in hot air oven. After

incubation collect the samples and cool it until it drops down to room temperature. Take 1 ml of above pure solution and is diluted to 5 ml with DMSO. Finally take absorbance readings at 645 and 663 nm using UV – VIS spectrophotometer. Total chlorophyll, chlorophyll- a and chlorophyll-b were calculated using the formula given by Lichtenthaler (1987) and expressed in milli gram per gram fresh weight.

$$\text{Chlorophyll 'a' (mg/g)} = \frac{(12.7 \times A663) - (2.69 \times A645) \times V \times D}{1000 \times W}$$

$$\text{Chlorophyll 'b' (mg/g)} = \frac{(22.7 \times A645) - (4.68 \times A663) \times V \times D}{1000 \times W}$$

$$\text{Total Chlorophyll (mg/g)} = \frac{(20.2 \times A645) + (8.02 \times A663) \times V \times D}{1000 \times W}$$

Where,

W = weight of fresh plant tissue (0.5 g)
V = final volume of chlorophyll extract (ml)
A = Absorbance at specific wave length
D = Dilution factor

2. Carotenoid content

2 g of flower petals was taken and cut into small pieces. Then, add 20 ml of acetone to the sample present in a beaker. Place the beakers in undisturbed condition for 24 hours. Next day, shake the beakers and kept it undisturbed for 5-10 minutes. Separate the sample through Whattmann filter paper and collect the mixture through separating funnel. Then add 10 ml of petroleum ether and 10 ml of 10% sodium sulphate to above sample. Mix the contents thoroughly and keep it undisturbed for 15-20 minutes in a separating funnel. Then the sample get separated into three layers (from bottom 1st layer- Acetone, 2nd layer- sodium sulphate and 3rd layer- petroleum ether). Take the absorbance values of carotenoids (petroleum ether layer consists of carotenoids) at 452 nm. Carotenoid content in petals was calculated using the formula given by Srivasthava and Kumar (2002) and expressed in milli gram per 100 gram weight.

$$\text{Carotenoid content}\left(\text{mg } 100\,\text{g}^{-1}\right) = \frac{\text{O.D.} \times 13.9 \times 10^4 \times 100}{\text{Weight of sample (g)} \times 560 \times 1000}$$

3. Peroxidase enzyme activity (O.D min $^{-1}$ g $^{-1}$ FW)

1 gram of fresh leaf tissue (leaf) was grinded in pre cooled mortar and pestle and extracted with 10 ml of 0.1 M phosphate buffer (pH 7.0). Then centrifuge the homogenate at (18,000 rpm) at 4°C for 15 minutes and the supernatant containing enzyme source is used within 2-4 hours and was stored on ice till the assay was carried out. Pipette out 2.4 ml of phosphate buffer, 0.2 ml of 20 mM guaicol solution. 0.2 ml of 0.042 % hydrogen peroxidise and 0.2 ml of enzyme extract in cuvettes and mix well. The same used as blank using 0.2 ml of distilled water instead of enzyme extract. Read the absorbance at 436 nm in UV-Vis Spectrophotometer. Sadasivam and Manickam (1992).

Peroxidase (O.D min^{-1} g^{-1}) = (Maximum absorbance – minimum absorbance) x 60 x2

4. Catlase enzyme activity (O.D. min $^{-1}$ g $^{-1}$ FW)

According to Aebi (1984), 0.5 g of fresh leaf tissue was homogenized and grinded with 0.05 M phosphate buffer and 1 ml of 1% poly vinyl pyrrolidine in a pre cooled mortar and pestle. Then centrifuge (10,000 rpm) at 4°c for 15 minutes and collect the supernatant containing enzyme extract. Pipette out 2 ml of 50 mM phosphate buffer, 0.95 ml of 0.03% hydrogen peroxide solution and 0.05 ml of enzyme extract in a cuvette and mix it well. The same used as blank using 0.05 ml of distilled water instead of enzyme extract. Read the absorbance at 240 nm in UV- Vis Spectrophotometer by kinetic method.

Catalase (O.D min^{-1} g^{-1}) = (Maximum absorbance – minimum absorbance) x 60 x2

Results and Discussion

1. Chlorophyll content in leaves (mg g^{-1} FW)

The data pertaining to chlorophyll content of leaf at 180 and 360 DAP, as influenced by nano and conventional micronutrients showed significant variation. At 180 DAP, T_9- ZnO @ 200 ppm + FeO @ 300 ppm recorded maximum chlorophyll-a, chlorophyll-b and total chlorophyll content in leaves (1.17 mg g^{-1} FW, 0.39 mg g^{-1} FW and 1.56 mg g^{-1} FW respectively) which was statistically on par with T_7- ZnO @ 300 ppm + FeO @ 300 ppm (1.15 mg g^{-1} FW, 0.37 mg g^{-1} FW and 1.52 mg g^{-1} FW respectively), T_3- FeO @ 300 ppm (1.10 mg g^{-1} FW, 0.34 mg g^{-1} FW and 1.44 mg g^{-1} FW respectively), T_4- FeO @ 200 ppm (1.07 mg g^{-1} FW, 0.32 mg g^{-1} FW and 1.39 mg g^{-1} FW respectively), T_8- ZnO @ 300 ppm + FeO @ 200 ppm (1.05 mg g^{-1} FW , 0.30 mg g^{-1} FW and 1.35 mg g^{-1} FW respectively) and T_{10}- ZnO @ 200 ppm + FeO @ 200 ppm (1.03 mg g^{-1} FW, 0.28 mg g^{-1} FW and 1.31 mg g^{-1} FW respectively), whereas minimum was recorded with T_{12}- control (0.70 mg g^{-1} FW, 0.12 mg g^{-1} FW and 0.82 mg g^{-1} FW respectively).

At 360 DAP, T_9- ZnO @ 200 ppm + FeO @ 300 ppm recorded maximum chlorophyll-a, chlorophyll-b and total chlorophyll content in leaves (1.28 mg g^{-1} FW, 0.42 mg g^{-1} FW and 1.69 mg g^{-1} FW respectively) which was statistically on par with T_7- ZnO @ 300 ppm + FeO @ 300 ppm (1.21 mg g^{-1} FW, 0.39 mg g^{-1} FW and 1.60 mg g^{-1} FW respectively), T_3- FeO @ 300 ppm (1.17 mg g^{-1} FW, 0.36 mg g^{-1} FW and 1.53 mg g^{-1} FW respectively), T_8- ZnO @ 300 ppm + FeO 200 @ ppm (1.14 mg g^{-1} FW , 0.35 mg g^{-1} FW and 1.49 mg g^{-1} FW respectively) and T_4- FeO @ 200 ppm (1.13 mg g^{-1} FW, 0.33 mg g^{-1} FW and 1.46 mg g^{-1} FW respectively) whereas, minimum was recorded with T_{12}- control (0.75 mg g^{-1} FW, 0.14 mg g^{-1} FW and 0.89 mg g^{-1} FW respectively).

Iron serves as a component of chlorophyll and is essential for its synthesis. Foliar application of iron increases the level of chlorophyll and other carotenoids in all plant species and leads to increase in photosynthesis. Additionally, the photosynthetic efficiency, the structure and function of the photosynthetic apparatus are heavily dependent on iron, directly or indirectly via the porphyrin biosynthesis pathway Briat *et al.* (2015). Deficiency of iron decreases all components of the electron transport chain. As iron is directly involved in protein synthesis, Fe deficiency decreases total proteins including ribulose-1,5-bisphosphate (rubisco), which plays a critical role in the carbon cycle of C_3 plants Prasad (2003).

In this study, chlorophyll content of gerbera leaves increased with increasing Fe_2O_3 concentration. Treatment of nano iron fertilizer in different concentrations causes significant increase in content of chlorophyll a, chlorophyll b and total chlorophyll content compared to bulk $FeSO_4$. Similar results were observed by Rombola *et al.* (2005) in beet root,

Moghadam *et al.* (2015) in cucumber, Rizwan *et al.* (2019) in wheat and Pouraghdam *et al.* (2019) in rosemary.

2. Carotenoid content in flower petals (mg 100 g^{-1})

Carotenoid content of gerbera flower petals recorded at 180 and 360 DAP as influenced by different concentrations of Fe and Zn in nano and bulk forms varied significantly. Maximum carotenoid content in flower petals was recorded in T_9- ZnO @ 200 ppm + FeO @ 300 ppm (6.73 mg 100 g^{-1} and 8.84 mg 100 g^{-1}) which stood statistically at par with T_7- ZnO @ 300 ppm + FeO @ 300 ppm (6.62 mg 100 g^{-1} and 8.67 mg 100 g^{-1}) and T_8- ZnO @ 300 ppm + FeO @ 200 ppm (6.57 mg 100 g^{-1} and 8.57 mg 100 g^{-1}) where as mimimum was recorded in T_{12}- control (2.61 mg 100 g^{-1} and 3.11 mg 100 g^{-1}) at 180 and 360 DAP respectively.

3. Catalase activity (O.D $min^{-1}g^{-1}$ FW)

The enzyme activity increases with the increase in age of the plant. At 180 DAP, the catalase activity showed non significant differences and found to be lowest in T_8- ZnO @ 300 ppm + FeO @ 200 ppm (0.17 O.D $min^{-1}g^{-1}$ FW) and highest in T_{12}- control (0.57 O.D $min^{-1}g^{-1}$ FW).

At 360 DAP, the catalase enzyme activity showed significant differences among the treatments and was recorded lowest in T_8- ZnO @ 300 ppm + FeO @ 200 ppm (0.36 O.D $min^{-1}g^{-1}$ FW) which was statistically similar with all other treatments in the study except control where as highest was recorded in T_{12}- control (1.67 O.D $min^{-1}g^{-1}$ FW).

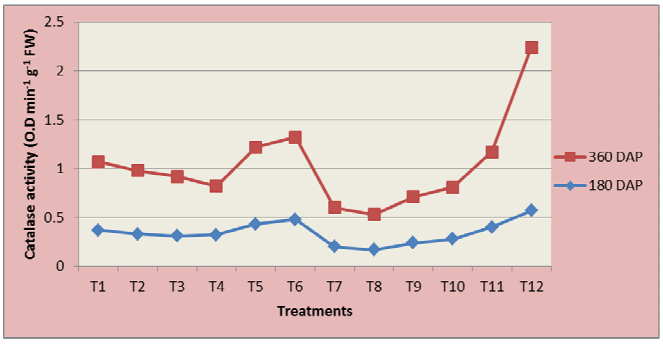

Fig. 1: Effect of nano and conventional micronutrients (Zn and Fe) on catalase activity (O.D $min^{-1}g^{-1}$ FW) of gerbera leaf at 180 and 360 DAP.

4. Peroxidase activity (O.D $min^{-1}g^{-1}$ FW)

Peroxidase activity exhibited similar trend as that of catalase activity. At 180 DAP, non significant differences were found with respect to peroxidise activity. Lowest peroxidase activity was observed in T_8- ZnO @ 300 ppm + FeO @ 200 ppm (0.17 O.D $min^{-1}g^{-1}$ FW) and highest in T_{12}- control (0.57 O.D $min^{-1}g^{-1}$ FW).

At 360 DAP, lowest peroxidise activity was recorded in T_8- ZnO @ 300 ppm + FeO @ 200 ppm (1.17 O.D $min^{-1}g^{-1}$ FW) which was statistically similar with all other treatments in the study except control where as highest was recorded in T_{12}- control (3.70 O.D $min^{-1}g^{-1}$ FW).

In the present study, plants treated with iron and zinc in both nano and bulk forms exhibited lower antioxidant enzyme activity (catalase and peroxidase) and it was also found to be highest in control plants indicating that application of iron and zinc in nano and bulk forms does not induce any additional stress to the plant system. In contrast, higher antioxidant enzyme activity (catalase and peroxidise) in control plants may be due to oxidative stress. During oxidative stress, antioxidant enzymes activity is reported to increase manifolds probably due to activation of defense mechanism. Increase in enzyme activity leads to more scavenging of reactive oxygen species and therefore, protecting the cells from damage Delaplace *et al.* (2009).

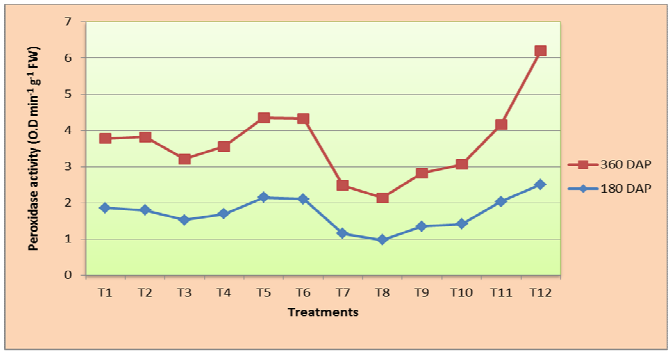

Fig. 2 : Effect of nano and conventional micronutrients (Zn and Fe) on peroxidase activity (O.D $min^{-1}g^{-1}$ FW) of gerbera leaf at 180 and 360 DAP.

Table 1 : Effect of nano and conventional micronutrients (Zn and Fe) on chlorophyll content (mg g^{-1} fw) of gerbera leaf at 180 and 360 DAP.

Treatments	Chlorophyll – a (mg g^{-1} fw)		Chlorophyll – b (mg g^{-1} fw)		Total chlorophyll (mg g^{-1} fw)	
	180 DAP	360 DAP	180 DAP	360 DAP	180 DAP	360 DAP
T_1- ZnO @ 300 ppm	0.90	1.00	0.19	0.22	1.10	1.22
T_2- ZnO @ 200 ppm	0.92	1.01	0.20	0.26	1.12	1.26
T_3- FeO @ 300 ppm	1.10	1.17	0.34	0.36	1.44	1.53
T_4- FeO @ 200 ppm	1.07	1.13	0.32	0.33	1.39	1.46
T_5- $FeSO_4$ @ 0.2%	0.93	0.98	0.21	0.25	1.14	1.23
T_6- $ZnSO_4$ @ 0.2%	0.81	0.87	0.18	0.23	0.99	1.10
T_7- ZnO @ 300 ppm + FeO @ 300 ppm	1.15	1.21	0.37	0.39	1.52	1.60
T_8- ZnO @ 300 ppm + FeO @ 200 ppm	1.05	1.14	0.30	0.35	1.35	1.49
T_9- ZnO @ 200 ppm + FeO @ 300 ppm	1.17	1.28	0.39	0.42	1.56	1.69
T_{10}- ZnO @ 200 ppm + FeO @ 200 ppm	1.03	1.03	0.28	0.28	1.31	1.31
T_{11}- $ZnSO_4$ @ 0.2% + $FeSO_4$ @ 0.2%	0.94	1.02	0.22	0.27	1.16	1.29
T_{12}- control (Water spray)	0.70	0.75	0.12	0.14	0.82	0.89
Mean	0.98	1.05	0.26	0.29	1.24	1.34
S.Em ±	0.02	0.03	0.02	0.01	0.03	0.03
C.D (P=0.05)	0.07	0.09	0.05	0.04	0.09	0.10

Table 2 : Effect of nano and conventional micronutrients (Zn and Fe) on carotenoid content (mg 100 g^{-1} fw) of gerbera flower petals at 180 and 360 DAP.

Treatments	Carotenoid content (mg $100g^{-1}$)	
	180 DAP	360 DAP
T_1- ZnO @ 300 ppm	4.01	4.91
T_2- ZnO @ 200 ppm	3.81	5.07
T_3- FeO @ 300 ppm	6.10	7.33
T_4- FeO @ 200 ppm	6.05	7.11
T_5- $FeSO_4$ @ 0.2%	5.07	6.31
T_6- $ZnSO_4$ @ 0.2%	3.67	4.57
T_7- ZnO @ 300 ppm + FeO @ 300 ppm	6.62	8.67
T_8- ZnO @ 300 ppm + FeO @ 200 ppm	6.57	8.57
T_9- ZnO @ 200 ppm + FeO @ 300 ppm	6.73	8.84
T_{10}- ZnO @ 200 ppm + FeO @ 200 ppm	6.06	7.16
T_{11}- $ZnSO_4$ @ 0.2% + $FeSO_4$ @ 0.2%	5.26	6.56
T_{12}- control (Water spray)	2.61	3.11
Mean	5.21	6.52
S.Em ±	0.14	0.19
C.D (P=0.05)	0.41	0.55

Table 3 : Effect of nano and conventional micronutrients (Zn and Fe) on catalase and peroxidise activity (O.D $min^{-1}g^{-1}$ FW) of gerbera leaves at 180 and 360 DAP.

Treatments	Catalase (O.D min^{-1} g^{-1} FW)		Peroxidase (O.D min^{-1} g^{-1} FW)	
	180 DAP	360 DAP	180 DAP	360 DAP
T_1- ZnO @ 300 ppm	0.37	0.70	1.86	1.92
T_2- ZnO @ 200 ppm	0.33	0.65	1.80	2.02
T_3- FeO @ 300 ppm	0.31	0.61	1.53	1.68
T_4- FeO @ 200 ppm	0.32	0.50	1.69	1.87
T_5- $FeSO_4$ @ 0.2%	0.43	0.79	2.15	2.20
T_6- $ZnSO_4$ @ 0.2%	0.48	0.84	2.10	2.23
T_7- ZnO @ 300 ppm + FeO @ 300 ppm	0.20	0.40	1.15	1.33
T_8- ZnO @ 300 ppm + FeO @ 200 ppm	0.17	0.36	0.97	1.17
T_9- ZnO @ 200 ppm + FeO @ 300 ppm	0.24	0.47	1.35	1.47
T_{10}- ZnO @ 200 ppm + FeO @ 200 ppm	0.28	0.53	1.41	1.65
T_{11}- $ZnSO_4$ @ 0.2% + $FeSO_4$ @ 0.2%	0.40	0.77	2.03	2.13
T_{12}- control (Water spray)	0.57	1.67	2.51	3.70
Mean	0.34	0.70	1.71	1.95
S.Em ±	-	0.17	-	0.37
C.D (P=0.05)	NS	0.49	NS	1.08

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