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Research Article
Effect of Mycorrhizal Fungi (AMF), Brassinosteroids and Sodium Silicate on Vegetative Growth, Flower Production and Pb Concentration of Zinnia (*Zinnia elegans*) Plant Under Pb Stress

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Abstract

Background and Objective: In recent years, heavy metal pollution is increasing rapidly, resulting in many environmental problems. This study was carried out to investigate the effect of lead soil pollution on the vegetative growth, flower production and enzyme activity of zinnia plant (*Zinnia elegans*) in the presence of mycorrhizal fungi, brassinosteroids and silicon. **Materials and Methods:** The Plants were treated with lead nitrate $Pb(NO_3)_2$ as a soil addition at 100 and 200 ppm Pb kg^{-1} soil, in addition 2 mycorrhiza treatments by using *Glomus mosseae* and *G. fasciculatum* at the rate of 500 and 1000 spores pot^{-1} [7 kg soil], foliar spray of Brassinosteroids by using 24-epibrassinolide at 10^{-6} and 10^{-8} M and sodium silicate at 50 and 100 ppm, in addition to the control. The statistical analysis was done by using ANOVA analysis, Least Significant Difference (LSD) was applied $p = 0.05$ probability level to compare the mean of the treatments. **Results:** The results indicated that the Pb at 200 ppm gave the lowest values for most studied characteristics, but increased Pb concentration in all organs, a/b ratio and peroxidase activity with high Pb level. The different treatments especially 24-epi at 10^{-8} M, sodium silicate at 50 ppm and then followed by mycorrhiza at 1000 spores increased all characteristics under Pb conditions, except Pb concentration and peroxidase activity decreased compared the untreated control plant. **Conclusion:** The data provided evidence that 24-epi, sodium silicate and mycorrhiza treatments reduced the adverse effects of Pb stress on zinnia plants and might play a key role in providing stress tolerance.

Key words: *Zinnia elegans*, zinnia, mycorrhiza, brassinosteroids, 24-epibrassinolide, sodium silicate, lead nitrate, growth, Pb concentration, pigments, enzyme activity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Soil contamination with toxic heavy metals has become a critical environmental concern because of its adverse effects on ecosystems. Elevated heavy metals can be attributed to a number of human activities and industrial processes such as smelting, mining, coal burning, electroplating and the production of leaded gasoline. The large amount of lead (Pb) derived from industrial activities has caused severe environmental pollution. As one of the most abundant and non-essential elements in soil, high dose of Pb probably results in metabolic disorders, growth inhibition and death for most plant species[1].

Ornamental plants are an important type of higher plants, which can be used to remedy contaminated soils from abundant plant species and types, they may bring economic benefits because they can beautify the environment at the same time. Zinnia (*Zinnia elegans*) an annual flowering plant of the genus Zinnia, belongs to the Asteraceae family and is native to Southwestern United States and Mexico to Central America[2].

Mycorrhizae (AMF) are of the most prominent soil microorganisms, engaging in mutual symbiosis with most terrestrial plants[3]. The AMF can enhance host plant heavy metal tolerance by influencing the fate of the metals in both plants and soil and by improving plant nutrient acquisition[4]. The extra radical mycelium of AMF acts as a plant root extension and can reach beyond the root depletion zone, enabling a thorough exploration of the soil for water and mineral nutrients[5]. However, the roles of AMF association in host plant heavy metal tolerance and phytostabilization efficiency are still broadly unknown.

Brassinosteroids have been suggested to increase the resistance of the plants to heavy metal stress. In this respect, 24-epibrassinolide (EBL) as a type of brassinosteroids has a protective role of the photochemical activity of photosystem, chlorophyll content, membrane lipids and proteins. The effects by 24-epibrassinolide were closely associated with EBL-induced changes in antioxidant contents and anti-oxidative enzyme activities and they proposed that EBL could improve plant growth under heavy metals stress. Also, accumulation of heavy metals (copper, cadmium, lead and zinc) under the influence of BR has been studied for different agricultural plants. It was indicated that the application of 24-epiBL significantly reduced the metal absorption[6].

Silicon has been shown to relieve metal toxicity in some species, though silicon is not an essential plant element nor it is included in traditional fertility programs, but it is typically abundant in soils and can be taken up in large amounts by plants. Silicon is known to have beneficial effects when added to plants. Richmond and Sussman[7] and Ma and Yamaji[8] have reported that this might be a beneficial result of adding Si on plant growth under stress conditions, because it is out of the way that Si affects the activity of antioxidant enzymes. Hossain *et al.*[9] indicated that the silicon applied, modifies the cell wall architecture, which may be responsible for the increase in the cell wall extensibility.

Therefore, this study was conducted to investigate the effect of mycorrhiza, sodium silicate or 24-epi at the 2 different rates of each, on the vegetative growth and flowering of zinnia (*Zinnia elegans*) plants grown under Pb stress. The information of this study may help in some extend to reducing the harmful effects of lead on zinnia plant.

MATERIALS AND METHODS

Two experiments had been carried out at the nursery area Faculty of Agriculture, Cairo University, Giza during 2014 and 2015 to study the effect of mycorrhizal fungi (AMF), brassinosteroids and sodium silicate on the vegetative growth, flower production, Relative Growth Rate (RGR), Net Assimilation Rate (NAR), Specific Utilization Rate (SUR), Pb concentration and pigments (chl a, b, total chl a+b, chl a/b ratio, total carotenoids and total chlo/total carotenoids) of zinnia plant (*Zinnia elegans*) under Pb conditions. On the 17th of Feb., 2014 and 2015 (in the 1st and 2nd season, respectively) seeds of zinnia (*Zinnia elegans*) were sown in clay pots and when the seedlings reached 10 cm height they were transplanted on the 20th April (2 seedling pot^{-1} carrying 3 pairs of leaves) in plastic pots (30 cm in diameter) filled with 7 kg of growing media [sand and clay at the ratio of (1:1, v/v)]. The recommended NPK fertilization using Crystalon 18:18:18, at the rate of 0.5 g pot^{-1} was applied twice in the soil every 21 days.

The soil was taken from Giza governorate and its particles size distribation was as follows: 35.27% coarse sand, 12.32% very coarse sand, 5.08% medium sand, 9.94% fine sand, 11.49% very fine sand, 13.04% silt and 12.86% clay. The soil chemical analysis was as followes: sandy loam, pH 7.7, Ec (Electrical conductivity) 0.76 (ds m^{-1}), Pb 9.86 (ppm) and 2.50, 2.1, 2.3, 1.5, 2.3, 0.6 (mEq L^{-1}) for chloride, sulphate, calcium, magnesium, sodium and potassium, respectively[10].

In both seasons, the established plants were treated with Pb as lead nitrate $Pb(NO_3)_2$, added as a soil application at the rates of 0, 100 and 200 ppm Pb kg^{-1} soil and supplied separately with different treatmeants, including Mycorrhiza as *Glomus mosseae* and *G. fasciculatum* as a soil addition at 500 or 1000 spores pot^{-1}, Sodium Silicate [Na_2SiO_3] as a

foliar spray at 50 or 100 ppm and Epibrassinolide as 24-epibrassinolide as a foliar spray at 10^{-6} or 10^{-8} M. The plants were sprayed with sodium silicate and 24-epibrassinolide 3 times started at the day 21 after transplanting (at 11th of May) and repeated every 15 days. In both seasons 3 plants sample were taken at 31, 56 and 81 days after transplanting and recorded the vegetative growth characters (plant height (cm), number of branches/plant, number of leaves/plant, stem diameter (cm), root length (cm), dry weight of shoot and root/plant (g), total dry weight of plant (mg), shoot/root dry weight ratio and total leaf area $(cm)^2$), pigments (Chlorophyll a, Chlorophyll b, Total carotenoids, a/b ratio, Total chlorophyll (a+b) and Total chlorophyll/Total carotenoides ratio), flowering characters (Number of inflorescences/plant, Inflorescences diameter (cm), flowering period (day) and dry weight of inflorescences (g/plant)) were recorded in the third sample (at 81 days after transplanting) in both seasons, Pb concentration was recorded at 56 and 81 days after transplanting (second and third samples of the 2nd season). Antioxidant enzyme activities, catalase (CAT), peroxidase (POD) and superoxide dismutase (SOD) were determined only at 56 days after transplanting of 2nd season. Moreover, from the dry weight and leaf area, the plant growth analysis between 31-56 days after transplanting and 56-81 days after transplanting were also recorded, e.g., Relative Growth Rate (RGR) mg g^{-1} day^{-1}, Net Assimilation Rate (NAR) mg $(cm^2)^{-1}$ day^{-1} and Specific Utilization Rate (SUR) mg g Pb^{-1} d^{-1}, according to the equation described by Hunt[11], as the following Eq:

$$RGR_{2-1} = \frac{Ln\ W_2 - Ln\ W_1}{T_2 - T_1}$$

$$RGR_{3-2} = \frac{Ln\ W_3 - Ln\ W_2}{T_3 - T_2}$$

where, W_1 and W_2 represent total plant dry weights of the first and second sample, respectively. times T_1 and T_2 the time at the first and the second sample, respectively.

$$NAR_{2-1} = \frac{W_2 - W_1}{T_2 - T_1} \times \frac{Ln\ L_{A2} - Ln\ L_{A1}}{L_{A2} - L_{A1}}$$

$$SUR_{3-2} = \frac{W_3 - W_2}{T_2 - T_1} \times \frac{Ln\ M_{Pb3} - Ln\ M_{Pb2}}{M_{Pb3} - M_{Pb2}}$$

Determinations of Pb were carried out on the dry material, according to the method described by Piper[12]. Lead concentrations were determined by using the Atomic Absorption Spectrophotometer (PerkinElmer 100 B, US).

Pigments determination: Chlorophyll a, b and carotenoids were determined in fresh leaf samples according to Saric *et al.*[13].

Extraction and assaying of antioxidant enzymes activities: The method adopted in enzyme extraction was that described by Mukherjee and Choudhuri[14].

Assay of catalase activity (CAT) EC 1.11.1.6: The CAT activity was assayed according to the method of Kar and Mishra[15].

Assay of superoxide dismutase activity (SOD) EC 1.15.1.1: The SOD activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described by Marklund and Marklund[16].

Assay of peroxidase activity (POD) EC 1.11.1.7: The POD activity was assayed following a modification for method of Kar and Mishra[15].

A factorial experiment was imposed in 3 replication in a completely randomized design, the treatments consist of 3 levels of Pb (0,100,200 ppm Pb kg^{-1} soil)×7 additive treatments (including the control). The combined analysis of the 2 growing seasons was done as the homogeneity test proved that both seasons followed a similar trend.

Statistical analysis: All data were analyzed using CoStat version 6.3.1.1 for windows and the statical analysis was done by using two-way of variance (ANOVA) analysis followed by Least Significant Difference (LSD) test was applied p = 0.05, probability level to compare the mean of the treatments[17].

RESULTS AND DISCUSSION

Growth characters: The data presented in Table 1-5 indicate that in the three samples the presence of lead (Pb) in the soil significantly decreased (p = 0.05) all of the studied growth characters (Plant height, branches number, leaves number, stem diameter, root length, total leaf area, dry weight of shoot and root and total dry weight) of zinnia plants, but increased shoot: root dry weight ratio. In all samples, the toxicity effect of Pb on the average of growth characters were slightly at the lowest concentration of Pb (100 ppm), but at the highest concentration (200 ppm), highly inhibitory effects were observed.

Table 1: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on plant height (cm), number of branches of *Zinnia elegans* plant in the first, second and third sample (combined of the 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Plant height (cm)				No. of branches			
First sample								
Control	21.25	19.41	16.83	19.17	1.00	1.00	1.16	1.05
AM_1	23.58	22.08	22.08	22.58	1.00	1.33	1.16	1.16
AM_2	24.58	23.08	23.00	23.55	1.33	1.66	1.33	1.44
E_1	23.83	22.50	21.50	22.61	1.16	1.16	1.00	1.11
E_2	25.16	23.83	23.33	24.11	1.50	1.16	1.00	1.22
Si_1	23.83	22.66	22.16	22.88	1.33	1.00	1.00	1.11
Si_2	21.83	21.50	20.16	21.16	1.16	1.00	1.00	1.05
Mean (A)	23.40	22.15	21.29		1.21	1.19	1.09	
LSD at 0.05 for	A 0.40	B 0.61	AB 0.88		A n.s	B 0.24	AB 0.35	
Second sample								
Control	48.83	41.17	40.17	43.38	3.16	1.50	1.33	2.00
AM_1	50.16	46.33	44.66	47.05	3.50	2.83	2.50	2.94
AM_2	54.00	49.73	48.83	50.85	4.00	3.16	3.16	3.44
E_1	59.33	56.83	53.66	56.61	3.83	3.33	3.16	3.44
E_2	64.00	60.33	58.50	60.94	5.16	4.33	3.66	4.38
Si_1	61.70	58.37	55.66	58.57	4.83	3.16	2.33	3.44
Si_2	57.33	54.88	50.93	54.38	3.83	3.16	2.66	3.22
Mean (A)	56.48	52.52	50.34		4.04	3.07	2.69	
LSD at 0.05 for	A 0.93	B 1.42	AB 2.06		A 0.23	B 0.35	AB 0.51	
Third sample								
Control	73.16	63.00	57.66	64.61	3.83	3.16	2.16	3.05
AM_1	76.00	70.00	63.33	69.77	4.10	3.83	3.51	3.81
AM_2	78.66	74.16	70.83	74.55	4.33	4.16	3.83	4.11
E_1	85.16	78.83	74.33	79.44	4.33	4.33	3.83	4.16
E_2	93.33	85.00	81.66	86.66	5.16	4.83	4.50	4.83
Si_1	91.00	82.34	79.83	84.39	4.83	4.83	4.50	4.72
Si_2	86.83	75.16	72.33	78.11	4.33	4.33	3.83	4.16
Mean (A)	83.45	75.50	71.42		4.41	4.21	3.74	
LSD at 0.05 for	A 0.88	B 1.35	AB 1.96		A 0.29	B 0.45	AB 0.66	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

Table 2: Effect of Pb, Mycorrhiza, 24-epibrassinolide and sodium silicate on number of leaves, stem diameter of *Zinnia elegans* plant in the first, second and third sample (combined of the 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	No. of leaves				Stem diameter (cm)			
First sample								
Control	14.00	12.50	12.33	12.94	0.42	0.35	0.31	0.36
AM_1	15.83	14.66	14.16	14.88	0.43	0.38	0.39	0.40
AM_2	17.00	16.33	15.66	16.33	0.43	0.41	0.39	0.41
E_1	14.83	13.83	12.33	13.66	0.41	0.41	0.31	0.38
E_2	15.33	14.33	13.50	14.38	0.43	0.41	0.34	0.39
Si_1	14.66	14.16	13.16	14.00	0.46	0.41	0.40	0.42
Si_2	13.33	12.66	12.33	12.77	0.43	0.41	0.40	0.41
Mean (A)	15.00	14.07	13.35		0.43	0.40	0.36	
LSD at 0.05 for	A 0.30	B 0.46	AB 0.66		A 0.01	B 0.02	AB 0.04	
Second sample								
Control	34.00	31.50	27.16	30.88	0.55	0.38	0.33	0.42
AM_1	39.66	36.66	33.66	36.66	0.58	0.43	0.40	0.47
AM_2	43.66	40.33	37.50	40.50	0.64	0.54	0.51	0.56
E_1	53.83	42.00	35.83	43.88	0.60	0.58	0.58	0.58

Table 2: Continue

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	No. of leaves				Stem diameter (cm)			
E_2	60.50	51.16	45.00	52.22	0.68	0.63	0.63	0.65
Si_1	57.83	45.50	40.83	48.05	0.65	0.61	0.56	0.61
Si_2	48.85	42.16	39.33	43.45	0.61	0.58	0.53	0.57
Mean (A)	48.33	41.33	37.04		0.61	0.53	0.50	
LSD at 0.05 for	A 0.80	B 1.22	AB 1.76		A 0.02	B 0.03	AB 0.04	
Third sample								
Control	51.66	38.66	34.33	41.55	0.68	0.64	0.55	0.62
AM_1	58.33	45.00	40.00	47.77	0.76	0.73	0.67	0.72
AM_2	64.00	48.33	44.66	52.33	0.81	0.77	0.71	0.76
E_1	82.16	65.00	60.83	69.33	0.80	0.72	0.71	0.74
E_2	85.66	70.83	66.16	74.22	0.95	0.79	0.76	0.83
Si_1	91.16	77.66	72.33	80.38	0.98	0.80	0.81	0.86
Si_2	82.83	66.16	61.66	70.22	0.85	0.77	0.74	0.79
Mean (A)	73.69	58.80	54.28		0.83	0.74	0.71	
LSD at 0.05 for	A 0.62	B 0.96	AB 1.38		A 0.02	B 0.03	AB 0.04	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

Table 3: Effect of Pb, Mycorrhiza, 24-epibrassinolide and sodium silicate on average root length, total leaf area of *Zinnia elegans* plant in the first, second and third sample (combined of the 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Root length (cm)				Total leaf area $(cm)^2$			
First sample								
Control	6.91	6.33	5.16	6.13	31.52	24.46	21.07	25.68
AM_1	7.58	6.75	6.75	7.02	43.24	37.30	29.92	36.82
AM_2	8.08	7.33	7.33	7.58	48.30	44.43	39.36	44.03
E_1	8.08	7.16	6.77	7.34	37.39	30.95	26.35	31.56
E_2	8.58	7.50	6.66	7.58	47.95	39.13	34.52	40.54
Si_1	10.41	8.83	7.33	8.86	49.52	41.08	31.39	40.66
Si_2	9.53	8.08	6.62	8.08	34.13	30.25	29.58	31.32
Mean(A)	8.45	7.42	6.66		41.72	35.37	30.31	
LSD at 0.05 for	A 0.24	B 0.37	AB 0.53		A 1.95	B 2.98	AB 4.31	
Second sample								
Control	10.41	8.58	7.91	8.97	158.8	109.6	80.49	116.3
AM_1	12.66	11.08	11.41	11.72	199.6	167.8	144.6	170.7
AM_2	14.33	12.83	11.66	12.94	247.8	195.2	173.5	205.5
E_1	12.58	12.08	11.58	12.08	352.9	240.0	192.3	261.7
E_2	13.00	12.83	12.25	12.69	478.9	341.6	269.5	363.3
Si_1	16.25	13.33	12.83	14.13	408.0	279.8	238.1	308.6
Si_2	12.83	11.00	10.33	11.38	315.2	238.0	194.1	249.1
Mean (A)	13.15	11.67	11.14		308.8	224.6	184.6	
LSD at 0.05 for	A 0.32	B 0.49	AB 0.72		A 8.04	B 12.29	AB 17.76	
Third sample								
Control	12.25	11.41	10.60	11.42	354.5	230.0	187.2	257.2
AM_1	14.00	12.75	12.50	13.08	427.6	301.2	246.5	325.1
AM_2	15.41	14.00	13.58	14.33	521.2	368.0	307.1	398.8
E_1	15.50	13.66	12.66	13.94	686.7	482.4	403.8	524.3
E_2	18.50	15.83	14.50	16.27	799.5	557.8	483.3	613.5
Si_1	17.33	14.33	13.58	15.08	794.3	600.2	510.1	634.9
Si_2	14.00	12.25	12.16	12.80	701.3	492.7	400.8	531.6
Mean (A)	15.28	13.46	12.80		612.2	433.2	362.7	
LSD at 0.05 for	A 0.36	B 0.56	AB 0.81		A 13.8	B 21.1	AB 30.5	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

Table 4: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on dry weight of shoot and dry weight of root of *Zinnia elegans* plant in the first, second and third sample (combined of the 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Dry weight of shoot (g)				Dry weight of root (g)			
First sample								
Control	0.81	0.74	0.60	0.72	0.20	0.16	0.12	0.16
AM_1	0.95	0.83	0.76	0.85	0.24	0.23	0.13	0.20
AM_2	1.01	0.88	0.83	0.91	0.27	0.21	0.18	0.22
E_1	0.85	0.82	0.65	0.77	0.20	0.16	0.14	0.17
E_2	1.46	1.12	0.88	1.15	0.29	0.17	0.16	0.21
Si_1	1.11	0.94	0.81	0.95	0.27	0.19	0.14	0.20
Si_2	0.98	0.76	0.68	0.81	0.22	0.17	0.12	0.17
Mean (A)	1.02	0.87	0.74		0.24	0.18	0.14	
LSD at 0.05 for	A 0.01	B 0.02	AB 0.03		A 0.01	B 0.01	AB 0.02	
Second sample								
Control	2.52	2.24	1.84	2.20	0.43	0.38	0.25	0.35
AM_1	2.82	2.71	2.52	2.68	0.52	0.44	0.36	0.44
AM_2	3.36	2.87	2.75	2.99	0.67	0.58	0.45	0.56
E_1	3.72	3.60	2.63	3.32	0.57	0.52	0.41	0.50
E_2	5.76	3.94	3.71	4.47	1.00	0.72	0.65	0.79
Si_1	4.71	3.95	3.32	3.99	0.80	0.62	0.48	0.63
Si_2	3.88	2.60	2.21	2.90	0.77	0.47	0.42	0.55
Mean (A)	3.82	3.13	2.71		0.68	0.53	0.43	
LSD at 0.05 for	A 0.07	B 0.10	AB 0.15		A 0.01	B 0.02	AB 0.04	
Third sample								
Control	6.20	5.68	4.73	5.54	0.77	0.61	0.58	0.65
AM_1	7.93	6.50	5.97	6.80	1.01	0.78	0.68	0.82
AM_2	8.91	7.57	7.12	7.86	1.21	0.95	0.80	0.98
E_1	8.79	8.68	8.78	8.75	1.00	0.84	0.78	0.88
E_2	15.69	10.85	10.13	12.2	1.47	1.19	1.13	1.26
Si_1	12.61	9.84	9.83	10.7	1.42	1.00	0.91	1.11
Si_2	11.91	8.90	8.31	9.71	1.25	0.90	0.83	0.99
Mean (A)	10.29	8.29	7.84		1.16	0.89	0.81	
LSD at 0.05 for	A 0.24	B 0.36	AB 0.53		A 0.03	B 0.05	AB 0.07	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si

Table 5: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on total dry weight (mg) and shoot/root dry weight ratio (g) of *Zinnia elegans* plant in the first, second and third sample (combined of the 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Total dry weight (mg)				Shoot/root dry weight ratio (g)			
First sample								
Control	1020	905	725	883.3	4.10	4.51	4.99	4.53
AM_1	1205	1065	895	1055.0	3.86	3.63	5.66	4.38
AM_2	1290	1105	1010	1135.0	3.74	4.19	4.57	4.16
E_1	1060	995	805	953.3	4.30	4.96	4.58	4.61
E_2	1750	1305	1035	1363.3	5.09	6.41	5.43	5.64
Si_1	1390	1135	960	1161.7	4.18	4.98	5.90	5.02
Si_2	1200	940	810	983.3	4.44	4.49	5.63	4.85
Mean (A)	1273.6	1064.3	891.4		4.24	4.74	5.25	
LSD at 0.05 for	A 17.6	B 26.9	AB 38.91		A 0.27	B 0.42	AB 0.61	
Second sample								
Control	2955	2525	1865	2448.3	5.83	5.86	7.27	6.32
AM_1	3465	3155	2890	3170.0	5.45	6.05	7.04	6.18
AM_2	4040	3455	3205	3566.7	4.99	4.95	6.21	5.38
E_1	4300	4200	3055	3851.7	6.49	6.89	6.37	6.58

Table 5: Continue

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Total dry weight (mg)				Shoot/root dry weight ratio (g)			
E_2	6770	5835	4370	5658.3	5.80	5.50	5.65	5.65
Si_1	5510	4575	3810	4631.7	5.89	6.33	6.84	6.35
Si_2	4665	3280	2590	3511.7	5.03	5.52	5.25	5.27
Mean (A)	4529.3	3860.7	3112.1		5.64	5.87	6.38	
LSD at 0.05 for	A 77.7	B 118.8	AB 171.7		A 0.26	B 0.40	AB 0.58	
Third sample								
Control	8900	6855	4415	6723.3	8.03	9.26	8.18	8.49
AM_1	12515	9650	8240	10135.0	7.86	8.28	8.82	8.32
AM_2	15275	11540	9675	12163.3	7.34	7.98	8.88	8.07
E_1	14285	12940	9710	12311.7	7.89	10.30	11.20	9.78
E_2	23650	17625	14600	18625.0	10.80	9.15	9.00	9.66
Si_1	20840	14800	12180	15940.0	8.90	9.92	10.80	9.87
Si_2	18090	11355	8540	12661.7	9.54	9.85	10.00	9.81
Mean (A)	16222.1	12109.3	9622.8		8.63	9.25	9.56	
LSD at 0.05 for	A 337.7	B 515.8	AB 745.6		A 0.53	B 0.81	AB 1.17	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

The effect on plant height with Pb treatments was also mentioned by Shivhare and Sharma[18] on Dahlia Jadia and Fulekar[19], Gopal and Khurana[20] on sunflower, number of branches of *Tagetes erecta*[21], number of leaves of *Zinnia marylandica*[22], on root length of *Zinnia elegans*[23], on dry weight of shoot and root species of Iris[24].

In this respect, Kosobrukhov *et al*.[25] on *Plantago major* reported that the application of lead (Pb) change photosynthesis efficiency through its effects on stomata or directly on mesophyll cells in which both photochemical and biochemical reactions were affected, Sharma and Dubey[1] and Gupta *et al*.[26] showed that the Pb impairs root elongation, plant growth, chlorophyll production and lamellar organization in the chloroplast and cell division. Stiborova *et al*.[27] indicated that the high levels of Pb in the soil decreased photosynthesis either through effects.

On the metabolites of the Calvin cycle or through decreased carboxylase activity, this caused reduction in plant height. The increase in the shoot: root dry weight ratio in plants that received the different levels of Pb may be attributed to a higher inhibition of root growth when compared with the inhibition of shoot growth due to the Pb treatments. Similar results and discussion were reported by Stobrawa and Lorenc-Plucinska[28], who indicated that lead caused a negative influence on root growth and morphological disorders of the roots as thickening and decreasing of root volume. The inhibition of root growth after exposure to Pb may be due to a decrease in Ca in the root tips, leading to a decrease in cell elongation or cell division[29].

The data in Table 1-5 also indicated that the application of all additive treatments had significant effects ($p = 0.05$) on the most of the studied growth characters of *Zinnia elegans* plants. The highest values were recorded by the plants treated with 24-epi at 10^{-8} M then followed by the plants sprayed with low concentration of sodium silicate at 50 ppm and then followed by plants treated with mycorrhiza at 1000 spores, while the lowest values were obtained by plants treated with the high level of Pb (200 ppm) alone without any additive treatments.

These results are in agreement with those obtained by Oklest *et al*.[30] they found that BRs have strong effects on plant growth and possess a unique combination of physiological actions such as cell division and elongation, increase of DNA and RNA polymerase activity, stimulate ethylene production, interact synergistically with auxins, Meir *et al*.[31] on *Eustoma grandiflorum*, Hosseinzadah *et al*.[32] on *Calendula officinalis* L., The foliar application of Si enhancing most of the studied growth characters under Pb toxicity. The might be induced due to the silicon not only contributes to cell wall rigidity and strengthening, but might also increase cell wall elasticity during extension growth. Moreover, the enhancement effect on shoot length of wheat plants supplied with Si might be induced through its role in both cell division and cell expansion by their effect on RNA and DNA synthesis. Silicon increased the thickness of the culm wall and the size of the vascular bundles preventing lodging in wheat plant, thereby enhancing the strength of the stem.[33] Joner and Leyval[34] reported that mycorrhiza fungi treatment increased plant height when the plants grown in polluted soil with Pb, because mycorrhiza could present a biological barrier for retention of heavy metals, so mycorrhiza was used most often to lower heavy metal concentration in the shoots of non hyperaccumulator plants.

Regarding the interaction between the effects of lead stress and the additive treatments on growth characters, the results in all samples recorded the lowest values of growth characters with high level of Pb (at 200 ppm) alone without any additive treatments. The results also showed that in all samples the highest values of growth characters were recorded by the untreated plants with Pb, but sprayed with 24-epi (at 10^{-8} M) on plant height, number of branches, stem diameter, dry weight of shoot and root and similar results detected with number of leaves in the second sample, total leaf area in the second and third samples and root length in the third sample. Also, the results indicated that the plants without Pb soil addition, but sprayed with the lower concentration of Si (50 ppm) recorded the highest values of root length in the second and third samples and on number of leaves in the third sample when *Zinnia elegans* plants were treated with Pb at 100 and 200 ppm Pb kg^{-1} soil, the highest values were recorded by the plants treated with 24-epi (E) at the lower rate (E_2), this treatment gave the highest values of plant height, dry weight of shoot and root, total dry weight and shoot: root dry weight ratio in all samples and found the same trend with number of branches, root length in the 2nd and 3rd samples, number of leaves, stem diameter, total leaf area in the 2nd sample and it is also noted that, spraying the plants with sodium silicate (Si_1) gave the highest values of number of leaves, stem diameter, total leaf area in the third sample then followed by plants treated with mycorrhiza at 1000 spores with all growth characters studied.

In this connection, the positive effect of 24-epi can be suggested that this reduction under Pb stress was associated with lesser ion uptake and accumulation and with the increasing activity of ATPase, an enzyme responsible for acid secretion and changes in membrane level[35]. The effect of Si is in harmony with Ghasemi *et al.*[36] using Si as a useful element for increasing the yield of plants and their resistance to environmental stresses, Metwally *et al.*[37] reported that exogenous application of Si increases plant tolerance to abiotic stress by decreasing generation of Reactive Oxygen Species (ROS), also plays an important role in the regulation of some physiological processes in plants such as effects on ion uptake and transport and membrane permeability and it has been found that Si has different effects on stress adaptation and damage development in plants. Moreover, Ahmed *et al.*[33] indicated that, Si could alleviate the effects of abiotic stresses including salt stress, metal toxicity and nutrient imbalance. these beneficial effects are mostly expressed through Si deposition in the leaves, stems and hulls. Gaill *et al.*[38] reported that the mycorrhiza possible retention of heavy metals to protection their host plants by the fungal mycelium involving adsorption to cell wall and fixation by polyphosphate granules.

Concerning the affect of lead soil addition on flowering characters: The data presented in Table 6 show that, the presence of lead (Pb) in the soil caused significant decrease ($p = 0.05$) in all of the studied flowering characters (number of inflorescences, inflorescences diameter, flowering period and dry weights of inflorescences). The plants treated with higher concentration of Pb (200 ppm), recorded the lowest values for all the studied flowering characters.

These results clearly indicated that the soil addition of Pb to zinnia plants had a negative effect on flower growth, which may be indirectly attributed to the adverse effect of lead on vegetative growth, which in turn makes the plant unhealthy and unable to produce large high-quality inflorescences. The decrease in flower development and growth as a result of using Pb was also clear in a number of ornamental plant species, including *Tagetes erecta* L.[21], sunflower[20] and *Zinnia marylandica*[22].

Morever, the results in Table 6 recorded that, additive treatments affected significantly ($p = 0.05$) on the flowering characters of *Zinnia elegans* plants. The plants sprayed with 24-epi at 10^{-8} M recorded the highest values of number of inflorescences, inflorescences diameter, flowering period and dry weights of inflorescences. The BRs affect by stimulating filament and pollen growth and modifying pollen properties[39]. This may be the reason for enhancing development of flowers and this was followed by the plants sprayed with Si, the cytokinin level in shoots, one of the most significant factors stimulating lateral bud development it was detected that foliar treatment with silicon may be increase the level of cytokinin in wheat plants[33] and then followed by plants treated with mycorrhiza, these results are in harmony with Sohn *et al.*[40] on *Chrysanthemum morifolium* and Perner *et al.*[41] on *Pelargonium peltatum*.

Concerning the interaction between the effects of Pb concentrations and additive treatments on the flowering characters, the results showed that the lowest values of flowering characters (inflorescences diameter and dry weights of inflorescences) were calculated from plants treated with the higher level of Pb (at 200 ppm) only without any additive treatments, however no significant effect was obtained between 100 or 200 ppm Pb in number of inflorescences and inflorescences diameter. The results also showed that the highest values of diameter and dry weights of inflorescences were recorded for the untreated plants with Pb but sprayed

Table 6: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on average number of inflorescences, inflorescences diameter, flowering period and dry weight of inflorescences of *Zinnia elegans* plant in third sample (combined of the 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	No. of inflorescences				Inflorescences diameter (cm)			
Third sample								
Control	1.83	1.50	1.16	1.50	5.37	5.15	4.78	5.10
AM_1	2.83	2.50	2.16	2.50	6.43	6.18	6.00	6.20
AM_2	3.66	3.23	2.66	3.18	7.04	6.47	6.17	6.56
E_1	2.83	2.66	2.83	2.77	6.92	6.34	6.31	6.52
E_2	4.66	3.66	3.33	3.88	7.61	7.12	6.58	7.10
Si_1	4.50	3.33	2.83	3.55	7.17	6.86	6.41	6.81
Si_2	4.00	2.50	2.23	2.91	6.29	6.01	5.81	6.04
Mean (A)	3.47	2.77	2.46		6.69	6.31	6.01	
LSD at 0.05 for	A 0.26	B 0.40	AB 0.58		A 0.13	B 0.20	AB 0.30	
	Flowering period (day)				Dry weight of inflorescences (g)			
Third sample								
Control	43.16	41.33	40.50	41.66	0.77	0.71	0.51	0.66
AM_1	52.66	51.83	51.83	52.11	1.26	0.94	0.73	0.98
AM_2	56.33	55.16	54.00	55.16	1.40	1.13	0.85	1.13
E_1	56.00	53.33	51.33	53.55	1.25	1.05	0.81	1.03
E_2	57.16	56.83	55.66	56.55	1.56	1.32	1.13	1.34
Si_1	56.83	56.16	55.33	56.11	1.46	1.22	1.07	1.25
Si_2	53.35	50.83	50.33	51.50	1.09	1.02	0.97	1.02
Mean (A)	53.64	52.21	51.28		1.25	1.06	0.87	
LSD at 0.05 for	A 0.63	B 0.97	AB 1.41		A 0.07	B 0.10	AB 0.15	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

with 24-epi (at 10^{-8} M), the same trend had been found in number of inflorescences for untreated plants with Pb but sprayed with 24-epi at 10^{-8} M or sprayed with sodium silicate at the lower concentration (50 ppm), the highest value of flowering period recorded with plants untreated with Pb but sprayed with (E_2) or sprayed with (Si_1) or by mycorrhiza soil addition at 1000 spores (AM_2).

Plant growth analysis: For plant growth analysis e.g., RGR, NAR and SUR the data presented in (Table 7 and 8) show that RGR, NAR and SUR were decreased with increasing Pb level of Pb (200 ppm), these results show inhibition of biosynthetic activity was estimated by a decline in photosynthesis, but its increased without Pb. This suggests that photosynthesis is the limiting factor for growth. Bandeh-Hagh *et al.*[42] reported that the NAR decreased reflects a decrease in the rate of photosynthesis or an increase in respiration with increasing lead concentrations. SUR decreased with increasing Pb concentration, because high concentrations of Pb are fast inhibition of root growth, decreased fresh and dry biomass of roots and shoots.

Moreover, from the results in Table (7 and 8) it is clear that, RGR was in a high value at the early growth stages (between the first and second sample) and lowered at the latest stages (between second and third sample) this trend was expected because at early stages plant cells are more active for division and elongation than later stages.

The application of all additive treatments also had increased RGR, NAR and SUR when compared with plants without any additive treatments.

The interaction between the effects of Pb concentrations and additive treatments the data revealed, that the lowest values of RGR, NAR and SUR were recorded at the higher level of Pb without any additive treatments. The highest values of RGR and SUR were obtained by the plants sprayed with 24-epi at 10^{-8} M, exogenous application of EBL improved the RGR of zinnia plants. These results are not surprising since brassinosteroids were implicated in cell elongation and differentiation [43] and found the same trend was detected by the plants sprayed with sodium silicate under Pb stress condition. The increase in RGR was attributed to the increase in the physiological growth parameter (NAR) rather than the morphological growth parameter, this results in harmony with Ali *et al.*[44], who reported that EBL increased growth in Indian mustard plants could be related to enhanced activity of antioxidative enzymes and proline level that protect the plants from oxidative damage. Therefore, photosynthesis which is a major controlling factor for plant growth and yield might have

Table 7: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on average relative growth rate (RGR mg g^{-1} d^{-1}) and net assimilation rate (NAR mg cm^2 d^{-1}) of *Zinnia elegans* plant during 2014 and 2015 seasons

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Relative growth rate (RGR_{1-2} mg g^{-1} d^{-1})				Relative growth rate(RGR_{2-3} mg g^{-1} d^{-1})			
Control	42	41	38	40.3	44	40	34	39.3
AM_1	42	43	47	44.0	51	45	42	46.0
AM_2	46	46	46	46.0	53	48	44	48.3
E_1	56	57	53	55.3	48	45	46	46.3
E_2	54	60	58	57.3	50	44	48	47.3
Si_1	55	56	55	55.3	53	47	46	48.7
Si_2	54	50	46	50.0	54	50	48	50.7
Mean (A)	49.9	50.4	49.0		50.4	45.6	44.0	
	Net assimilation rate (NAR_{1-2} mg $(cm^2)^{-1}$ d^{-1})				Net assimilation rate (NAR_{2-3} mg $(cm^2)^{-1}$ d^{-1})			
Control	0.997	1.146	1.027	1.057	0.976	1.071	0.803	0.9500
AM_1	0.900	0.975	1.114	0.996	1.214	1.154	1.126	1.1648
AM_2	0.903	0.935	0.988	0.942	1.226	1.238	1.167	1.2106
E_1	0.926	1.263	1.091	1.094	0.790	1.022	0.9474	0.9198
E_2	1.081	1.314	1.169	1.188	1.080	1.066	1.1270	1.0910
Si_1	0.969	1.123	1.119	1.071	1.058	0.976	0.9404	0.99177
Si_2	1.100	0.909	0.822	0.944	1.133	0.939	0.8339	0.9684
Mean (A)	0.983	1.095	1.047		1.068	1.066	0.992	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si

Table 8: Effect of Pb stress, mycorrhiza, 24-epibrassinolide and sodium silicate on specific utilization rate (SUR_{2-3} mg g Pb^{-1} d^{-1}) of *Zinnia elegans* plant

	Pb (ppm)			
Treatments	0	100	200	Mean (B)
(SUR_{2-3} mg g Pb^{-1} d^{-1})				
Control	1.8	1.0	0.6	1.1
AM_1	3.3	1.8	1.6	2.2
AM_2	4.3	2.6	1.8	2.9
E_1	2.5	2.4	2.2	2.4
E_2	4.6	2.5	3.0	3.4
Si_1	4.7	2.9	2.5	3.4
Si_2	3.5	2.2	1.6	2.4
Mean (A)	3.5	2.2	1.9	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si

been increased due to EBL application[45]. Also, the results indicated that the highest values of NAR under Pb conditions in the (NAR_{2-1}) were obtained by the plants sprayed with (E_2) and in the (NAR_{3-2}) by plants treated with mycorrhiza (AM_2).

Pb concentration in shoot, root and inflorescences: Data in Table 9 and 10 of the 2 samples(60 and 85 days) of shoot and root, as well as inflorescence in third sample, showed that lead (Pb) soil addition caused significantly increased ($p = 0.05$) on Pb concentration in all organs of zinnia plants. Pb concentration in roots was higher than in the other organs (roots>shoots>inflorescence). The highest values of Pb concentration in all organs recorded with high concentration of Pb (200 ppm). These results are in harmony with Nowak[46] on salvia, Bosiacki[21] on marigold and He *et al.*[47] on *Zinnia elegans*.

Low transport of heavy metals to shoots may be due to saturation of root metal uptake, when internal metal concentrations are high[48]. Metal concentrations, therefore, may rise as leaves age simply due to the continued passive metal transport into leaf tissues. Movement of metals into older leaves is a way that some plants have to eliminate some of their metal excess[49]. This result disagrees with that of Marschner[50], who attributed a refuse in dry matter mineral content as plants age to an increase in the proportion of structural material (cell wall and lignin) and storage compounds.

The application of all additives treatment decreased Pb concentration in all organs (in all samples). The lowest values of Pb concentration in shoot (in 2nd sample were obtained by the plants sprayed with Si_1 or E_2 and in 3rd sample the lowest values were detected by plants sprayed with E_1 followed E_2). In root showed the lowest value of Pb concentration was detected (in the 2nd and 3rd samples) by plants sprayed with E_2. And in inflorescence the lowest values were obtained by plants sprayed with Si_1 or E_2 then follow by plants treated with mycorrhiza. This result agreement with Bajguz[35], who found that the 24-epi at the concentration of 10^{-8} M in combination with heavy metals blocked metal accumulation in *Chlorella vulgaris* cells and Wu *et al.*[51], who suggested the mechanisms for Si reducing active heavy metal ions in growth

Table 9: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on Pb concentration in the shoot and root of *Zinnia elegans* plant during 2015 season

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
	Second sample				Third sample			
Treatments	Pb (ppm) in shoot				Pb (ppm) in shoot			
Control	19.20	32.30	36.8	29.4	24.7	35.9	44.25	35.0
AM_1	15.00	25.50	27.6	22.7	17.5	29.2	34.3	27.0
AM_2	13.00	20.00	22.0	18.3	15.4	22.1	25.9	21.1
E_1	18.00	23.00	26.0	22.3	19.0	23.6	24.8	22.5
E_2	10.50	16.21	17.0	14.6	11.5	17.0	18.0	15.5
Si_1	11.30	13.00	16.5	13.6	13.3	18.0	21.5	17.6
Si_2	14.87	21.00	25.3	20.4	18.0	24.4	27.9	23.4
Mean (A)	14.50	21.60	24.4		17.0	24.3	28.1	
LSD at 0.05 for	A 0.97	B 1.48	AB 2.14		A 0.40	B 0.62	AB 0.89	
	Pb (ppm) in root				Pb (ppm) in root			
Control	30.8	35.5	60.5	42.3	38.2	41.5	65.5	48.4
AM_1	25.9	27.2	38.0	30.4	27.0	32.1	43.3	34.1
AM_2	18.5	20.0	28.3	22.3	22.0	25.2	33.5	26.9
E_1	21.0	24.5	35.3	26.9	25.9	35.5	38.4	33.3
E_2	13.0	15.5	17.5	15.3	16.0	19.0	21.5	18.8
Si_1	15.0	20.2	25.7	20.3	17.5	23.5	28.4	23.1
Si_2	16.2	26.1	32.2	24.8	20.0	28.2	34.5	27.6
Mean (A)	20.0	24.1	33.9		23.8	29.3	37.9	
LSD at 0.05 for	A 0.50	B 0.76	AB 1.11		A 1.15	B 1.77	AB 2.56	

Table 10: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on Pb concentration in inflorescence of *Zinnia eleganes* plant during 2015 season

	Pb (ppm)			
	0	100	200	Mean (B)
	Third sample			
Treatments	Pb concentration in inflorescence			
Control	20	28.0	47.0	31.7
AM_1	8.0	13.1	21.0	14.0
AM_2	5.0	8.00	14.5	9.20
E_1	9.0	12.0	15.0	12.0
E_2	4.0	6.50	8.00	6.20
Si_1	4.0	9.00	9.00	7.30
Si_2	6.0	16.0	17.0	13.0
Mean (A)	8.0	13.2	18.8	
LSD at 0.05 for	A 0.59	B 0.90	AB 1.30	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

media, reducing heavy metal transport to the shoot and showed that mycorrhiza decreased Pb concentration in both shoots and roots in maize plants[52].

The interaction between the effects of Pb concentrations and additive treatments, indicated that the highest values of Pb concentration in all organs (in all samples) were recorded with high level of Pb without any additive treatments. On the other hand the lowest values of Pb concentration under Pb conditions in shoot, root and inflorescence were recorded by plants sprayed with 24-epi at 10^{-8} M (E_2), then followed by plants treated with (Si_1), then followed by plants treated with mycorrhiza (AM_2).

The protective effect of epibrassinolide, may be induce due to the mechanism involved in reducing the toxicity to the chelation of the metal ion by a ligand. Such ligands include organic acids, amino acids, peptides or polypeptides[53,54] and Bajguz and Hayat[55] reported that the application of 24-epiBL significantly reduced ($p = 0.05$) the metal absorption and Liu *et al.*[56] concluded that Si application reduced Pb concentration in shoots of rice plants.

Plant pigments: Concerning the effect of lead soil addition on chemical constituents, the data presented in Table 11,12 and 13 indicate that at the three samples the presence of lead (Pb) in the soil caused significant decreases ($p = 0.05$) of all pigments compared with lead untreated plants (chlorophyll a, chl b, total chlorophyll (a+b) and total carotenoids) in all samples. These results are in agreement with that finding by Lambers *et al.*[57] they reported that the process of photosynthesis is adversely affected by Pb toxicity, Plants exposed to Pb ions show a decline in photosynthetic rate, which results from distorted chloroplast ultrastructure, restrained synthesis of chlorophyll, plastoquinone and carotenoids, obstructed electron transport, inhibited activities of Calvin cycle

Table 11: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on chlorophyll a and chlorophyll b concentration (mg g^{-1} F.W.) of *Zinnia elegans* plant in the first, second and third samples (combined of 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Chlorophyll a				Chlorophyll b			
First sample								
Control	0.43	0.34	0.27	0.35	0.25	0.20	0.16	0.20
AM_1	0.47	0.50	0.40	0.46	0.26	0.23	0.24	0.24
AM_2	0.64	0.60	0.46	0.56	0.24	0.25	0.25	0.25
E_1	0.64	0.50	0.57	0.57	0.23	0.22	0.20	0.22
E_2	0.75	0.67	0.65	0.69	0.28	0.24	0.23	0.25
Si_1	0.70	0.64	0.60	0.65	0.28	0.25	0.23	0.25
Si_2	0.64	0.60	0.57	0.60	0.24	0.21	0.20	0.22
Mean (A)	0.61	0.55	0.50		0.25	0.23	0.22	
LSD at 0.05 for	A 0.03	B 0.05	AB 0.08		A 0.02	B 0.03	AB 0.04	
Second sample								
Control	0.71	0.63	0.52	0.62	0.30	0.19	0.14	0.21
AM_1	0.94	0.68	0.55	0.72	0.42	0.28	0.23	0.31
AM_2	1.09	0.82	0.76	0.89	0.48	0.29	0.25	0.34
E_1	1.12	0.69	0.67	0.83	0.49	0.27	0.24	0.33
E_2	1.25	0.89	0.80	0.98	0.54	0.30	0.28	0.37
Si_1	1.12	0.86	0.78	0.92	0.48	0.29	0.25	0.34
Si_2	1.01	0.74	0.71	0.82	0.47	0.25	0.23	0.32
Mean (A)	1.03	0.76	0.69		0.45	0.27	0.23	
LSD at 0.05 for	A 0.04	B 0.06	AB 0.10		A 0.02	B 0.03	AB 0.04	
Third sample								
Control	0.68	0.43	0.39	0.50	0.23	0.13	0.12	0.16
AM_1	0.75	0.57	0.50	0.61	0.31	0.21	0.17	0.23
AM_2	0.79	0.69	0.63	0.70	0.32	0.26	0.21	0.26
E_1	0.83	0.68	0.60	0.70	0.35	0.24	0.20	0.26
E_2	0.96	0.78	0.71	0.82	0.40	0.28	0.24	0.31
Si_1	0.95	0.80	0.72	0.82	0.40	0.26	0.28	0.32
Si_2	0.87	0.68	0.67	0.74	0.35	0.23	0.22	0.27
Mean (A)	0.83	0.66	0.60		0.34	0.23	0.20	
LSD at 0.05 for	A 0.04	B 0.06	AB 0.09		A 0.01	B 0.02	AB 0.03	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

Table 12: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on total chlorophyll (a+b) and total carotenoid concentrations (mg g^{-1} F.W.) of *Zinnia elegans* plant in the first, second and third samples (combined of 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Total chlorophyll (a+b)				Total carotenoid			
First sample								
Control	0.69	0.55	0.43	0.55	0.29	0.25	0.20	0.25
AM_1	0.73	0.73	0.65	0.70	0.30	0.27	0.26	0.27
AM_2	0.88	0.86	0.71	0.82	0.34	0.29	0.29	0.31
E_1	0.88	0.72	0.78	0.79	0.33	0.31	0.28	0.31
E_2	1.04	0.91	0.89	0.95	0.39	0.31	0.30	0.33
Si_1	0.98	0.89	0.84	0.90	0.39	0.32	0.29	0.33
Si_2	0.89	0.81	0.77	0.82	0.31	0.29	0.26	0.29
Mean (A)	0.87	0.78	0.72		0.34	0.29	0.27	
LSD at 0.05 for	A 0.05	B 0.07	AB 0.11		A 0.02	B 0.04	AB 0.06	
Second sample								
Control	1.02	0.82	0.67	0.84	0.34	0.26	0.19	0.27
AM_1	1.36	0.96	0.79	1.04	0.36	0.35	0.29	0.33
AM_2	1.57	1.12	1.02	1.24	0.42	0.37	0.34	0.38
E_1	1.61	0.96	0.91	1.16	0.48	0.40	0.38	0.42

Table 12: Continue

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	Total chlorophyll (a+b)				Total carotenoid			
E_2	1.80	1.20	1.08	1.36	0.53	0.48	0.44	0.48
Si_1	1.61	1.15	1.04	1.27	0.49	0.46	0.39	0.45
Si_2	1.48	0.99	0.95	1.14	0.45	0.43	0.36	0.41
Mean (A)	1.49	1.03	0.92		0.44	0.39	0.34	
LSD at 0.05 for	A 0.05	B 0.08	AB 0.12		A 0.02	B 0.04	AB 0.06	
Third sample								
Control	0.92	0.56	0.51	0.67	0.42	0.35	0.27	0.35
AM_1	1.07	0.78	0.68	0.84	0.47	0.42	0.38	0.42
AM_2	1.12	0.95	0.84	0.97	0.49	0.47	0.45	0.47
E_1	1.18	0.92	0.81	0.97	0.51	0.45	0.42	0.46
E_2	1.37	1.06	0.95	1.13	0.53	0.48	0.46	0.49
Si_1	1.36	1.06	1.00	1.14	0.45	0.43	0.43	0.44
Si_2	1.23	0.92	0.89	1.01	0.44	0.38	0.36	0.39
Mean (A)	1.18	0.89	0.81		0.47	0.42	0.40	
LSD at 0.05 for	A 0.04	B 0.07	AB 0.10		A 0.02	B 0.04	AB 0.06	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

Table 13: Effect of Pb, mycorrhiza, 24-epibrassinolide and sodium silicate on a/b ratio and total chlorophyll/total carotenoid ratio of *Zinnia elegans* plant in the first, second and third samples (combined of the 2014 and 2015 seasons)

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	a/b ratio				Total chlorophyll/total carotenoid ratio			
First sample								
Control	1.75	1.69	1.83	1.76	2.39	2.16	2.21	2.25
AM_1	1.86	2.24	1.64	1.91	2.49	2.73	2.55	2.59
AM_2	2.60	2.39	1.79	2.26	2.65	3.00	2.60	2.75
E_1	2.82	2.31	2.85	2.66	2.67	2.37	2.78	2.61
E_2	2.73	2.77	2.81	2.77	2.69	2.89	3.09	2.89
Si_1	2.58	2.69	2.62	2.63	2.51	2.80	2.97	2.76
Si_2	2.61	2.80	2.91	2.77	2.90	2.85	2.91	2.88
Mean (A)	2.42	2.41	2.35		2.61	2.68	2.73	
LSD at 0.05 for	A n.s	B 0.35	AB 0.50		A n.s	B 0.42	AB 0.61	
Second sample								
Control	2.38	3.41	3.64	3.14	2.73	3.04	3.39	3.05
AM_1	2.25	2.49	2.40	2.38	3.35	2.56	2.62	2.84
AM_2	2.28	2.86	3.00	2.71	3.39	2.65	2.77	2.94
E_1	2.27	2.63	2.77	2.55	3.41	2.44	2.38	2.74
E_2	2.28	2.95	2.97	2.73	3.43	2.55	2.42	2.80
Si_1	2.33	2.96	3.06	2.78	3.30	2.49	2.71	2.83
Si_2	2.15	2.92	3.02	2.70	3.27	2.31	2.67	2.75
Mean (A)	2.98	2.89	2.28		3.27	2.58	2.71	
LSD at 0.05 for	A 0.22	B 0.34	AB 0.49		A 0.20	B 0.31	AB 0.45	
Third sample								
Control	2.87	3.20	3.27	3.11	2.20	1.69	1.90	1.93
AM_1	2.41	2.75	3.04	2.73	2.29	1.89	1.80	1.99
AM_2	2.48	2.67	2.97	2.70	2.27	2.03	1.88	2.06
E_1	2.39	2.77	2.93	2.70	2.32	2.03	1.92	2.09
E_2	2.37	2.74	2.94	2.69	2.55	2.32	2.07	2.32
Si_1	2.35	3.01	2.55	2.64	2.78	2.46	2.39	2.54
Si_2	2.44	2.98	3.11	2.84	2.71	2.42	2.47	2.53
Mean (A)	2.97	2.87	2.47		2.45	2.12	2.06	
LSD at 0.05 for	A 0.19	B 0.30	AB 0.44		A 0.16	B 0.24	AB 0.35	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

enzymes, as well as deficiency of CO_2 as a result of stomatal closure of *Zinnia elegans*[47,58] and *Helianthus annus* L[59].

Also, the results in Table 11-13 indicated that with increasing Pb soil addition the a/b ratio and total chlorophyll/ total carotenoid in the 2nd and 3rd samples were increased of the plants treated with Pb alone without any additives treatment, but in the 1st sample the effect was non-significant between the some treatments. The increase in the Chl a/b ratio as a result of increasing the Pb supply suggests that the Pb treatments caused much more reduction on Chl b than of Chl a, resulting in increased Chl a/b ratio[60]. Pb effects have been described for both donor and acceptor sites of photosynthesis-2 (PS II), the cytochrome b/f complex and photosynthesis-1 (PS I). It is largely accepted that PS I electron transport is less sensitive to inhibition by Pb than photosynthesis-2 (PS II)[61,62].These results are in agreement with those obtained by Djukic *et al*. [63] on *Ailanthus altissima* and Dey and Mondal[64].

Also, data in Table 11, 12 and 13 revealed that application of additive treatments also showed significant effects ($p = 0.05$) on the plant pigments of *Zinnia elegans* plants. The plants sprayed with 24-epi at 10^{-8} M recorded the highest values of chl a, chl b and total chl (a+b) in all samples followed by plants sprayed with sodium silicate at 50 ppm and AM_2 (1000 spores), but in chl a/chl b ratio (in the 1st sample) the highest value indicated by plants sprayed with sodium silicate at 100 ppm and 24-epi at 10^{-8} M, in the 2nd and 3rd samples the highest value in plants without any additive treatments. Concerning the ratio between total chl/total carotenoid, the results in the 1st and 2nd samples indicated no significant differences could be recorded between various treatments, but in the third sample the highest total chl/total carotenoid ratio were obtained by plants sprayed with Si_1 and Si_2 followed by plants sprayed with 24-epi at 10^{-8} M. Effect of 24-epi application via activation of enzymes participating in chlorophyll biosynthesis or an induction of their synthesis and thus improving the photosynthetic efficiency[65].

The positive effect of Si may be due to the fact that Si protects photosynthetic apparatus through increasing the ability of cell antioxidation and new proteins. synthesis[66,67].

Concerning carotenoids the data indicated that highest value in the first sample was obtained by plants sprayed with Si_1, E_2 or E_1 and AM_2, but in the second and third samples the highest value were recorded by the plants sprayed with E_2.

The interaction between the effects of Pb soil addition and additive treatments, it is clear the plants with 200 ppm Pb recorded the lowest value of all plant pigments except a/b ratio. Under Pb condition (100 and 200 ppm) show the highest value of (chlo a, chlo b, total chlo (a+b) and total chlo/total carotenoid) with plants sprayed with (E_2), (Si_1) and (AM_2), the same trend of result found in total carotenoid concentration in the second sample, but in the first and third samples had no significantly effects. The result favourable effect of plants treated with mycorrhiza on plant pigments concentration was in harmony with this reported by Nowak[68] who suggested that the increased photosynthetic activity of mycorrhizal plants was connected with the increased ratio of variable to maximum chlorophyll fluorescence (Fv/Fm), which is directly proportional to the maximum quantum yield of primary photochemistry of PS II.

Antioxidant enzymes activities: The results in Table 14 and 15 revealed effect of Lead soil addition on antioxidant enzymes, SOD and CAT activity decreased with increasing Pb level, this enzyme activity usually decreases in living tissues exposed to stress environments[69], but increased POD activity.

The SOD is a metallo-enzyme present in various cellular compartments, functioning at the first step of ROS generation, i.e., superoxide formation, superoxide radicals can act as a precursor to other ROS, SOD dismutates two superoxide radicals to H_2O_2 and oxygen and thus maintains superoxide radicals in a steady state level[70]. In this study, the decline in SOD activity at 200 ppm Pb indicated that the oxygen scavenging function of SOD was impaired.

The CAT is a universally present oxidoreductase that decomposes H_2O_2 to water and molecular oxygen and it is one of the key enzymes involved in the removal of toxic peroxides. Decline CAT activity at higher concentration of Pb in this study might be attributed to inactivation of enzyme by ROS, change in assembly of its subunits or decrease in synthesis of enzyme[71].

The POD catalyzes H_2O_2-dependent oxidation of substrate. POD activity is also considered a beneficial biomarker for sublethal metal toxicity in plant species. Morever, POD participating in lignin biosynthesis can build up a physical barrier against toxic heavy metals. Other studies in plants have reported increases, decreases and no changes in POD activity in response to exposure of heavy metal[72]. The results show increased of POD activity at higher Pb concentrations and a decline without any Pb soil addition (control). Tanyolac *et al*.[73] indicated that the POD activity was found to be sufficiently high to enable the plants to protect themselves against oxidative stress.

The result in Table 14, 15 also indicated that the application of additive treatments without stress or interaction with Pb conditions had significant effects ($p = 0.05$) on the enzymes activity of *Zinnia elegans* leaves. The highest

Table 14: Effect of Pb stress, mycorrhiza, 24-epibrassinolide and sodium silicate on SOD and CAT unit g^{-1} fresh weight in fresh leaves of second sample of *Zinnia elegans*

	Pb (ppm)				Pb (ppm)			
	0	100	200	Mean (B)	0	100	200	Mean (B)
Treatments	SOD unit g^{-1} fresh weight				CAT unit g^{-1} fresh weight			
Second sample								
Control	4.66	3.66	2.39	3.57	35.28	34.20	22.65	30.71
AM_1	4.95	3.75	2.55	3.75	38.76	37.44	37.08	37.76
AM_2	6.03	3.86	2.61	4.17	49.35	45.81	42.70	45.95
E_1	5.54	3.72	2.70	3.99	38.85	38.01	36.16	37.67
E_2	5.75	4.10	3.30	4.38	51.72	46.92	43.48	47.37
Si_1	5.72	4.97	3.28	4.66	50.85	46.16	43.31	46.77
Si_2	6.09	4.98	3.25	4.77	45.40	40.35	36.75	40.83
Mean (A)	5.53	4.14	2.87		44.31	41.27	37.44	
LSD at 0.05 for	A 0.42	B 0.64	AB 0.93		A 2.89	B 4.42	AB 6.39	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

Table 15: Effect of Pb stress, mycorrhiza, 24-epibrassinolide and sodium silicate on POD, unit g^{-1} fresh weight in fresh leaves of second sample of *Zinnia eleganes*

	Pb (ppm)			
Treatments	0	100	200	Mean (B)
POD unit g^{-1} fresh weight				
Control	64.38	78.33	83.81	75.51
AM_1	52.92	64.42	73.25	63.53
AM_2	49.38	59.29	58.95	55.87
E_1	51.72	60.52	68.72	60.32
E_2	47.78	56.30	60.87	54.98
Si_1	49.85	53.28	72.10	58.41
Si_2	61.68	69.28	73.90	68.29
Mean (A)	53.96	63.06	70.23	
LSD at 0.05 for	A 4.25	B 6.50	AB 9.39	

AM_1: 500 spores, AM_2: 1000 spores, E_1: 10^{-6} M, E_2: 10^{-8} M, Si_1: 50 ppm Si, Si_2: 100 ppm Si, A: Pb, B: Treatments, AB: Interaction

activity of SOD and CAT activity were recorded by the plants sprayed with Si (at 50 or 100 ppm) then followed by the plants sprayed with 24-epi at 10^{-8} M and then followed by plants treated with mycorrhiza at 1000 spores but POD recorded the highest activity by the plants treated with high level of Pb without any additive treatments. These results agree with the results mentioned by Garg and Kaur[74] on *Cajanus cajan* L. and Liang[69] on barly they suggested that silicon may affect the structure and integrity of plasma membranes by influencing the stress-dependent peroxidation of membrane lipids. Ghasemzadeh and Jaafar[75] on *Zingiber officinale* indicated that the positive effect of foliar Si to increased SOD activity was accompanied by increases in CAT and POD activities because of the high demands of H_2O_2 quenching. By supporting photosynthetic rate in response to enhanced antioxidant enzyme activities. Hajipour and Jabbarzadeh[76] reported that the foliar application of sodium silicate on chrysanthemum increasing antioxidant enzymes activity, Si may increase CAT by affecting catalase production path in peroxisomes. Morever Sadhana[77] suggested that the mycorrhiza *Glomus mosseae* application can be enhanced the enzyme activity, which develops resistance in host plants, farther more Vazquez *et al.*[54] and Ahmed *et al.*[78] reported that the application of BRs modified antioxidant enzymes activities under different abiotic stresses.

Thus, it can be suggested that, the application of different additive treatments might be decreased the stress induce by lead in plant through increasing antioxidant enzymes SOD and CAT, while POD activity was decreased.

CONCLUSION

It can be concluded that the exogenous application of 24-epi and sodium silicate or addition mycorrhiza to zinnia plant resulted in improvements of growth and flower characters. Chlorophyll a, b, total chlorophyll, total carotenoids, SOD and CAT activity was increased and Pb concentration and POD activity was decreased during Pb stress as compared to untreated control plants, especially that 24-epi at 10^{-8} M, sodium silicate at 50 ppm and mycorrhiza at 1000 spores.

SIGNIFICANCE STATEMENT

Lead soil pollution usually affects many metabolic aspects of zinnia plant and induces changes in its physiology. It is worthy here to mention that using mycorrhiza, brassinosteroid and sodium silicate may correct to some extent the negative effect of lead soil pollution on zinnia plants. These treatments possibly alleviate lead soil toxicity such as its applied concentrations and level of lead soil addition.

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