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RESPONSE OF *Ficus nitida* PLANTS TO SOME TREATMENTS FOR DECREASING THE HARMFUL EFFECT OF LEAD POLLUTION

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ABSTRACT

Lead concentrations (0, 500, 1000, 2000 ppm) in soil caused changes in botanical traits and biochemical structure. Decrease in plant height, root length, shoots fresh-dry weights and roots fresh-dry weights were obtained. Also lead caused decrement in chl.a, chl.b, total carotenoids and catalase enzyme activity, while increment in both peroxidase and Superoxide dismutase (SOD) activity were observed in plant leaves under lead treatment compared with control.

Increasing in plant height, root length, shoots fresh-dry weight, roots fresh-dry weight, chl.a,b, total carotenoids and catalase enzyme activity, also decrement in peroxidase and SOD enzymes were noted response to applying mycorrhiza fungi, EDTA-Fe and ascorbic acid treatments.

Keywords: *Ethylene Diamine Tetra-acetic Acid, Ficus nitida, Lead, Mycorrhizal fungi, , Ascorbic acid.*

INTRODUCTION

Ficus trees specially grown at environmentally contaminated sites in Egypt could take up and accumulate metals at high concentrations that are toxic to plants.

Heavy metals are taken into plant tissue either *via* the soil, or through leaves (Kovacs, 1992). Some heavy metals perform important biological functions, such as being structural, and catalytic constituents of enzymes and other proteins (Hall, 2002), While in excess heavy metals such as lead (Pb), are toxic to plant cells and may inhibit growth (Steffens, 1990; Csintalan and Tuba, 1992).

The enters of Pb inside the cells even in small amounts produce a wide range of adverse effects on physiological processes. Pb phytotoxicity leads to inhibition of enzyme activities, disturbed mineral nutrition, water imbalance, change in hormonal status and alteration in membrane permeability (Ernst, 1998; Seregin and Ivanov, 2001and Solange *et al.*, 2006).

According to the previous investigations, lead element has harmful effect for plant so, this study of some treatments such as mycorrhiza fungi, EDTA-Fe and ascorbic acid was used to low the harmful effects.

MATERIALS AND METHODS

A pot experiment was carried out at the Floriculture Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Egypt, during 2005-2006.

Ficus nitida plants were transplanted on 1st June 2005 in 30 cm plastic pots and each pot contained 7.5 kg soil.
After 30 days of transplanted plants, lead (Pb) was added to the soil (0, 500, 1000 and 2000 ppm.) in the form of lead acetate $(CH_3COO)_2Pb.3H_2O$.

After that the treatments were carried out using mycorrhizal fungi (M.F.) which added to soil at the concentrations (M1= 500 spores and M2= 1000 spores/ pot), iron salt of Ethylene Diamine Tetra-acetic Acid (Fe-EDTA) was sprayed to plants in the form of Foliafeed (chelated iron 13%) at the two concentrations of iron (Fe1= 130 and Fe2= 260 ppm) and ascorbic acid (vitamin C) was sprayed at the rates of (AsA1= 250 and AsA2= 500 ppm). After a year from transplanting (June 1st 2006) data of growth characteristics and biochemical analysis were recorded.

Plant height, root length (cm), shoots fresh weight, shoots dry weight, roots fresh weight and roots dry weights (g) were recorded. For leaves pigments determination, fresh leaves were extracted with dimethyl formamide (D.M.F.) solution $[HCON(CH_3)_2]$ in which the leaves were placed overnight at cool temperature (5° C), then cholorophyll a and b as well as carotenoids were measured by spectrophotometer Beckman Du 7400 at wave lengths 663 , 647 ,and 470 nm respectively according to the equation described by Nornai (1982). Catalase was assayed by measuring the decrease in absorbance due to disappearance of H_2O_2 at 240 nm according to Chance and Maehly (1955), Peroxidase was assayed spectrophotochemically according to Amako *et al* (1994) and SOD was determinated by photochemical method as described by Ginnopolitis and Ries (1977).

RESULTS AND DISCUSSION

1.Botanical traits:

a.Effect of lead (Pb) on growth traits:

Data in tables (1, 2 and 3) indicated that, the presence of lead concentrations (500, 1000, 2000 ppm) in the soil caused a significant decrease in all of the studied growth characters (plant height, roots length, fresh and dry weights of shoots and roots of plants) with some exceptions. The toxicity of Pb on the previous growth parameters was slightly at the lowest concentration of Pb (500 ppm). In this respect, the reduction percentages on plant height, root length, shoots fresh and dry weights and roots fresh and dry weights were 7.2, 1.3, 11.0, 9.0, 1.0 and 5.0 % respectively, but at the highest concentration of Pb (2000 ppm), highly inhibitor effects were observed. The reduction percentages were 16.2, 6.9, 14.0, 13.0, 10.0 and 13.0%, respectively comparing with control.

These results are in agreement with the results obtained by Kosobrukhov *et al.*(2004), who has been found that lead (Pb) can alter photosynthesis efficiency through its effects on stomata or directly on mesophyll cells in which both photochemical and biochemical reactions were affected. Przymusinski *et al.* (1991) reported that the decrement in root length due to Pb might be the result of disturbation either in cell division or cell elongation within the root meristem. Also, Stobrawa and Lorenc (2007)

working on pointed out that lead caused a negative impact on root growth. Piechalak *et al* (2002) showed a few unfavorable changes on treated roots with lead such as growth inhibition and decrease biomass growth.

The depressive effect on plant height due to Pb treatments was also mentioned by Aery and Jagetiga (1997) on Barley (*Hordeum vulgare*), Sayed (1999) on safflower (*Carthamus tinctorius)* plants and Liu *et al.* (2000) on *Brassica juncea.*

b.Effect of treatments on growth traits:

Data in tables (1, 2 and 3) illustrated that mycorrhiza fungi, EDTA-Fe and ascorbic acid treatments, caused a significant increment on plant height, roots length, fresh and dry weights of shoots and roots resulting at all these treatments comparing with the control untreated plants.

The highest values were recorded due to applying the highest concentration of EDTA-Fe (260 ppm), where the height plant and roots length were 90 and 50 cm compared with 74 and 40 cm in control treatment respectively, while Mycorrhiza treatment (1000 spores) recorded the highest values in fresh- dry weights of shoots and dry weight of roots (227.1, 75.1 and 52.9 g.), compared with 195.5, 69.8 and 41.0 g. in control respectively.

In this respect, many authors suggested that mycorrhizal fungi increased plant height when the plants grown in polluted soil with Pb, because mycorrhizal fungal and hyphae, could present a biological barrier for retention of heavy metals so mycorrhizae used to decrease heavy metal concentrations in the shoots of nonhyperaccumulator plants, (Joner and Leyval, 2001). Moreover, Andrade *et al.* (2003) concluded that the inoculation of arbuscular mycorrhizal fungus improved Pb uptake and produced shoots with Pb concentrations 30% higher than those of non-mycorrhizal plants, at the highest Pb concentration added to the soil.

Mechanisms of protection against heavy metals provided by mycorrhizae to their host plants are not clear, a possible retention of heavy metals by the fungal mycelium involving adsorption to cell wall and fixation by polyphosphate granules (Galli *et al.* 1994). The abundance of external mycelium produced by the mycorrhiza funfi (MF) can be important for heavy metal–fixing ability of the fungi and consequently for their plant–protecting action.

Moreover, Rabie (2005) investigated the role of arbuscular mycorrhizal fungal inoculation in the tolerance of red kidney (*Phaseolus vulgaris*) and wheat (*Triticum aestivum*) to heavy metals in soil artificially contaminated with high concentrations of zinc, copper, lead and cadmium. Metals accumulated by mycorrhizal wheat plants were mostly distributed in root tissues, suggesting that an exclusion strategy for metal tolerance widely exists. Mycorrhizal red kidney plants accumulated relatively high metal concentrations in their shoots which indicated that internal detoxification metal tolerance mechanisms are also included. From a number of physiological indices measured in this study, mycorrhizal symbiosis significantly increased root and shoot dry weight, chlorophyll content and total lipid in wheat plants.

Fe-EDTA treatment reduced the depressive effect of lead on plant height whereas Fe-EDTA an excellent' source of iron for sunflower (Weinstein *et al.*1953).

Moreover, Guerinot and Yi, (1994) observed that iron is an essential element required for respiration, photosynthesis, and many other cellular functions such as DNA synthesis, nitrogen fixation, and hormone production. Although abundant in nature, iron often is unavailable because it forms insoluble ferric hydroxide complexes in the presence of oxygen at neutral or basic pH. In addition, Blylock *et al* (1997) reported that adding EDTA at a rate of 10 mM /kg soil, stimulated Pb accumulation in maize to levels as high as 1.6 % of the shoot dry weight.

Tables 1, 2 and 3. Plant height, roots length (cm), shoots fresh and dry weights and roots fresh and dry weights of ficus plants as effect of different levels of mycorrhiza, EDTA-Fe and ascorbic acid under different levels of lead in soil.

	Plant height (cm)					Roots length (cm)				
	Pb concentrations									
Treatments	0	500	1000	2000	Mean (B)	0	500	1000	2000	Mean (B)
Control	90.3	73.3	70.3	65.0	74.8	46.3	42.7	37.3	34.7	40.3
M1	93.0	90.0	81.7	78.7	85.8	51.0	54.0	51.7	43.0	49.0
M2	93.3	90.3	83.7	82.7	87.5	53.0	58.7	50.3	44.7	50.1
EDTA-Fe1	93.7	86.0	83.3	80.3	85.8	49.0	54.0	47.0	45.7	47.5
EDTA-Fe2	95.0	92.0	89.0	84.0	90.0	52.7	54.7	48.3	49.0	50.4
AsA1	93.0	86.3	79.7	77.0	84.0	51.3	53.7	47.7	44.7	48.5
AsA2	94.3	87.7	81.7	79.0	85.7	51.3	54.3	47.7	45.0	48.7
Mean (A)	93.2	86.5	81.3	78.1		50.7	49.4	47.1	43.8	
L.S.D at 5%	A = 8.6 B = 11.4 AB = 22.7			A = 3.4 B = 4.5 AB = 8.9						A = 3.4 B = 4.5 AB = 8.9

(A) lead concentrations. (B) [M1 (mycorrhiza =500 spores), M2 (mycorrhiza =1000 spores), EDTA-Fe1(ethylenediaminetetraacetic acid –irin = 130 ppm), EDTA-Fe2 =260 ppm, AsA.1(ascorbic acid =250 ppm) and AsA.2 (ascorbic acid =500ppm] AB (interaction).

	Shoots fresh weight (g)					Shoots dry weight (g)				
	Pb concentrations									
Treatments	0	500	1000	2000	Mean (B)	0	500	1000	2000	Mean (B)
Control	229.0	188.3	182.7	182.0	195.5	79.3	68.0	66.7	65.0	69.8
M1	244.3	224.3	215.7	214.0	224.6	79.0	75.3	72.0	71.7	74.5
M2	246.3	237.3	217.7	207.0	227.1	78.7	78.3	72.3	71.0	75.1
EDTA-Fe1	245.7	203.0	202.0	202.7	213.3	79.7	69.0	70.3	69.3	72.1
EDTA-Fe2	255.7	214.7	210.3	211.0	222.9	80.0	71.7	72.0	69.7	73.3
AsA1	224.7	219.3	209.0	205.7	214.7	79.0	73.0	71.7	68.3	73.0
AsA2	228.7	207.7	208.7	210.3	213.8	79.3	71.7	70.0	69.3	72.6
Mean (A)	239.2	213.5	206.6	204.7		79.3	72.4	70.7	69.2	
L.S.D at 5%	A =13.7 B = 18.2 AB =36.3		A =2.8 B = 3.7 AB = 7.4						A = 3.4 B = 4.5 AB = 8.9	

	Roots fresh weight (g)					Roots dry weight (g)				
	Pb concentrations									
Treatments	0	500	1000	2000	Mean (B)	0	500	1000	2000	Mean (B)
Control	83.0	77.3	93.0	80.0	83.3	44.0	41.0	40.0	39.0	41.0
M1	92.3	89.0	87.3	82.7	87.8	51.7	49.0	51.7	48.0	50.1
M2	102.7	99.3	87.0	83.3	93.1	59.7	57.3	48.0	46.7	52.9
EDTA-Fe1	51.7	74.0	68.3	68.3	65.6	42.5	40.0	42.0	39.0	40.9
EDTA-Fe2	81.3	76.3	69.3	70.0	74.2	43.3	39.7	47.3	39.0	42.3
AsA1	91.3	89.3	86.3	74.3	85.3	49.3	48.7	49.0	44.0	47.8
AsA2	100.7	90.0	87.0	85.7	90.9	56.7	54.3	50.7	48.0	52.4
Mean (A)	86.1	85.0	82.6	77.8		49.6	47.1	47.0	43.4	
L.S.D at 5%	A =7.7 n.s.	B = AB = 20.4	A =5.9 7.7	B = AB =15.5					A = 3.4 B = 4.5 AB = 8.9	

Ascorbic acid has numerous and diverse roles in plant metabolism, regulation of photosynthesis, and control of the partitioning of antioxidative enyzmes. The effect of ascorbic acid on plant growth was investigated by Foyer *et al.* (2006).

Moreover, Hall and Cuppet (1997) noted that among antioxidant ascorbic acid, glutathione and phenolic acids are particularly important. Damage to tissue occurs when the capacity of detoxification of the natural antioxidative systems becomes lower than the rate of reactive oxygen species (ROS) production (Sgherri *et al.*, 2003

2.Biochemical analysis.

a.Effect of lead (Pb) on plant pigments and enzymes activity.

The results concerning the effect of lead concentrations on plant pigments and enzymes activity the results in tables 4, 5 and 6 indicated that, there were significant differences in pigments concentrations (chlorophyll a, b and carotenoides) and enzymes activity as result of applying lead concentrations.

Generally it could be mentioned from tabulated data that, lead concentrations caused a significant decrement in pigments concentrations with some exceptions, whereas the reduction percentage of chlorophyll a reached to 17% in the highest level of lead concentrations (2000 ppm), while these decreases reached to 36% and 19% in both chlorophyll b and total carotenoids respectively at the same concentration of lead (2000 ppm).

Also, the results revealed that the highest rate of lead applications (2000 ppm) caused significant decrement in catalase enzyme activity (6.9) comparing with 9.9 in control, while it caused a significant increment in both peroxidase and SOD enzymes activity, the highest values were 2.5, and 6.9 at the highest rate of lead concentration (2000 ppm) comparing with 1.5 and 4.4 in control respectively.

These results are in agreement with those reported Paivoke (1983) who studied the effects of lead (0.01 – 1.0 mM) in nutrient solution on growth and development, chlorophyll content and nitrogen fixation of the garden pea. They reported that, at higher levels of Pb, the chlorophyll a and b contents of the leaves were decreased.

Burzynsk (1987) reported that, Pb inhibits chlorophyll synthesis by causing impaired uptake of essential elements such as Mg and Fe by plants.

In this connection, an enhancement of chlorophyll degradation occurs in Pb-treated plants due to increased chlorophyllase activity (Drazkiewicz, 1994). Chlorophyll b is reported to be more affected than chlorophyll a by Pb treatment (Vodnik *et al.*, 1999).

In addition Levina (1972) reported that, the inhibition exerted by Pb on enzyme activity results from the interaction of Pb with enzyme –SH groups. Pb interacts with free –SH groups that are present in the active site of the enzyme and essential for enzyme activity as well as with –SH groups that are necessary for the stabilization of enzyme tertiary structure. Besides the reaction with –SH groups, blockage of –COOH groups with Pb ions also appears to play a major role in inhibition of enzyme activity under Pb treatment.

The principal harmful oxygen species-scavenging enzyme in plant is catalase, which decomposes hydrogen peroxide to water and molecular oxygen, thus maintains the redox balance during oxidative stress (Bowler *et al.*, 1992).

A decline in the activity of catalase has been observed in Pb-stressed plants (Verma and Dubey, 2003). Moreover, the authors mentioned that, decreased intensity of two isoenzymic forms of catalase in shoots of Pb-stressed seedlings, consistent with decreased activity of the enzyme under Pb treatment.

The decreasing in catalase appears to be due to a decline in enzyme synthesis or a change in the assembly of enzyme subunits (Hertwig, *et al.* 1992).

In addition, Verma and Dubey (2003) showed increased activities of the antioxidative enzymes such as superoxide dismutase, and peroxidase on rice plants grown for 20 days in sand cultures containing 0.5 mM and 1 mM $Pb(NO_3)2$. However, activities of the antioxidative metalloenzymes decline when Pb displaces metals that are an essential part of enzyme.

b.Effect of treatments on plant pigments and enzymes activity.

Tables 4, 5 and 6 showed that treatments (mycorrhiza, EDTA-Fe and ascorbic acid) caused reduction in the hazard effects of lead in both pigments and enzymes activity of leaf, where treatments induced significantly increasing on chl.a, total carotenoids and catalase activity, the highest values were 0.58, 0.31 in EDTA-Fe treatment (260 ppm) and 9.1 in mycorrhiza treatment (1000 spores) comparing with 0.52, 0.27 and 6.8 in control respectively, while they caused decrement in both peroxidase and SOD enzymes activity the lowest values were 1.4 and 5.1 in AsA.2 treatment (500 ppm) comparing with 3.6 and 7.0 in control untreated plants, respectively.

Ulrich *et al.* (1994) found that Ecto- and endomycorrhizal symbiosis play a crucial role in protecting plant from heavy metals (HMS). EDTA-Fe1 (260 ppm of Fe) treatment played an important role for increment pigments because this treatment contained iron (Fe) which is essential element affecting on structure of chlorophyll.

Tables 4, 5, and 6. Chlorophyll a, b, carotenoids, catalase, peroxidase and superoxide dismutase (SOD) of ficus plants as effect of different levels of mycorrhiza, EDTA-Fe and ascorbic acid under different levels of lead in soil.

	Chlorophyll a mg / g f.w.					Chlorophyll b mg / g f.w.				
	Pb concentrations									
Treatments	0	500	1000	2000	Mean (B)	0	500	1000	2000	Mean (B)
Control	0.57	0.53	0.49	0.47	0.52	0.32	0.29	0.25	0.16	0.26
M1	0.59	0.55	0.51	0.49	0.53	0.32	0.30	0.25	0.21	0.27
M2	0.60	0.56	0.53	0.50	0.55	0.33	0.30	0.26	0.21	0.28
EDTA-Fe1	0.61	0.59	0.55	0.51	0.57	0.33	0.29	0.26	0.22	0.28
EDTA-Fe2	0.63	0.60	0.56	0.53	0.58	0.34	0.30	0.27	0.23	0.29
AsA1	0.59	0.57	0.55	0.51	0.56	0.32	0.28	0.24	0.20	0.26
AsA2	0.60	0.57	0.53	0.51	0.55	0.33	0.29	0.25	0.21	0.27
Mean (A)	0.60	0.57	0.53	0.50		0.33	0.29	0.25	0.21	
L.S.D at 5%	A = 0.04 B =0.05 AB = 0.11			A = 0.05 B = n.s. AB = 0.13						A = 3.4 B = 4.5 AB = 8.9

(A) lead concentrations. (B) [M1 (mycorrhiza =500 spores), M2 (mycorrhiza =1000 spores), EDTA-Fe1(ethylenediaminetetraacetic acid –irin = 130 ppm), EDTA-Fe2 =260 ppm, AsA.1(ascorbic acid =250 ppm) and AsA.2 (ascorbic acid =500ppm] AB (interaction).

	Total carotenoids mg / g f.w.					Catalase activity units/mg protin				
	Pb concentrations									
Treatments	0	500	1000	2000	Mean (B)	0	500	1000	2000	Mean (B)
Control	0.31	0.27	0.26	0.23	0.27	8.4	7.4	6.2	5.1	6.8
M1	0.32	0.30	0.27	0.25	0.29	10.1	9.4	8.4	7.1	8.8
M2	0.32	0.31	0.28	0.26	0.29	10.1	10.0	9.0	7.2	9.1
EDTA-Fe1	0.33	0.31	0.29	0.27	0.30	10.3	9.1	8.1	7.5	8.8
EDTA-Fe2	0.33	0.32	0.30	0.28	0.31	10.7	9.4	8.2	7.7	9.0
AsA1	0.31	0.29	0.27	0.24	0.28	9.9	8.3	7.9	6.9	8.3
AsA2	0.32	0.30	0.28	0.26	0.29	9.9	8.6	8.0	7.1	8.4
Mean (A)	0.32	0.30	0.28	0.26		9.9	8.9	8.0	6.9	
L.S.D at 5%	A = 0.03 B = 0.04 AB = 0.07			A =1.1 B = 1.4 AB = 2.8						A = 3.4 B = 4.5 AB = 8.9

	Peroxidase activity units/mg protin					SOD activity units/mg protin				
	Pb concentrations									
Treatments	0	500	1000	2000	Mean (B)	0	500	1000	2000	Mean (B)
Control	1.34	3.12	3.92	5.93	3.58	3.5	5.1	6.3	7.7	5.7
M1	2..14	1.73	2.11	1.87	1.90	3.5	3.8	4.3	5.3	4.2
M2	1.84	1.47	1.85	1.81	1.74	3.5	3.8	4.3	5.3	4.2
EDTA-Fe1	1.14	1.41	1.78	2.22	1.64	3.4	3.8	4.3	5.3	4.2
EDTA-Fe2	1.14	1.10	1.70	2.01	1.49	3.4	3.7	4.3	5.3	4.2
AsA1	1.99	1.24	1.32	2.06	1.65	3.2	3.6	4.2	5.2	4.1
AsA2	1.24	1.18	1.18	1.83	1.36	3.2	3.5	4.2	5.2	4.0
Mean (A)	1.45	1.61	1.98	2.53		3.4	3.9	4.6	5.6	
L.S.D at 5%	A =0.18 B =0.27 AB = 0.54			A =0.9 B =0.9 AB =1.8						A = 3.4 B = 4.5 AB = 8.9

Gupta *et al.* (1999) reported that antioxidants mobile in the plant to react enzymatically such as superoxide dismutase, peroxidase, catalase and enzymes ascorbate-glutathione or non-enzymatically such as ascorbate and glutathione with these toxic molecular species making them less harmful.

CONCLUSION

1. Lead concentrations caused a significant decrements in plant height, root length, shoots fresh and dry weights, roots fresh and dry weights, chl.a, b, carotenoids and catalase enzyme. But peroxidase and superoxide dismutase enzymes were increased under stress of lead.
2. The harmful effects of lead on botanical traits and biochemical structure were decreased by applying mycorrhiza, EDTA-Fe and ascorbic acid treatments.

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ستجابة نباتات الفيكس (*Ficus nitida*) لبعض المعاملات لتخفيض الأثر الضار للتلوث بالرصاص.

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إن زراعة شتلات نبات الفيكس(*Ficus nitida*) في تربة تحتوي على عنصر الرصاص بتركيزات (٠، ٥٠٠، ١٠٠٠، ٢٠٠٠ جزء بالمليون) سببت تغييرات في صفات النمو حيث أدى التلوث بالرصاص إلى إنخاض معنوي في كل من إرتفاع النبات، طول الجذور ،الوزن الطازج والجاف لكل من المجموع الخضري والجذري. كما سببت أيضا تغييرات في الصفات الكيميائية للنبات كإنخفاض تركيز كل من الكلورفيل أ والكلورفيل ب والكاروتينويدات إضافة إلى التأثير على النشاط الأنزيمي في اوراق النبات حيث سبب الرصاص إنخفاض معنوي في نشاط إنزيم الكاتليز بعكس كل من إنزيمي البيروكسيديز والسوبراوكسيد ديسميوتيز (Superoxide) dismutase اللذان ازداد نشاطهما بشكل معنوي. كما ان كل من الزياده أوالنقصان في كل الصفات السابقة تأثرت بشكل كبير بزيادة تركيز الرصاص في التربة حيث أزداد التأثير السلبي للرصاص على كل من صفات النمو والصفات الكيميائية بزيادة تركيز الرصاص .

أدت معاملة النباتات بفطر الميكورايزا والحديد المخلبي وفيتامين ج , دورا مهما في الحد من التأثير الضار للرصاص حيث ازداد معنويا كل من ارتفاع النبات وطول الجذر والوزن الجاف والرطب لكل من المجموع الخضري والجذري , كما ازداد تركيز كل من كلوروفيل أ وكلورفيل ب والكاروتينويدات وأزداد نشاط انزيم الكاتليز كما انخفض نشاط كل من إنزيمي البيروكسيديز والسوبر اوكسيد ديسميوتيز(SOD) وبشكل معنوي مقارنة بالكونترول.كما ان تأثير المعاملات كان أكثر فعالية في تركيزاتها العليا.