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ORIGINAL ARTICLE

STUDIES ON THE ENZYMATIC ACTIVITIES OF MERCURY CHLORIDE TREATED ORNAMENTAL PLANT *Zinnia elegans* (L.)

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ABSTRACT

Heavy metals are among the most general contaminants in the environment. Mercury (Hg) has been used for minimum the past 2500 years caused by its unique chemical and physical properties. The distribution of large quantities of mercury into the environment has concluded in its widespread occurrence in the whole food chain through plants. *Zinnia elegans* are well known garden plants, and can also be used as flowering potted plant. They have attractive capitula with a wide variety of colors, shape and size, long bloom period, easy propagation, fast growth. *Zinnia* ornamental plant species (*Zinnia elegans*), select the six concentration of mercury chloride (40, 100, 200, 300, 400 and 800 mg kg^{-1}) treatment in pot culture method. The testing periods is 15 days, 30 days, 45 days, 60 days, 75 days and 90 days. Enzyme activities of mercury chloride treated *Zinnia elegans*, to analyze the activity range of enzymes, α-amylase, β-amylase, catalase, peroxidase and polyphenol-oxidase. Mercury chloride treatment at all concentration levels (40, 100, 200, 300, 400 and 800 mg kg^{-1}) the enzyme activities are more than the control plant. The maximum enzyme activity recorded in the 800 mg kg^{-1} than in other concentrations.

Keywords: *Zinnia elegans*. Mercury chloride, Ornamental plant, enzyme activity.

1.INTRODUCTION

Mercury is also known to generate an antioxidant protective mechanism including the enzymatic and non-enzymatic constituents to resist oxidative damage in stressed plant tissue (Zhou *et al*., 2009). Inhibition of enzymes of different metabolic pathways has also been reported by mercury toxicity (Morch *et al*, 2002). Starch-degrading enzymes were found to be sensitive to increased heavy metal concentrations in *in vitro* and *in vivo* (Losch, 2004). α-amylase and β-amylase activity of germinating pea seeds were approximately halved under the influence of chromium ions (Dua and Sawhney, 1991). α-amylase and β-amylase activity of germinating pea seeds were approximately halved under the influence of cadmium ions (0.5 and 1 mM) (Chugh and Sawhney, 1996). The role of the antioxidative enzyme system was investigated in relation to nickel and cadmium stress (Schicker and Caspi, 1999). The activity of the antioxidative enzymes, including that of catalase has been found to increase in response to heavy metal treatment. Under copper stress, the growth of lettuce and bean seedling was inversely proportional to the induction of peroxidase activity (Van Assche and Clijsters, 1983). Karataglis *et al*., (1991) reported copper increased the activities of antioxidative enzyme (peroxidase) in wheat plants. Copper toxicity had been associated with an increase in antioxidative enzymes (Savoure *et al*., 1999). Peroxidase activity in wheat leaves increased in relation to the increase of leaf copper content (Mocquot *et al*., 1996). Chen *et al*. (2000) examined that copper treatment resulted in an increase in the activities of peroxidase in rice seedlings. Hg at 50 mM concentration in the nutrient medium increased the antioxidant enzyme activity of *Lolium perenne* (Bonnet *et al*., 2000). Apart from the mercury, excess supply of other heavy metals such as cadmium in *Vigna unguiculata* (Bhattacharya and Chowdhuri, 1994), lead and cadmium in rice (Bhattacharya and Chowdhuri, 1994) also produced different enzymatic activities.

The present study has been aimed to find out the enzyme (α-amylase, β-amylase, catalase, peroxidase and polyphenol-oxidase) activity of mercury chloride treated ornamental plant *Zinnia elegans* with six different concentration (40, 100, 200, 300, 400 and 800 mg kg^{-1}) level of the soil for the testing periods (15, 30, 45, 60, 75 and 90 days) also record the maximum activity of enzymes in these mercury chloride concentration level in the soil.

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2.MATERIALS AND METHODS

α – Amylase activity assay

α-Amylase activity was assayed by following the method of Bernfeld (1955). One gram of the material was homogenized in a pre-chilled mortar and pestle with 10ml of chilled distilled water. The homogenate was centrifuged at 15,000rpm for 30 min at 4 ºC in a refrigerated centrifuge. The supernatant was saved and it was used as the enzyme source. To 5ml of the enzyme extract 3 mM calcium chloride was added and heated for 5min at 70 ºC to inactivate α-amylase. α-Amylase digests the soluble starch into reducing sugars. 0.5ml of the enzyme extract, 1ml of 0.1M citrate buffer (pH 5.0) and 0.5ml of 2 per cent soluble starch were added. The reaction was allowed for 5min after addition of starch at 30 ºC. After 5min the reaction was stopped by adding 2ml of colour reagent. The mixture was heated for 5min in a water bath at 50 ºC. After cooling, the final volume of the solution was made up to 10ml. the absorbance was read at 540nm in a spectrophotometer. A calibration curve established with maltose was used to convert the calorimeter readings into milligrams of maltose. α-amylase activity was expressed in terms of mg of maltose liberated min^{-1} mg^{-1} protein. One gram of 3,5-dinitrosalicylic acid, 200mg of crystalline phenol and 50mg of sodium sulfide were dissolved in 20ml of 1 per cent NaOH. To this mixture, 40g of sodium potassium tartarate was added. The final volume was made upto 100ml with distilled water and filtered. Fresh reagent was used for each experiment.

β-amylase activity assay

Both extraction and estimation of enzyme activity were similar to that of α-amylase. But the crude enzyme extract was treated with EDTA and 2-mercaptoethanol to inactivate the α-amylase (Tarrago and Nicolas, 1976). To 5 ml of the enzyme extract, 1mM EDTA and 5mM 2-mercaptoethanol were added. The extract was incubated at 30 ºC for 30min and then was used for the assay of β-amylase activity. Assay procedure was followed as described in α-amylae. The activity was expressed in terms of mg of maltose liberated min^{-1} mg^{-1} protein.

Catalase activity assay

Catalase activity was measured by the method of Machly and Chance (1967). One gram of leaf sample was homogenized in 10ml of 0.1M sodium phosphate buffer pH 7 and centrifuged at 4 ºC for 10min at 10,000rpm. An aliquot of 1ml of the supernatant of the enzyme extract was added to the reaction mixture containing 1 ml of 0.01 M H_2O_2 and 3ml of 0.1M sodium phosphate buffer. The reaction was stopped after an incubation of 5min at 20 ºC by adding 10ml of 1% H_2SO_4. The acidified medium without or with the enzyme extract was titrated against 0.005N $KMnO_4$ and catalase activity was expressed as n moles of H_2O_2 utilized g^{-1} fresh $water^{-1}$.

Peroxidase activity assay

One gram of fresh plant material was homogenized with 20ml of ice cold extraction medium containing 2mM $MgCl_2$, 1 mM EDTA, 10mM β-mercaptoethanol, 7 per cent PVP and 10mM sodium metabisulphate. The homogenate was strained through two layers of cheese cloth and centrifuged at 10,000rpm for 15min. the supernatant was made up to 20ml with the same buffer and it was used as the source of enzyme. Peroxidase activity was assayed by the method of Kumar and Khan (1982). Assay mixture of peroxidase contained 2ml of 0.1M phosphate buffer pH 6.8, 1ml of 0.001M pyrogallol, 1ml of 0.005M hydrogen peroxide and 0.5ml of enzyme extract. The solution was incubated for 5min at 25 ºC after which the reaction was terminated by adding 1ml of 2.5N sulphuric acid. The amount of purpurogallin formed was determined by reading the absorbance at 420nm against a blank prepared by adding the extract after the addition of 2.5N sulphuric acid. The activity was expressed in unit = 0.1 absorbance min^{-1} mg^{-1} protein.

Polyphenoloxidase activity assay

Polyphenoloxidase activity was assayed by the method of Kumar and Khan (1982). Assay mixture for polyphenoloxidase contained 2ml of 0.1M phosphate buffer (pH 6.0), 1ml of 0.1M catechol and 0.5ml of enzyme extract. This was incubated for 5min at 25 ºC, after which the reaction was stopped by adding 1ml of 2.5N sulphuric acid. The absorbance of the purpurogallin formed was recorded at 495nm. The enzyme activity was expressed in units. One unit is defined as the amount of purpurogallin formed, which raised the absorbance by 0.1 min^{-1} under the assay condition.

Statistical Analysis

The statistical analysis of the experimental data was carried out as per the procedure given by Gomez and Gomez (1984).

3.RESULTS

α-Amylase activity

The α-Amylase activity of *Zinnia elegans* plants at various level of mercury chloride at different stages of growth is furnished in Table 1. Maximum α-Amylase activity of *Zinnia elegans* leaves was found at control (viz., 12.874, 18.696, 20.786, 18.976, 16.866 and 15.643) in all the sampling days. There was a gradual decrease in the α-Amylase activity with further increase of mercury chloride concentration (40, 100, 200, 300, 400 and 800 mg kg^{-1}) in the soil. A decrease α-Amylase activity of *Zinnia elegans* leaf (viz., 8.745, 14.664, 16.365, 14.755, 12.656 and 10.446) was observed at 800 mg kg^{-1} mercury chloride level of the soil in all the sampling days. The α-Amylase activity showed a progressively trend up to 45^{th} day and it gradually declined afterwards. Variance of mean values for treatment and sampling days are significant at 1 per cent level in mercury chloride treated *Zinnia elegans* plants.

β-Amylase activity

The β-Amylase activity was monitored in the experimental plant under different mercury chloride concentrations and the results are presented in the Table 2. The higher β-Amylase activity was observed at control plant leaves (viz.,

Table 1. Effect of Mercury chloride on α-amylase activity (µg min^{-1} mg^{-1} protein) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	**30**	**45**	**60**	**75**	**90**
0	12.874	18.696	20.786	18.976	16.866	15.643
40	12.278	18.223	20.545	18.186	16.166	14.754
	(-10.67)	(-5.64)	(-10.45)	(-11.34)	(-10.34)	(-9.65)
100	11.878	17.765	19.565	17.576	15.565	13.364
	(-15.75)	(-18.34)	(-17.75)	(-15.15)	(-16.96)	(-19.43)
200	10.978	16.575	18.566	16.766	14.646	12.743
	(-26.75)	(-21.26)	(-20.73)	(-17.57)	(-21.65)	(-19.73)
300	9.574	15.745	17.676	15.865	13.754	11.765
	(-35.75)	(-25.93)	(-21.73)	(-27.62)	(-23.83)	(-31.12)
400	8.745	14.664	16.365	14.755	12.656	10.446
	(-23.11)	(-34.74)	(-31.06)	(-25.74)	(-21.64)	(-27.73)
800	6.757	12.654	13.766	12.756	10.767	8.476
	(-41.73)	(-31.83)	(-28.83)	(-38.73)	(-37.73)	(-40.78)

Comparison of significant effects	F test
Mercury chloride level	139.41**
Sampling days	345.27**

Figures in parentheses represent per cent reduction (-) over control

Table 2. Effect of Mercury chloride on β-amylase activity (µg min^{-1} mg^{-1} protein) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	**30**	**45**	**60**	**75**	**90**
0	14.44	22.86	25.37	18.84	16.36	14.85
40	14.38	22.84	25.94	17.89	16.56	14.85
	(-9.75)	(-7.84)	(-4.75)	(-3.33)	(-4.66)	(-7.78)
100	13.65	21.74	24.21	16.61	15.20	13.34
	(-14.64)	(-13.84)	(-14.83)	(-11.38)	(-16.27)	(-19.67)
200	12.67	20.38	23.74	15.05	14.74	12.94
	(-20.89)	(-19.37)	(-14.77)	(-17.84)	(-16.67)	(-21.03)
300	11.57	19.94	22.84	14.38	13.85	11.74
	(-26.27)	(-25.55)	(-19.36)	(-20.63)	(-27.98)	(-28.44)
400	10.95	18.57	21.38	13.95	12.84	10.64
	(-19.65)	(-16.95)	(-17.43)	(-18.78)	(-15.85)	(-20.89)
800	8.84	16.84	19.93	11.26	10.95	8.83
	(-29.64)	(-28.77)	(-23.85)	(-27.74)	(-36.57)	(-37.85)

Comparison of significant effects	F test
Mercury chloride level	68.37**
Sampling days	241.21**

Figures in parentheses represent per cent reduction (-) over control

Table 3. Effect of Mercury chloride on catalase activity (units min^{-1} g^{-1} fresh weight) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	**30**	**45**	**60**	**75**	**90**
0	1.27	1.45	1.73	1.85	1.64	1.49
40	1.45	1.58	1.89	1.92	1.74	1.60
	(+3.68)	(+5.48)	(+4.84)	(+2.38)	(+6.93)	(+7.27)
100	1.54	1.63	2.04	2.04	1.86	1.75
	(+12.86)	(+17.94)	(+16.84)	(+9.22)	(+10.83)	(+13.85)
200	1.62	1.89	2.48	2.37	1.97	1.84
	(+25.27)	(+23.94)	(+18.57)	(+17.86)	(+15.27)	(+18.98)
300	1.73	2.03	2.69	2.48	2.09	1.92
	(+28.74)	(+33.86)	(+29.26)	(+26.95)	(+25.58)	(+21.37)
400	1.85	2.46	2.76	2.68	2.27	2.11
	(+35.38)	(+34.68)	(+35.48)	(+25.05)	(+26.03)	(+21.56)
800	1.93	2.89	2.86	2.76	2.65	2.37
	(+29.36)	(+21.74)	(+31.95)	(+28.35)	(+27.59)	(+28.48)

Comparison of significant effects	F test
Mercury chloride level	102.87**
Sampling days	147.27**

Figures in parentheses represent per cent reduction (-) over control

Table 4. Effect of Mercury chloride on peroxidase activity (units min^{-1} mg^{-1} protein) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	30	45	60	75	90
0	0.175	0.245	0.355	0.366	0.266	0.210
40	0.196 (+7.48)	0.267 (+9.85)	0.376 (+8.37)	0.394 (+3.75)	0.290 (+4.37)	0.257 (+8.07)
100	0.264 (+15.06)	0.289 (+12.45)	0.398 (+8.86)	0.425 (+9.47)	0.314 (+8.47)	0.283 (+12.36)
200	0.279 (+16.484)	0.302 (+20.85)	0.423 (+12.84)	0.452 (+13.73)	0.358 (+15.73)	0.297 (+21.75)
300	0.316 (+27.38)	0.347 (+26.75)	0.437 (+21.38)	0.478 (+18.27)	0.375 (+17.83)	0.325 (+27.72)
400	0.337 (+31.27)	0.363 (+36.85)	0.476 (+26.86)	0.497 (+19.75)	0.387 (+21.09)	0.365 (+31.68)
800	0.386 (+24.78)	0.402 (+27.86)	0.486 (+21.75)	0.523 (+27.77)	0.404 (+30.67)	0.387 (+29.37)

Comparison of significant effects	F test
Mercury chloride level	42.37**
Sampling days	385.43**

Figures in parentheses represent per cent reduction (-) over control

Table 5. Effect of Mercury chloride polyphenol-oxidase activity (units min^{-1} mg^{-1} protein) of ornamental plant, *Zinnia elegans* (L.)

Mercury chloride added in the soil (mg kg-1)	Sampling days					
	15	30	45	60	75	90
0	0.268	0.375	0.453	0.517	0.377	0.273
40	0.287 (+7.76)	0.389 (+6.66)	0.482 (+5.64)	0.549 (+6.22)	0.397 (+6.74)	0.294 (+10.85)
100	0.317 (+12.76)	0.412 (+11.68)	0.497 (+14.35)	0.573 (+18.37)	0.425 (+16.98)	0.324 (+17.45)
200	0.346 (+19.76)	0.436 (+18.47)	0.508 (+17.88)	0.592 (+15.66)	0.468 (+19.86)	0.359 (+21.53)
300	0.368 (+23.42)	0.465 (+18.66)	0.546 (+17.07)	0.624 (+17.65)	0.482 (+19.53)	0.379 (+21.76)
400	0.386 (+27.43)	0.478 (+26.77)	0.586 (+026.33)	0.664 (+22.65)	0.532 (+21.45)	0.391 (+24.43)
800	0.421 (+31.86)	0.498 (+28.76)	0.612 (+26.64)	0.694 (+32.33)	0.632 (+34.56)	0.421 (+28.76)

Comparison of significant effects	F test
Mercury chloride level	167.24**
Sampling days	798.24**

Figures in parentheses represent per cent reduction (-) over control

14.44, 22.86, 25.37, 18.84, 16.36 and 14.85) in various sampling days. There was a gradual decrease in the β-Amylase activity with further increase of mercury chloride concentration (40, 100, 200, 300, 400 and 800 mg kg^{-1}) in the soil. A decrease β-Amylase activity of *Zinnia elegans* leaf (viz., 8.84, 16.84, 19.93, 11.26, 10.95 and 8.83) was observed at 800 mg kg^{-1} mercury chloride level of the soil in all the sampling days. At all β-amylase activity was always higher than α-amylase activity. F-values were significant at 1 per cent level in mercury chloride treated *Zinnia elegans* plants.

Catalase activity

The catalase activity of *Zinnia elegans* plants at various level of mercury chloride at different stages of growth is furnished in Table 3. The lowest catalase activity of leaf was observed at control *Zinnia elegans* plant (viz., 1.27, 1.45, 1.73, 1.85, 1.64 and 1.49) in all the sampling days. There was a gradual increase in the catalase activity with further increase of mercury chloride concentration (40, 100, 200, 300, 400 and 800 mg kg^{-1}) in the soil. The highest catalase activity of *Zinnia elegans* leaf (viz., 1.93, 2.89, 2.86, 2.76, 2.65 and 2.37) was recorded at 800 mg kg^{-1} mercury chloride level of the soil. In the mercury chloride treatment catalase activity is higher on the 60^{th} day than the 75^{th} and 90^{th} day. The F test values were significant at 1 per cent level for treatment and sampling days in mercury chloride treated *Zinnia elegans* plants.

Peroxidase activity

Peroxidase activity of leaves of *Zinnia elegans* under mercury chloride stress is represented in Table 4. The peroxidase activity also showed a similar trend as that of the catalase activity. There was a gradual increase in the peroxidase activity with increase Mercury chloride level. The peroxidase activity of leaf was minimum at control *Zinnia elegans* plant (viz., 0.175, 0.245, 0.355, 0.366, 0.266 and 0.210) in all the sampling days. The increase of Mercury chloride level in the soil (40, 100, 200, 300, 400 and 800 mg kg^{-1}), increased the peroxidase activity of *Zinnia elegans* in all the sampling days. Maximum peroxidase activity of *Zinnia elegans* leaf (viz., 0.386, 0.402, 0.486, 0.523, 0.404

and 0.387) was observed at 800 mg kg^{-1} mercury chloride level of the soil. The peroxidase activity of mercury chloride treated plants was higher at the 60^{th} day than the 75^{th} and 90^{th} day. The F test values were significant at 1 per cent level for treatment and sampling days in mercury chloride treated *Zinnia elegans* plants.

Polyphenol oxidase activity

The polyphenol oxidase activity also showed similar increasing trend as that of the catalase and peroxidase activity is represented in Table 5. There was a gradual increase in the polyphenol oxidase activity with increase Mercury chloride level (40, 100, 200, 300, 400 and 800 mg kg^{-1}) of the soil. The polyphenol oxidase activity of leaf was minimum at control *Zinnia elegans* plant (viz., 0.268, 0.375, 0.453, 0.517, 0.377 and 0.273) in all the sampling days. The increase of Mercury chloride level in the soil (40-800 mg k^{-1}), increased the polyphenol oxidase activity of *Zinnia elegans* in all the sampling days. The maximum polyphenol oxidase activity of *Zinnia elegans* leaf (viz., 0.421, 0.498, 0.612, 0.694, 0.632 and 0.421) was observed at 800 mg kg^{-1} mercury chloride level of the soil. The polyphenol oxidase activity of mercury chloride treated plants was higher at the 60^{th} day than the 75^{th} and 90^{th} day. The statistical analysis was significant at 1 per cent level for treatment and sampling days in mercury chloride treated *Zinnia elegans* plants.

4.DISCUSSION

Mercury chloride stimulation of both α and β amylase activity analyzed the *Zinnia elegans* plants in the different concentration level 40, 100, 200, 300, 400 and 800 mg kg^{-1} in the soil. At the highest concentration of mercury chloride (800 mg kg^{-1}) the activity was declined in 45^{th} day, which agrees with the result of Dua and Sawhney (1991) in chromium and Chugh and Sawhney (1996) and Bansal *et al*. (2001) in cadmium. Starch degrading enzymes such as α and β amylase activities were found to be sensitive to increased heavy metal concentrations. Further, the reduced release of sugars from the storage macromolecules under the influence of heavy metals might also be the reason for reduced rate of amylase enzyme (Losch, 2004).

Higher catalase activity is observed in the increased concentration of mercury chloride than the control plant *Zinnia elegans*. This can be compared with earlier reports of Luna *et al*. (1994) in oat leaves, Weckx and Clijsters (1996) in *Phaseolus vulgaris*, Savoure *et al*. (1999) in *Nicotiana plumbaginifolia* under copper treatment. Cadmium (Bhattacharya and Chowdhuri, 1994) in *Vigna unguiculata* and lead and cadmium (Bhattacharjee *et al*., 1996) in rice plants. In the present study, higher levels of mercury chloride treatment results in an increase in the activities of catalase, which can be considered as an indirect evidence for enhanced production of free radicals. The stimulation of catalase synthesis by excess heavy metals may be due to the increased synthesis of the protein moiety of catalase and also the increased synthesis of iron-porphyrin for the formations of catalase also confirm the results (Agarwal *et al*., 1961).

Peroxidase activity also increased with the increase in mercury chloride level. The increase of peroxidase has also been reported under copper treatment in wheat (Karataglis *et al*., 1991), in maize (Mocquot *et al*., 1996) and in rice (Chen *et al*., 2000) The increase in peroxidase activity is known to play an important role in reducing oxidative stress by catalyzing the reduction of H_2O_2 (Weckx and Clijsters, 1996). The increase in peroxidase activity is likely to be related to oxidative reactions corresponding to an increase in free radicals in the plant cell (Van Assche and Clijsters, 1990). The protective mechanisms adopted by plants to scavenge free radicals and peroxides include several antioxidative enzymes such as catalase and peroxidase. The antioxidative enzymes are important components in preventing the oxidative stress in plants as is based on the fact that the activity of one or more of these enzymes are generally increased in plants when exposed to stressful condition (Allen, 1995). These results are also evident with the observation of various metals such as Shaw and Rout (1998) in mercury and cadmium, Subhadra *et al*. (1991) in mercury and Schicker and Caspi (1999) in nickel and cadmium. Kariev (1969) in zinc and nickel and Vlasyuk and Galinskaya (1970) in rubidium, nickel and cesium.

5.CONCLUSION

High polyphenol-oxidase activity under stress indicates its ability to oxidize and to degrade the toxic substances such as phenolic components, which are generally reported to be accumulated during metal stress. It may, therefore, be inferred that increase in catalase, peroxidase and polyphenol oxidase activity in *Zinnia elegans* are the best indicators of stress and the measurement of activity of these enzymes may lead to the knowledge of invisible injuries caused to plants under heavy metal mercury chloride stress in all the concentrations (40. 100. 200, 300, 400 and 500 mg kg^{-1}).

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