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## Effect of Lead on Germination, Growth and Activity of Catalase and Peroxidase Enzyme in Root and Shoot of Two Cultivars of *Brassica napus* L.

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**Abstract:** In this project the different concentration of lead [(NO<sub>3</sub>)<sub>2</sub>Pb] (0, 100, 200, 400, 600, 800 and 1000 μM) on germination and the effect of nutrient solution with and without lead (0, 100, 200 and 400 μM), in hydroponics culture were examined. The growth parameters and activity of enzymes catalase and peroxidase on *Brassica napus* cv. Pf 7045 91 and Hyola 401 were studied. Lead caused a decrease on germination in two cultivars (p<0.05). By increasing lead concentration more decreasing in germination on Pf than the Hyola has been shown. Growth parameters on both cultivars have been decreased by lead effect. The enzyme activity of catalase and peroxidase in root and shoot on both cultivars by increasing lead concentration has been increased. The activity of root peroxidase has shown significant decrease only in high lead concentration on Pf (p<0.05). The results show that the germination on Pf was more resistant than the Hyola and at vegetative stage in Hyola has more resistance than the Pf.

**Key words:** Lead, germination, growth, catalase, peroxidase

### INTRODUCTION

Lead is a danger heavy metal, which plotted the environment. Most of present lead in environment is from the paint, petrol battery production industry, additives, pesticides, car exhaust, soldering and compost (Erick *et al.*, 1999).

High lead concentration in soil, decrease the germination and has a harmful effect on growth and metabolism in plant (Geebelen *et al.*, 1999; Pang *et al.*, 2002).

Toxicity of lead on plants caused inhibition of root growth (MáthéGáspár and Anton, 2002; Verma and Dubey, 2003; Yang *et al.*, 2000). Different environmental stress, for example, salinity, high temperature, drought and heavy metal caused oxidative effect and high production of different oxygen reactive (ROS) in plants (Asad, 1994). Reactive oxygen is super oxide, hydroxyl radical group and peroxide hydrogen. They produced during the activity of membrane electron transport chain reaction and some metabolic pathway (Assad, 1992). This oxygen is dangerous for membrane lipids, proteins, chloroplast, enzymes and nucleic acids (Shah *et al.*, 2001). The plants have a defense system against oxidation. This system has catalase enzymes, peroxidase, superoxide

mutase and nonenzyme part like tocopherol, ascorbate and glutathione and neutralized the oxygen (Sairam *et al.*, 2002).

H<sub>2</sub>O<sub>2</sub> produced from photorespiration and fatty acids •-oxidations have removed by catalase. H<sub>2</sub>O<sub>2</sub> produced from organics and inorganic substrate's oxidation has used by peroxidase. Heavy metal like Cd, Pb, Al, Zn has a role on production of ROS. They also induced oxidative stress on plants. In this project germination, growth and activity of catalase and peroxidase in two cultivars of rape seed (PF7045.91 and Hyola 401) has been studied.

### MATERIALS AND METHODS

**Experimental technique of germination:** Solutions of lead nitrate (0, 100, 200, 400, 600, 800 and 1000 μM) were prepared. About 50 seeds (for each sample) put on filter paper and cover it with another filter paper and each plate labeled and then added 5 mL of different concentrations of lead nitrate. Closed the top of plates and leaved them in germinator and set the temperature on 25°C. After two days the number of germination in related to root appearance counted. Then counting has repeated every day till 9th days.

**Experimental stage on green house with hydroponic**

**method:** Seeds of 2 cultivars of *Brassica napus* were cultured in depth of 1.5 cm with equal distance on washed sand. Sand was washed with hydrochloric acid 3% for 24 h followed by washing with plenty of water. Finally it washed with distilled water to remove all unwanted materials. Seeds were grounded at 25°C temperature.

They feed with 0.5% Hogland's solution (Table 1) and enough water till cotyledon slipped and first leaves have been appeared. Seeding were selected and three of them moved to plastic container with 400 mL of Hogland's nutrient solution, then close the container and made three holes (0.5 cm diameter) on the cap. Used 4 containers for each cultivars and each treatment (total 32 containers). Plants were on a room (2×3 m dimensions) equipped with air condition.

Room temperature was 25±1°C in a day and 18±1°C in a night. The length of light and darkness was 14 and 10 h, respectively. Light was sunlight, enforcement with 4 fluorescents tube (400 and 40 w) tube. The plant was studied for 16 days.

Nutrient solution weathered five times and each time takes half and hours every day. Plants leaved in Hogland's solution for 6 days. After 6 days the solution was refreshed. At this time solution was mixed with different solutions of lead nitrate (0, 100, 200 and 400 µM). The plants leave to growth in this solution for two days.

**Determination of growth parameters:** Plant was removed from nutrient solution after 16 days (6 days in Hogland's nutrient and 10 days on Hogland's nutrient solution mixed with different concentrations of lead nitrate). Roots were washed with water, separate them from aerial organ and weighted. The aerial organ was also weighted and the area of leaves was measured. To measure the dry weight of root and shoot, they incubated in 90°C for 24 h, then immediately the dry samples were weighted.

**Determination of peroxidase activity:** Activity of peroxidase was determined with Koroi's method (Koroi, 2003). One gram of fresh organ were grinded with 4 mL of extraction solution, containing 1.2 g tris, 2 g ascorbic acid, 3.8 g borax, 2 g EDTA Na<sub>2</sub>, 50 g polyethyleneglycol 2000 in 100 mL D.water. The solution was left at 4°C temperature for 24 h. Then it was centrifuged at 400 g for 30 min.

Added 0.1 mL of enzyme extract to a mixture of 1 mL acetate tampon solution (0.2 M, pH = 5), 0.4 mL hydrogen peroxidase (%3) and 0.2 mL benzydin:alcohol (0.01 M).

Then the absorbance on 530nm wavelength was read. The enzyme activity was calculated (O. D.g<sup>-1</sup>.Fw. min<sup>-1</sup>).

Table 1: Hogland's nutrient solution

Concentration	Salt	Concentration	Salt
5 mM L <sup>-1</sup>	Ca(NO <sub>3</sub> ) <sub>2</sub> .4H <sub>2</sub> O	5 mM L <sup>-1</sup>	KNO <sub>3</sub>
2 mM L <sup>-1</sup>	MgSO <sub>4</sub>	1 mM L <sup>-1</sup>	KH <sub>2</sub> PO <sub>4</sub>
108 mM L <sup>-1</sup>	MnCl <sub>2</sub> .4H <sub>2</sub> O	3 g L <sup>-1</sup>	H <sub>3</sub> BO <sub>3</sub>
1 mg L <sup>-1</sup>	CuSO <sub>4</sub> .5H <sub>2</sub> O	0.2 mg L <sup>-1</sup>	ZnSO <sub>4</sub> .7H <sub>2</sub> O
0.24 mM L <sup>-1</sup>	EDTA	20 mg L <sup>-1</sup>	H <sub>2</sub> MOO <sub>4</sub> .H <sub>2</sub> O
		0.24 mM L <sup>-1</sup>	FeSO <sub>4</sub> .7H <sub>2</sub> O

**Determination of catalase:** Catalase activity was obtained by Chance's and Maehly Method (1995). Five milliliter of solution containing 300 µM phosphate buffer (pH = 6.8) and 100 µM hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was prepared and 1 mL of enzyme extraction (2 times diluted) added and left at 25°C for 1 min. Then 10 mL sulfuric acid (2%) was added and titrated with potassium permanganate (0.01 N) till pink color was achieved. Enzyme activity was decomposition of 1 µM H<sub>2</sub>O<sub>2</sub> in 1 min.

**Static calculation:** Variation and F-test was obtained by Spss software and all graphs were drawn by excel software.

**RESULTS**

**Effect of lead on germination:** In both cultivars, percentage of germination was decreased by increased lead concentration. In Hyola treated with different lead concentration (200, 400, 600, 800 and 1000 µM) the percentage of germination was decreased, compared to the control sample (Fig. 1). Decreasing of germination in PF treated with different lead concentration (200, 400, 600, 800 and 1000 µM) the change of germination was not significant. Percentage of decrease of germination of Hyola was higher than the PF (Fig. 2).

**Effect of lead on growth parameters**

**Dry and fresh weight:** Dry and fresh weight of root and shoot on both cultivars treated with lead was decreased. Weight of fresh root of Hyola treated with moderate and high lead concentration was decreased significantly. Weight of fresh root of Pf treated with only high lead concentration was shown significant reduction (p<0.05) (Fig. 3).

Fresh weight of shoot of Hyola and PF was decreased significantly only in presence of high Lead concentration (p<0.05, Fig. 4), Dry weight of roots of Hyola and PF treated with high Lead concentration was significantly reduced (p<0,05, Fig. 5). Dry weight of shoot on both cultivars was reduced significantly p<0.05, Fig. 6). Percentage of reduction of dry and fresh weight of root and shoot on Pf was higher than the Hyola. Percentage reduction of shoot fresh weight on Hyola was higher than the Pf on sample treated with low lead concentration.

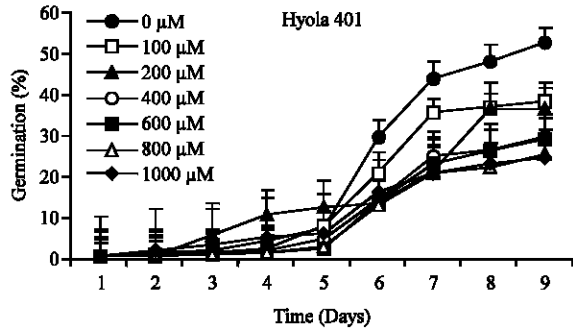


Fig. 1: Percentage of germination of Hyloa in different lead concentration

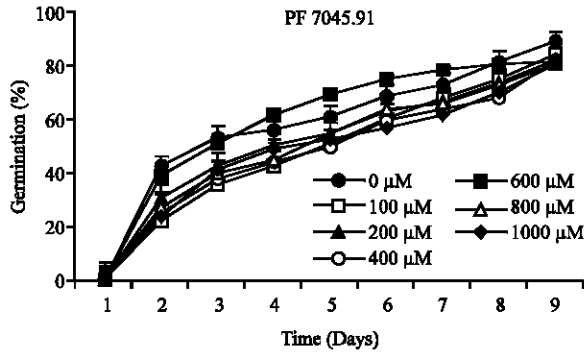


Fig. 2: Percentage of germination of PF 7045.91 in different lead concentration

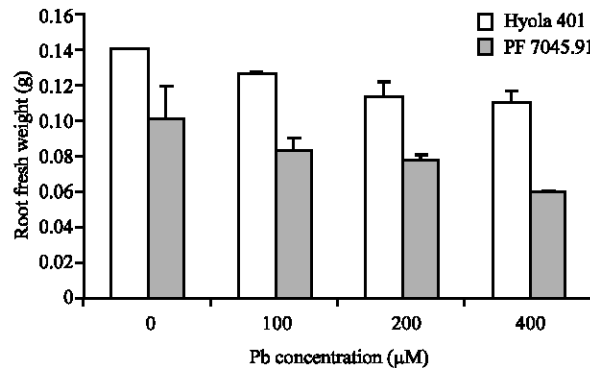


Fig. 3: Fresh weight of root in Hyloa 401 and PF 7045.91 after 10 days treated in Hogland's nutrient solution

**Root length:** Root length on both cultivars was reduced by increasing lead concentration. Root length of Hyloa treated with high and moderate lead concentration was decreased significantly ( $p < 0.05$ ). Root length of PF treated with lead concentration was decreased ( $p < 0.05$ , Fig. 7). Percentage reduction of root length on Pf was higher than the Hyloa.

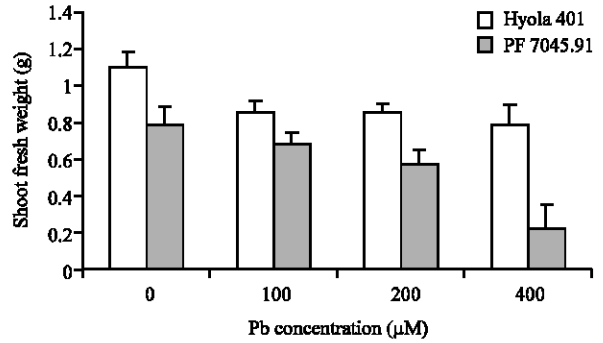


Fig. 4: Fresh weight of shoot in Hyloa 401 and PF 7045.91 after 10 days in Hogland's nutrient solution treated with different lead concentration

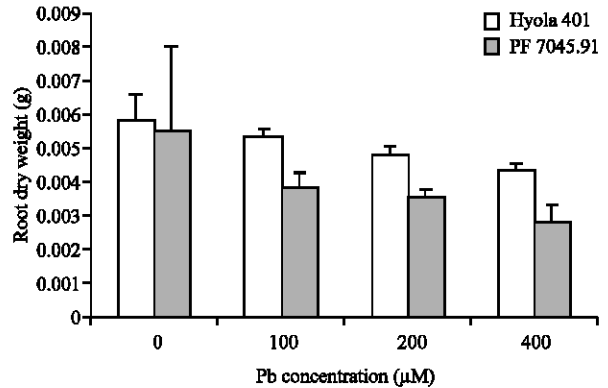


Fig. 5: Dry weight of root in Hyloa 401 and PF 7045.91 after 10 days in Hogland's nutrient solution treated with different lead concentration

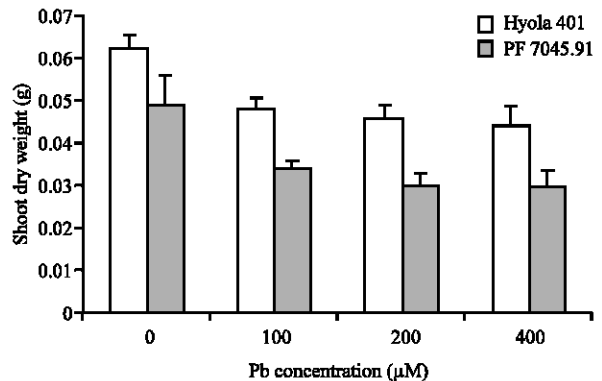


Fig. 6: Dry weight of shoot in Hyloa 401 and Pf 7045.91 after 10 days in Hogland's nutrient solution treated with different lead concentration

**Leaf area:** Increasing lead concentration reduced leaf area of both cultivars. Only Hyloa treated with high lead concentration was shown significant reduction ( $p < 0.05$ ). Pf treated with high and medium lead concentration was

shown reduction ( $p < 0.05$ , Fig. 8). Percentage reduction of leaf area on Pf was higher than the Hyola.

**Effect of lead on catalase activity:** On both cultivars catalase activity on root and shoot at higher lead

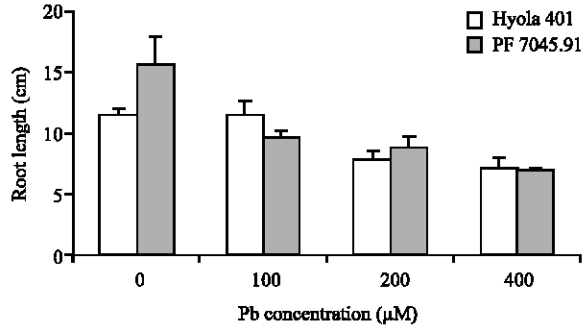


Fig. 7: The length of root in Hyola 401 and Pf7045.91 after 10 days in Hogland's nutrient solution treated with different lead concentration

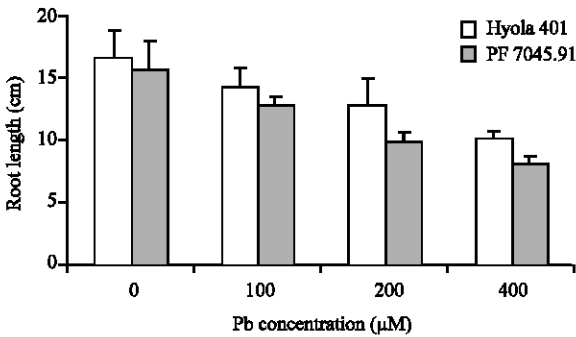


Fig. 8: The leaf area in Hyola 401 and Pf7045.91 after 10 days in Hogland's nutrient solution treated with different lead concentration

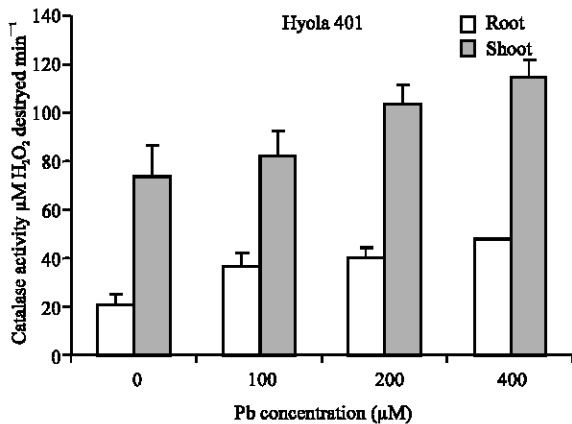


Fig. 9: Catalase activity on root and aerial organs of Hyola 401 after 10 days in Hogland's nutrient solution treated with different lead concentration

concentration was increased. Catalase activity on roots of Hyola treated with all of lead concentration was significantly increased. Shoot of Hyola in presence of high and moderate lead concentration were effectively increased. Also the catalase activity of Hyola's root treated with high lead concentration compare to sample treated with low lead concentration was significantly increased. ( $p < 0.05$ , Fig. 9).

Catalase activity was increased on root of PF treated with moderate and high lead concentration. The activity was also increased on shoot. In samples treated with all lead concentration was examined ( $p < 0.05$ , Fig. 10). Percentage of catalase activity on root and shoot of Hyola was higher than the Pf. Percentage of catalase activity on low lead concentration was increased on shoot of Pf compare to Hyola percentage of catalase activity on root of both cultivars was higher than the shoot.

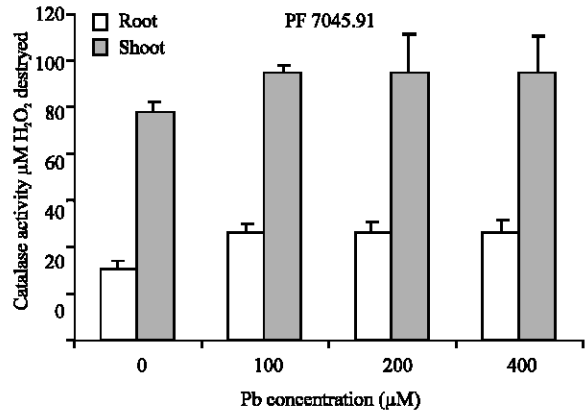


Fig. 10: Catalase activity on root and shoot of Pf 7045.91 after 10 days in Hogland's nutrient solution treated with different lead concentration

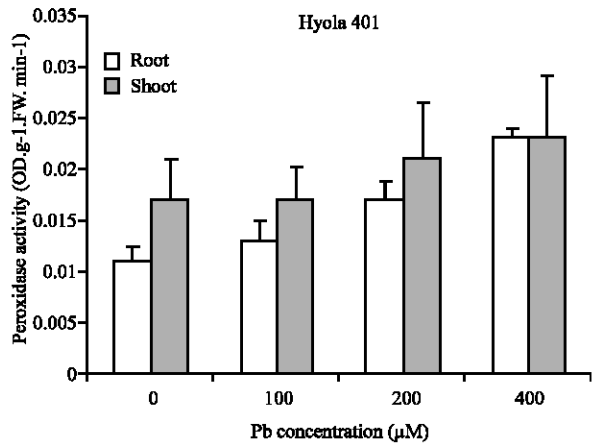


Fig. 11: Peroxidase activity on root and aerial organs of Hyola 401 after 10 days in Hogland's nutrient solution treated with different lead concentration

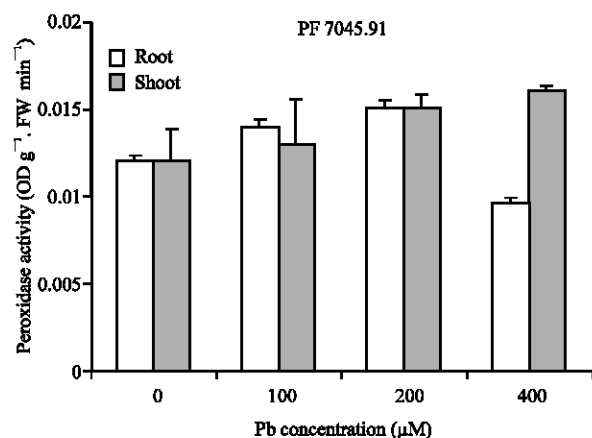


Fig. 12: Peroxidase activity on root and aerial organs of Pf 7045.91 after 10 days in Hogland's nutrient solution treated with different lead concentration

**Effect of lead on peroxidase activity:** Peroxidase activity on root and shoot of both cultivars by increasing lead concentration was increased. Peroxidase activity on root of Hyola with moderate and high lead concentration was significantly increased. The activity also on sample treated with high lead concentration compare to moderate and low concentration was significantly high. The activity on shoot of Hyola treated with all lead concentration was not significant increase in presence of low and medium lead concentration. But in high lead concentration the enzyme activity was reduced significantly compare to control and the other lead concentrations (Fig. 11). On shoot of Pf the activity of enzyme was not shown significant change ( $p < 0.05$ , Fig. 12). Catalase activity on root's Hyola was higher than the Pf. But the enzyme activity of shoot of Pf was higher than Hyola. In the presence of high lead concentration, enzyme activity on shoot of Hyola was increased related to Pf.

**Enzyme activity on root of both cultivars was higher than the shoot:** Peroxidase activity on shoot of Pf treated with high lead concentration was higher than the root enzyme.

## DISCUSSION

**Effect of lead on germination:** Lead concentrations (200, 400, 600, 800 and 1000 μM) decreased germination percentage. On Pf the decrease of percentage is not significant. Barman and Bera (2002) have been studied toxicity of Al on growth of seedlings and germination percentage of Mung Bean. They have been shown that negative relationship between germination percentage and Al concentration. The effects of heavy metals [Cd(•), Cr(•), Cu(•), Ni(•), Zn(•)] on germination of *Medicago*

*sativa* have been studied by Peralta *et al.* (2000). They suggested that on presence of Cd(•) and Cr(•) concentration (10 ppm) and Cu(•) and Ni(•) concentration (20 ppm) the germination percentage on alfalfa decreased.

In presence of Cd(•), Cr(•), Cu(•), Ni(•) at 40 ppm concentrations, germination decreased by 55, 45, 40 and 25% respectively.

The lead has been shown no permeability through seed membrane in first step of water absorption. At the second step lead shows permeability. Entrance of lead to seed caused delay on germination. The results show that the seed membrane has selective permeability on lead ions (Wierzbicka and Obidziska, 1998).

**Effect of lead on growth parameters:** Root length, leaf area, root dry weight and shoot weight in each cultivar decreased by increasing lead concentration on solutions. Geebelen *et al.* (1999) have been studied the effect of lead on pea and reported that root length in presence of lead 80 μM concentration has been decreased. Decreasing root length has affected of unbalanced microtubules that was suggested by Yang *et al.* (2000).

The results showed that, by increasing lead concentration (usually more than 100 μM), growth parameters on both *Brassica napus* L. Cultivars have been reduced. In presence of lead ( $10^{-3}$  M concentration) the reduction of root and shoot growth in *Brassica juncea*, has been suggested by Liu *et al.* (2000).

Mathe-Gaspar and Anton (2002) have been reported the effect of lead on two cultivars of radish shown reduction (percentage) in dry Weight of root and shoot.

Yang *et al.* (2000) also have been studied the effect of lead on 229 cultivars of rice and they reported that the dry weight of root on sensitive cultivars has been reduced about 10 times than the resistant cultivars. These results were coincided with our results. Chantachon *et al.* (2002) have been reported that the high concentration of lead (about 9-11 g L<sup>-1</sup>) reduced the growth rate on *Vetiveria zizanioides* and necrosis on *Vetiveria nemoralis* after one week.

**The effect of lead on catalase activity:** Increasing lead concentration increased the catalase activity on root and shoot in both cultivars. This activity in root was higher than the shoot Bittel *et al.* (1974) have been reported the same results on corn's roots.

Luna *et al.* (1994) on seedling of wheat have been reported the same results.

Catalase is an antioxidant that broken H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> catalase is a key enzyme which removes toxic peroxide (Lin and Kao, 2000).

The effect of long stress of lead on pea has been reported by Malecka *et al.* (2001) that catalase activity on pea's root has been increased. Increasing catalase activity was observed on cytosol and peroxisome. It seems that the activity of antioxidant enzymes like catalase in plants at environment stress is a reason at protein synthesis of de novo enzyme (Lozano *et al.*, 1996).

**Effect of lead on peroxidase activity:** Peroxidase activation increased by increasing lead concentration in two cultivars. The activity in root was more than the leaf. Peroxidase activity in high lead concentration in roots of Pf decreased in comparison to the control. This was the same as the results, which reported for *Sonchus oleraceus* by Xiong (1997).

Mohan and Hosetti (1997) reported in *Lemna minor* the peroxidase activity in lead stress. Lipid peroxidation increased in roots of wheat rather than the leaves (Pang *et al.*, 2002). Lead damaged roots more than the shoot. Lead can bind to nucleic acids and caused concentrated chromatin and stabilized the double helix of DNA. Therefore, it has been inhibited the transcription and translation. Environmental stress caused reduction on growth of roots by production of free radicals, which caused increasing peroxidase activity. Yamamoto *et al.* (2002) explained that aluminum caused induction of active oxygen on roots of peas, which synchronized with root's reduction. The plants have a defense mechanism to neutralize the active oxygen caused from heavy metals. Vetiver grass which exposed to heavy metals such as lead, showed increasing catalase and peroxidase activity in root and shoot. There were confliction between peroxidase and catalase activity of roots and stems of vetiver grass.

Catalase activity on shoot was higher than the roots, but peroxidase activity on roots were higher. Differences between the activities of these two enzymes are indicated that different defense mechanism to neutralize active oxygen in different part of vetiver grass can be understood (Pang *et al.*, 2003). There also has been reported the induction of peroxidase activity on plants which are under stress of toxic metal such as Al, Cu, Cd, Zn (Shah *et al.*, 2001; Chaoui *et al.*, 1997; Cakmak and Horst, 1991).

Lead toxic concentration caused oxidative stress on seedling of rice. This caused production of active oxygen and also activation of peroxidase. Peroxidase is located in cytosol, cell wall, vacuoles and cells space. Increasing the peroxidase activity on seedling of rice exposed to lead may caused by released peroxide from cell wall (Verma and Dudev, 2003).

It seems that the increasing of antioxidant enzymes on plants under environmental stress caused new synthesis of enzyme proteins (Lozano *et al.*, 1996).

Verma and Dubey (2003) reported that increasing in peroxidase activity in seed of rice exposed to lead were internal defense mechanism of seed. This mechanism caused plant resistance against damage of infused peroxidation induced by lead. Sairam *et al.* (2002) show that in different genotype of sensitive and resistant wheat, the peroxidase activity increased specially in resistant genotype. It seems, increasing peroxidase activity caused resistance in *Hyola* compare to Pf.

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