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**Ecotoxicological effects of iron on the activities of antioxidant enzymes in Safflower (*Carthamus tinctorius* L.) seedlings**

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**ABSTRACT**

*Iron is an essential metal at micro-concentration for the growth of plants and their metabolic activities. Excess Fe<sup>2+</sup> concentration generate oxidative stress within sub cellular compartments of the plant cell which are derived from the metabolism of oxygen. To combat the oxidative damage plants have the antioxidant defense system to remove, neutralize and scavenge the Reactive Oxygen Species (ROS) which is produced during heavy metal stress. The paper examines the effect of excess of FeSO<sub>4</sub> on the activities of some antioxidative enzymes, catalase and peroxidase in roots and shoots of Safflower seedlings. Fe<sup>2+</sup> solution treated Safflower seeds with different concentrations were sown in plastic pots and incubated at greenhouse conditions. Fresh roots and shoots were collected at different intervals to evaluate the enzyme activity. Protein estimation and enzyme assay were carried out using standard methods. The activity of peroxidase (POX) and catalase (CAT) of *Carthamus tinctorius* treated with Fe<sup>2+</sup> mainly displayed biphasic responses with increased metal concentration. In the present investigation, the excess of Fe<sup>2+</sup> (100-200 mg.l<sup>-1</sup>) induced toxicity in root and shoot of *C. tinctorius*. The results indicated an enhancement in the activity of peroxidase in shoots, suggesting that this enzyme serves as an intrinsic defense tool to resist Fe-induced oxidative damage in safflower plants, while catalase did not appear to be an efficient scavenger of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) than POX. However, results indicated that the exposure of *C. tinctorius* to iron provoke pronounced responses of antioxidative systems which protects the plants to some extent against oxidative damage.*

**Key words:** Antioxidant, Catalase, Peroxidase, Toxicity, Biphasic.

**INTRODUCTION**

It is well documented that iron is an essential metal at micro concentrations; however, excess concentrations are believed to generate oxidative stress as understood by an increase in the steady state concentration of reactive oxygen species (ROS) within subcellular compartments of the plant cell which are derived from the metabolism of oxygen. Reactive oxygen species which include superoxide anion radical (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (OH<sup>-</sup>) and singlet oxygen (<sup>1</sup>O<sub>2</sub>), are amongst the most reactive compounds known to be produced during heavy metal stress<sup>10</sup>.

To combat the oxidative damage plants have the antioxidant defense system comprising of enzymes - catalase (EC 1.11.1.6), peroxidases (EC 1.11.1.7), superoxide dismutases (SOD, EC 1.15.1.1) and the nonenzymic constituents alpha-tocopherol, ascorbate and reduced glutathione which remove, neutralize and scavenge the ROS<sup>21</sup>. Catalases are involved in scavenging H<sub>2</sub>O<sub>2</sub> generated during the photo-respiration and beta-oxidation of fatty acids<sup>18</sup>. Peroxidases are heme containing proteins that utilize H<sub>2</sub>O<sub>2</sub> in the oxidation of various organic and inorganic substrates<sup>1</sup>. Peroxidases utilizing guaiacol as electron donor in vitro are guaiacol peroxidases and participate in developmental processes, lignification, ethylene biosynthesis, defense, wound healing, etc<sup>2</sup>.

The other groups of peroxidases scavenge  $H_2O_2$  in cell and utilize glutathione, Cytochrome, pyridine nucleotide and ascorbate as electron donors *in vitro*<sup>2</sup>. Guaiacol peroxidases are glycoproteins, located in cytosol, vacuole, cell wall and in extracellular space, while the other group is non glycosylated and localized in chloroplasts and cytosol<sup>2</sup>.

Safflower (*Carthamus tinctorius* L.) is a tap-rooted annual crop which can tolerate environmental stresses including salinity and water stress<sup>13</sup>. After the commercialization of less expensive synthetic dyes (aniline), safflower has been grown predominantly for its seed oil<sup>12</sup>. Since safflower is more drought and salt tolerant than some other oilseed plants such as sunflower, it is especially suitable for dry and salty areas where other oilseeds are difficult to grow<sup>24</sup>. In previous studies, Safflower appeared to be tolerant to heavy metal stress<sup>14,15</sup>. In the present paper, we have conducted a study of the effect of excess  $FeSO_4$  on the activities of some antioxidative enzymes, catalase and peroxidase, in roots and shoots of safflower (*Carthamus tinctorius* L.) seedlings.

## MATERIALS AND METHODS

### **Plant material**

Seeds of Safflower (*Carthamus tinctorius* L.) were collected from the agricultural fields of Gajanur Sakrebyle, Shimoga district, Karnataka, India. The seeds were surface-sterilized with 1% sodium hypochloride solution for 10 minutes and thoroughly rinsed with sterile distilled water for three times and used throughout the experiments.

### **Stress conditions**

In order to determine the effect of iron on activity of antioxidant enzymes, catalase and peroxidase, surface sterilized Safflower seeds were soaked for 24 h in different concentration (0, 50, 100, 150 and 200 mg/l) of  $Fe^{2+}$  solutions on a rotary shaker at 150 rpm under room temperature. Seeds immersed only in distilled water served as control. To evaluate the effect of iron on catalase and peroxidase activities in shoot and root of safflower treated and control seeds were sown in plastic pots each filled with sterilized coirpith (coconut compost). Each pot sown with 3 seeds is incubated at greenhouse conditions and watered regularly. Seedlings raised from control and treated seeds were carefully uprooted without causing any damage to root tissues at different time intervals, 7, 14, 21 and 28 days after sowing. Fresh roots and shoot parts were washed in running tap water, blot dried and used to evaluate the enzyme activity.

### **Enzyme extraction**

The plant roots and shoots (3:1, buffer volume: fresh weight) were homogenized in a pre-chilled pestle and mortar with 100 mM cold potassium phosphate buffer (pH 6.8). The homogenate was centrifuged at  $12,000 \times g$  for 10 min at 4°C. The supernatant was transferred to a micro tube for further analysis.

### **Protein Estimation**

The protein contents of the extracts were determined by using the standard procedure of Bradford (Bradford, 1976) with Bovine Serum Albumin (BSA) (sigma, USA) as standard.

### **Enzyme assay**

Catalase activity was determined from safflower seedlings using the method of Beers and sizer (1951) with minor modification. The 2.4 ml reaction mixture contained 100 mM K-phosphate buffer (pH 6.8) and 30% (w/v)  $H_2O_2$  and the reaction was initiated by adding 100  $\mu$ l of extracted enzyme. The decrease in absorbance at 240 nm was measured for 3 min. The specific activity of catalase was expressed as change in optical density (OD) at 240 nm/min/mg protein. Experiments were conducted in triplicates and repeated in four weeks.

The substrate for peroxidase (POX) assay was prepared by adding 125  $\mu$ l of guaiacol and 153  $\mu$ l of  $H_2O_2$  (30%) in 50 ml 100 mM potassium phosphate buffer (pH 6.8). The reaction was initiated by adding 100  $\mu$ l of crude enzyme extract to 2.4 ml of substrate buffer. Increase in absorbance was recorded spectrophotometrically for 3 min at 470 nm. Peroxidase specific activity was calculated as change in absorbance units/min/mg protein. Experiments were conducted in triplicates and repeated in four weeks.

**Statistical analysis**

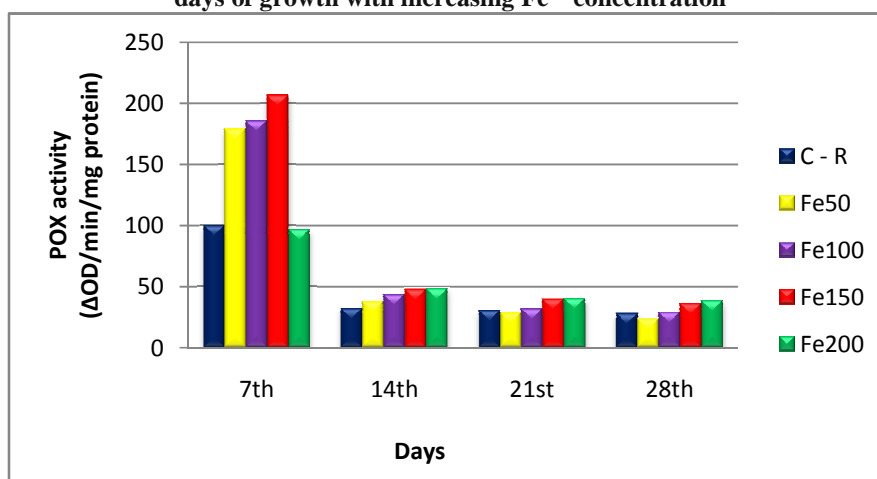
All data from laboratory and greenhouse experiments were analyzed separately for each experiment and were subjected to analysis of variance (ANOVA) (MINITAB, version 14). Significant effects of treatments were determined by the F values ( $P \leq 0.01$ ). Treatment means were separated using Tukey's HSD test.

**RESULTS****Effects of iron on peroxidase activity**

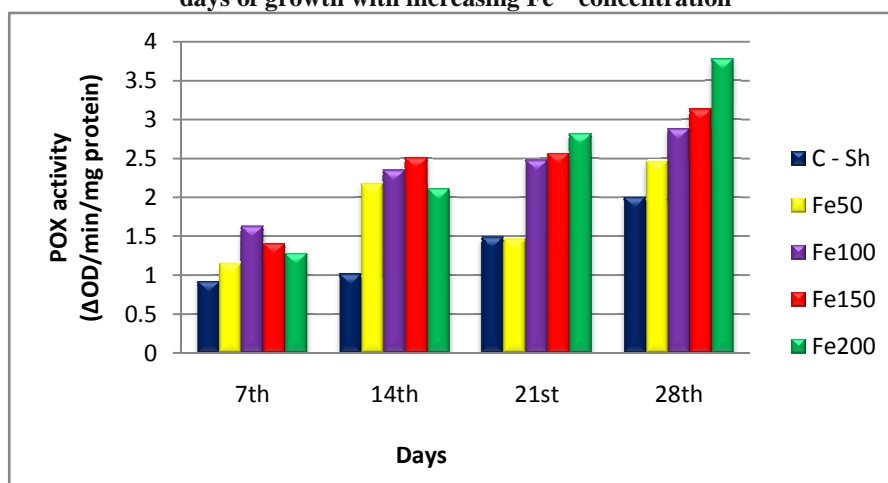
The activity of peroxidase showed a significant decrease ( $p \leq 0.01$ ) from 7<sup>th</sup> to 14<sup>th</sup> day of growth with increase in  $Fe^{2+}$  treatment levels (Fig.1) and then was maintained at relatively constant values from 14<sup>th</sup> day to 28<sup>th</sup> day of growth. Roots showed higher level of enzyme activity than shoots in Fe- treated as well as control plants.

peroxidase activity increased in shoots at all levels of  $Fe^{2+}$  treatments from 7<sup>th</sup> to 28<sup>th</sup> day. A 200 mg.l<sup>-1</sup>  $Fe^{2+}$  treatment led to about 1.3- 3.0 fold increase in peroxidase activity in shoots of safflower seedlings at 28<sup>th</sup> day of growth (Fig.2).

**Fig.1: Activity of peroxidase ( $\Delta OD/min/mg$  protein) in roots of *Carthamus tinctorius* after 7, 14, 21 and 28 days of growth with increasing  $Fe^{2+}$  concentration**



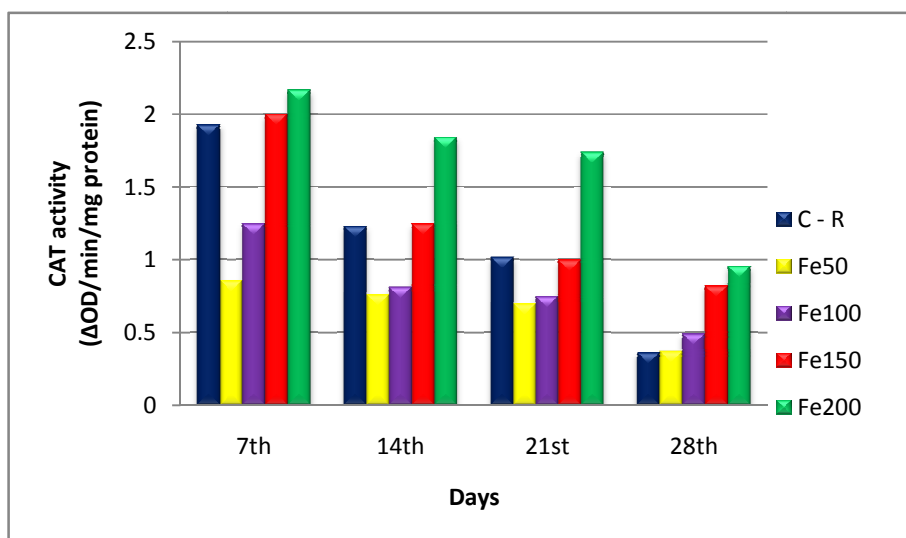
**Fig.2: Activity of peroxidase ( $\Delta OD/min/mg$  protein) in shoots of *Carthamus tinctorius* after 7, 14, 21 and 28 days of growth with increasing  $Fe^{2+}$  concentration**



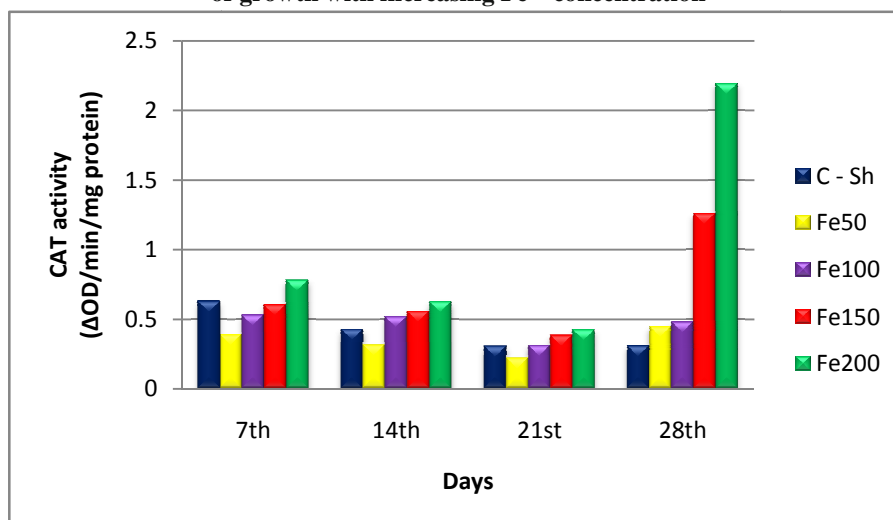
**Effects of iron on CAT activity**

In Fe- treated seedlings a decrease in catalase activity was observed in roots during 28 days period (Fig.3). In shoots, up to 21<sup>st</sup> day, the activity of CAT gradually decreased whereas at 28<sup>th</sup> day a significant ( $p \leq 0.01$ ) increase in enzyme activity was noticed (Fig.4).

**Fig.3: Activity of catalase ( $\Delta$ OD/min/mg protein) in roots of *Carthamus tinctorius* after 7, 14, 21 and 28 days of growth with increasing Fe<sup>2+</sup> concentration**



**Fig.4: Activity of catalase ( $\Delta$ OD/min/mg protein) in shoots of *Carthamus tinctorius* after 7, 14, 21 and 28 days of growth with increasing Fe<sup>2+</sup> concentration**



**DISCUSSION**

Plants growing in heavy metal-contaminated condition need to develop some degree of tolerance to metal toxicity for surviving. Induction in the activities of antioxidative enzymes is a general strategy for plants to overcome oxidative stress<sup>25</sup>. The antioxidant enzymes and certain metabolites present in plants play an important role leading to adaptation and ultimate survival of plants during periods of stress<sup>8,16</sup>. The present study suggested that Fe toxicity induces some of the key enzymes of antioxidant defense system such as catalase and peroxidase in roots and shoots of *C. tinctorius*.

POX belongs to enzymes involved in growth, development and senescence processes of plants. They affect lignin and ethylene synthesis, decomposition of indole-3-acetic acid (IAA), and involves in resistance against pathogens and wound healing<sup>2</sup>.

Catalase (CAT) belongs to most important enzymes scavenging the active oxygen species in plant cells. CAT participates in the main defense system against accumulation and toxicity of hydrogen peroxide and can play the role in controlling H<sub>2</sub>O<sub>2</sub> level in cells. It acts on H<sub>2</sub>O<sub>2</sub> and converts it to water and oxygen<sup>19</sup>. The activity of POX and CAT of *C. tinctorius* treated with Fe<sup>2+</sup> mainly displayed biphasic responses with increased metal concentration. When safflower seeds were faced with low-level metal stress, it could activate POX and CAT activities, which lead to strengthening of seedlings to scavenge ROS while the activities decreased distinctly under too acute stress which overloaded cellular defense system of plants. Also the decline in the activities of POX and CAT might be due to the formation of protein complex with metals, to change the structure or integrity of proteins<sup>7,17</sup>. For seeds treated with 200 mg.l<sup>-1</sup> of Fe<sup>2+</sup>, CAT and POX activities appeared to be inhibited.

Increased Fe<sup>2+</sup> concentrations intensified development of iron toxicity symptoms on roots and shoots. It has been demonstrated that excess Fe<sup>2+</sup> treatment induces oxidative stress in plant tissues<sup>11,20,23</sup>. In the present investigation, the excess of Fe<sup>2+</sup> (100- 200 mg.l<sup>-1</sup>) induced toxicity in root and shoot of *Carthamus tinctorius*. These results support the possibility of FeSO<sub>4</sub> -induced toxicity mediated through oxidative stress. The results indicate an enhancement in the activity of peroxidase in shoots, suggesting that this enzyme serves as an intrinsic defense tool to resist Fe-induced oxidative damage in safflower plants. Peroxidases are widely accepted as 'stress enzymes'<sup>9</sup>. As guaiacol peroxidases are located in cytosol, cell wall, vacuole and in extracellular spaces, increased peroxidase activity in Fe<sup>2+</sup> stressed seedlings might be possibly due to increased release of peroxidases localized in the cell walls. Under sub-lethal salinity and metal toxicity conditions, level of peroxidase activity has been used as potential biomarker to evaluate the intensity of stress<sup>20,21</sup>. The results show significant decreased POX activity in Fe-treated roots of safflower seedlings from 7<sup>th</sup> to 14<sup>th</sup> day, revealing efficient break down of peroxides up to 14<sup>th</sup> day of growth in safflower seedlings. The POX activity remained almost unchanged from 14<sup>th</sup> to 28<sup>th</sup> which support the possibility of efficient detoxification.

In the present work, CAT activity was inhibited in roots from 7<sup>th</sup> day to 28<sup>th</sup> day of growth under Fe<sup>2+</sup> toxicity especially at higher concentrations, while it decreased gradually in shoots from 7<sup>th</sup> to 21<sup>st</sup> day and then increased at 28<sup>th</sup> day. The decline in CAT activity in Fe-treated safflower plants might be due to inhibition of enzyme synthesis or due to a change in the assembly of enzyme subunits under such conditions. Wei-Ching *et al.*,<sup>23</sup> reported no change in CAT activity under iron toxicity in rice leaves, while in some other plants a decline (sunflower) or enhancement (*Nicotina plumbagnifolia*) of CAT activity has been found<sup>11,22</sup>. Thus, in the present experiments, CAT did not appear to be an efficient scavenger of H<sub>2</sub>O<sub>2</sub> than POX. These inconsistent results regarding CAT activity might be due to differences in the plant organs studied, the durations, concentrations of metals utilized and the plant species under investigation.

In conclusion, our results indicated that the exposure of *C. tinctorius* to iron provoke pronounced responses of antioxidative systems which protects the plants to some extent against oxidative damage, but the direction of response was dependent on the intensity of the stress.

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#### REFERENCES

1. Asada, K. Production and action of active oxygen species in photosynthetic tissues, in: C.H. Foyer, P. Mullineaux (Eds.), Causes of Photo-Oxidative Stress and Amelioration of Defense Systems in Plants, CRC Press, Boca Raton, FL, pp. 77-104. (1994)
2. Asada, K. Ascorbate peroxidase/a hydrogen peroxide scavenging enzyme in plants, Plant Physiol. **85**: 235-241 (1992)

3. Asch, F. Becker. M. Kpomgor, D.S. A quick and efficient screen for resistance to iron toxicity in lowland rice. *J Plant Nutr Soil Sci* **168**: 764–773 (2005)
4. Bradford, M. A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding" *Anal. Biochem.* **72**: 248-254 (1976)
5. Comba, M.E. Benavides, M.P., Tomaro M.L. Effect of salt stress on antioxidant defence system in soybean root nodules, *Aust. J. Plant Physiol.* **25**: 665-671 (1998)
6. Gholamabbas Sayyad, Majid Afyuni, Sayed-Farhad Mousavi & Karim C.. Effects of Cadmium, Copper, Lead, and Zinc Contamination on Metal Accumulation by Safflower and Wheat. *Soil & sedim. Contam.* **18(2)**: 216-228 (2009)
7. Florence, T.M. & Stauber, J.L. Toxicity of copper complexes to the marine diatom *Nitzschia closterium*, *Aquat. Toxic.* **8**: 11-26 (1986)
8. Foyer, C.H. Lopez-Delgado, H. Dat, J.F & Scott, I.M. Hydrogen peroxide and glutathione-associated mechanisms of acclimatory stress tolerance and signaling. *Physiol. Plant.* **100**: 241- 254 (1997)
9. Gaspar, T. Penel, C. Hagege, D. & Greppin, D. Peroxidase in plant growth, differentiation and development processes, in: J. Labor- zewsky, H. Greppin, C. Penel, T. Gaspar (Eds.), *Biochemical, Molecular and Physiological Aspects of Plant Peroxidases*, University M. Curie, Sklodowska, 249-280 (1991)
10. Halliwell, B. Gutteridge, J.M.C. *Free Radicals in Biology and Medicine*. Clarendon Press, Oxford (1993)
11. Karlheinz Kampfenkel, Marc Van Montagu, and Dirk Inzé. Effects of Iron Excess on *Nicotiana plumbagnifolia* Plants : Implications to Oxidative Stress. *Plant Physiol.*, **107**: 725-735 (1995)
12. Knowles, P.F. Safflower. In: G. R " obbelen, R.K. Downey and A. Ashri (Eds.), *Oil* (1989)
13. Lovelli, S. Perniola, M. Ferrara, A. Di Tommaso, T. Yield response factor to water (Ky) and water use efficiency of *Carthamus tinctorius* L. and *Solanum melongena* L. *Agric. Water Manage.* **92**: 73–80 (2007)
14. Madaan, N. and Mudgal, V. Phytotoxic effect of selenium on the accessions of wheat and safflower. *Res. J. Environ. Sci.*, **5**: 78-82 (2009)
15. Madaan, Nidhi, mudgal & Varsha. Differential tolerance behaviour of safflower accessions to some heavy metals. *Ap. Environ. Sci.* **91**: 2215 (2009)
16. Malecka, A. Jarmuszkiewicz, W. & Tomaszewska, B. Antioxidative defense to lead stress in subcellular compartments of pea root cells. *Acta Biochim. Polon.* **48**: 687-698 (2001)
17. Mohan, B.S. & Hosetti, B.B. Potential phytotoxicity of lead and cadmium to *Lemna minor* grown in sewage stabilization ponds. *Environ. Pollut.* **98 (2)**: 233-238 (1997)
18. Morita, S. Tasaka, M. Fujisawa, H. Ushimaru, T. Tsuji, H. A. cDNA clone encoding a rice catalase isozyme, *Plant Physiol.* **105**: 1015-1016 (1994)
19. Mstte's, J.M. Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, **153**: 83-104 (2000)
20. Sarita Sinha & Rohit Saxena. Effect of iron on lipid peroxidation, and enzymatic and non-enzymatic antioxidants and bacoside-A content in medicinal plant *Bacopa monnieri* L. *Chemosphere*, **62**: 1340-1350 (2006)
21. Shah, K. Kumar, R.G. Verma, S. & Dubey, R.S. Effect of cadmium on lipid peroxidation, superoxide anion generation and activities of antioxidant enzymes in growing rice seedlings. *Plant Sci.* **161**: 1135-1144 (2001)
22. Susana M. Gallego, Maria P. Benavides & Maria L. Tomaro. Effect of heavy metal ion excess on sunflower leaves: evidence for involvement of oxidative stress. *Plant Science*, **121**: 151-159 (1996)
23. Wei-Ching Fang, Jen-Wu Wang, Chuan Chi Lin and Ching Huei Kao. Iron induction of lipid peroxidation and effects on antioxidative enzyme activities in rice leaves. *Plant Growth Reg.*, **35**: 75-80 (2001)
24. Weiss, E.A. *Oilseed Crops*. 2nd Edition, Victoria-Australia, Blackwell Sci. Ltd; 364 (2000)
25. Zaman, M.S. & Zereen, F. Growth responses of radish plants to soil cadmium and lead contamination. *Bull. Environ. Contam. Toxicol.* **61**: 44–50 (1998)