Responses of antioxidative defense system and photosynthetic pigment composition of *Brassica juncea* L. to imidacloprid stress.

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Abstract:

The effect of insecticide imidacloprid on photosynthetic pigments and antioxidative enzymes of *Brassica juncea* L. cv. alankar was investigated. Forty-day-old pot plants were exposed to different concentrations of insecticide imidacloprid, ranging from 0 to 40 gram active ingredient hectare^{-1} (g.a.i ha^{-1}) through foliar spray. Analyses were done at Day 3, Day 07, and Day 15 after the treatment. Lipid peroxidation rate, proline content, ascorbate and glutathione content and antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and glutathione reductase (GR) were all assayed. Imidacloprid enhanced malondialdehyde (MDA) and proline content over their respective controls at all doses at all the observed growth stages, attaining maximum with 40 gram active ingredient hectare^{-1} at Day 15 after treatment. The antioxidative enzyme activity (SOD, Apx and GR) showed increasing trend in a dose dependent manner, attaining maximum at 40 g.a.i ha^{-1} of imidacloprid treatment at 7 days after treatment (DAT) followed by 3 and 15 DAT respectively. On the contrary, the CAT activity was increased at lower concentrations of imidacloprid but showed dose dependence decline at higher concentations of insecticide at all the observed growth stages, with maximum decrease under 40 g.a.i ha^{-1} at 15 DAT followed by 3 and 7 DAT respectively. Among the non enzymatic antioxidants a dose dependent decline was observed in Asc, dehydroascorbate (DHA) and reduced glutathione (GSG) levels. The photosynthetic pigments showed slight increase over their respective controls at lower concentrations of insecticide at all the growth stages, but at higher concentrations (30 and 40 g.a.i ha^{-1}), both the pigments were decreased considerably

at all the observed growth stages. These results suggested that the lower concentration of imidacloprid applied on the mustard might have no severe effect on the mustard plant because of the regulation of anti- oxidative enzyme system and MDA, but provoked the state of oxidative stress when applied at higher dosages. Therefore pesticide application at higher concentrations can be considered as a kind of stress factor similar to other stresses such as salt, drought and low or high temperature.

Key words: growth; insecticide; mustard; malondialdehyde; proline; stress.

1. Introduction

Pesticides were introduced to agriculture to fulfill the increased food needs of the growing world population. The use of synthetic insecticides has made the production of higher yields of crop possible in areas where insects would have otherwise damaged the crop (Gonias et al. 2008). On the other hand, uptake of many pesticides in plants also causes chemical shock, which results in interference of growth and metabolism by triggering secondary responses such as oxidative damage by producing highly reactive oxygen species (ROS) (Halliwell 1987). Cellular systems scavenge these active oxygen species by invoking an increase in antioxidative machinery, composed of enzymes and non-enzymatic components. The enzymatic components include ROS scavengers like superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) (Khan and Kour 2007; Ganguly et al. 2010). Such enzyme based anti-oxidative system has been one of the important strategies for plants to respond to environmental stresses. In most cases, toxic organic compounds can give rise to the increased activities of antioxidant enzymes such as SOD, POD or ascorbate peroxidase (APX), which reflect not only the degree of toxicity but the ability to tolerate the stress as well (Mishra et al. 2009). Additionally, nonenzymatic (e.g., glutathione, tocopherols, ascorbate, proline and

carotenoids) defense systems protect cells from damage by scavenging ROS (Bashir et al. 2007a; Jan et al. 2012a).

Brassica juncea L. is the major oil seed crop cultivated in entire Indian sub-continent, but infestation of this crop with insect pests is the major problem in the region (Rehman et al. 2013). Farmers deal with the issue of insect pests by spraying various insecticides and pesticides. However, some insect pests develop resistance to the conventionally used insecticides. Imidacloprid [1-(6-chloropyridin-3-yl)-N-nitroimidazolidin-2-ylideneamine], a nicotinoid insecticide, has been found very effective against these resistant pests (Conway et al. 2003) and therefore is used widely in areas where mustard crop is attacked by insects.

Although, large amount of work relating to the pesticide and insecticide induced effects on growth and photosynthetic pigment contents has been done. But to the best of our knowledge, there is little information available so far about the effect of Imidacloprid especially on ROS system of plants. Keeping in view the importance of oil seed crops, the present investigation was carried out to find out whether Imidacloprid induces oxidative stress in plant cells which might affect various enzymatic and non-enzymatic antioxidants involved in Ascorbate-Glutathione cycle in plants and to find out the optimum level of insecticide imidacloprid for efficient growth and photosynthetic pigments of *B. juncea*.

2. Material and Methods:

The present experiment was performed in a net house of the Department of Botany, Aligarh Muslim University, Aligarh, India, under natural conditions. The seeds of mustard (*B. juncea* L. Czern & Coss) Cv. Alankar were surface sterilized with 0.5% sodium hypochlorite for 20 min, rinsed and soaked overnight in double distilled water (DDW) for 12 h at 4 ^{o}C for

uniform germination. The seeds were then transferred to 23-cm-diameter earthen pots filled with 4 kg of reconstituted soil (sand: clay: peat; 70: 20: 10, by dry weight). After two weeks of sowing, thinning was done and four healthy plants of uniform size were maintained in each pot. Plant pots were watered every alternate day and watering schedule was adjusted throughout the experimental duration in order to prevent leaching. Forty day-old plants were subjected to foliar application of five levels of commercial insecticide imidacloprid 17.8% SL (trade name Ultimo^{TM} 200 SL), ranging from 0 to 40 g a.i ha^{-1} with hand held sprayer (adjustable nozzle). Doses were prepared by dissolving the required amount of imidacloprid solution in double distilled water. Each experiment was repeated three times with four replicates in a completely randomised blocks. Plant samples were collected 03, 07 and 15 days after the treatment (DAT) to analyse the effect of different concentrations of insecticide on photosynthetic pigments, proline content, and activities of enzymes ascorbate peroxidase (APX) (EC 1.11.1.11), superoxide dismutase (SOD) (EC 1.15.1.1), glutathione reductase (GR) (EC 1.6.4.2) and catalase (CAT) (EC 1.11.1.6).

2.1. Measurement of proline and MDA content

The proline content in control and Imidacloprid treated plants was estimated following the method of Bates et al. (1973). The absorbance of the chromophore extracted in toluene was taken at 520 nm. The concentration of proline was estimated using the standard curve prepared from L-proline (0-100 µg/ml). Proline concentration was expressed in µg g^{-1} of fresh weight of sample.

Lipid peroxidation was estimated measuring the formation of malondialdehyde (MDA), a breakdown product of lipid peroxidation, with 2-thiobarbituric acid (TBA) according to De Vos et al. (1989). The absorbance of resulting supernatant was taken at 532 and 600 nm.

MDA content was determined by subtracting absorbance of supernatant at 600 nm from that of 532 nm and using an absorbance coefficient of 155 $mM^{-1}cm^{-1}$ and was expressed as nmol g^{-1} fresh weight of sample.

2.2. Extraction and estimation of antioxidative enzymes

For enzyme extraction leaf samples of *B. juncea* L. were homogenized in pre-cooled mortar and pestle in 5 ml extraction mixture (1mM EDTA, 0.05% Triton X- 100, 2% PVP, 1 mM ascorbate in 50mM phosphate buffer of pH 7). The homogenate was centrifuged at 15000 rpm for 20 min at $4^{o}C$. Supernatants obtained were used for enzyme determinations.

2.3. Superoxide dismutase activity

SOD activity was assayed according to the method of Beauchamp and Fridovich (1971) in terms of ability to inhibit the photochemical induction of nitroblue tetrazolium (NBT) to formazon at 560 nm. The amount of reduced NBT was calculated using the absorbance coefficient 100 $mM^{-1}cm^{-1}$. One unit of SOD was defined as the amount of enzyme required to cause 50% inhibition of the reduction of NBT monitored at 560 nm by a spectrophotometer (T70 UV/VIS Spectrometer).

2.4. Catalase activity

CAT activity was measured following Aebi (1984). 0.1 ml of enzyme extract was added to 2.8 ml 50 mM phosphate buffer (pH 7.0) and absorbance was taken at 240 nm. To this 0.1 ml of H_2O_2 was added and again absorbance was taken at the same wavelength. The extinction coefficient value of 0.039 $mM^{-1}cm^{-1}$ was used for calculations.

2.5. Ascorbate peroxidase activity

For the estimation of APX activity, 0.1 ml of enzyme extract was added to 2.8 ml reaction mixture composed of 0.5mM ascorbic acid in 50 mM phosphate buffer (pH 7.0). Changes in absorbance at 290 nm were recorded after addition of 0.1 ml of H_2O_2 (Asada 1984).

2.6. Glutathione reductase activity

The leaf samples for estimation of GR were extracted in 100mM potassium phosphate buffer (pH 7.0) containing 1mM Na_2-EDTA and 4% polyclar AT (w/v). Activity of GR was determined following the method of Sgherri et al. (1994) by measuring decrease in absorbance at 340nm and using extinction coefficient of 6.2mM^{-1} cm^{-1}.

2.7. Estimation of Chlorophyll and Carotenoids

Chlorophyll and carotenoids were estimated by the method of Arnon (1949).

2.8. Ascorbate content

Fresh leaf (0.05 g) was ground in 2 ml of 0.1 M Na-phosphate buffer (pH 7) and 1 mM EDTA and centrifuged at 10,000 rpm for 15 minutes. Ascorbate (Asc) and dehydroascorbate (DHA) were estimated by a method of Law et al. (1983).

2.9. Glutathione content

Fresh leaf tissue (0.05 g) was homogenized in 2 ml of 5 % sulphosalicylic acid at 4 ^{o}C. The homogenate was then centrifuged at 10,000 rpm for 15 minutes. Reduced (GSH) and oxidised (GSSG) glutathione were determined by the method of Anderson (1985).

Statistical analysis

The data were analyzed by employing analysis of one-way variance (ANOVA) followed by a Duncan's multiple range test (DMRT) to determine whether the values were significantly different from the control. Analysis of variance (ANOVA) for all the measured variables was performed by SPSS Ver. 10, Inc., Chicago, USA. The data are expressed as mean ± SE (n = 4).

3. Results

3.1. Lipid peroxidation rate.

The MDA content was increased with increase in the imidacloprid concentration as well as with plant age as observed at 3, 7 and 15 DAT. The maximum accumulation of MDA took place at 40 g.a.i ha^{-1} imidacloprid, compared to the respective control (fig 1). The MDA content at day 3 after treatment was 14.25%, 36.2%, 90% and 181.1% higher in the plants exposed to 10, 20, 30 and 40 g.a.i ha^{-1} imidacloprid respectively than in the control plants. Interestingly, except for the 10 g.a.i ha^{-1} of imidacloprid, the MDA contents at 20, 30 and 40 g.a.i ha^{-1} were 50%, 99% and 143.1% higher at day 7 after treatment of imidacloprid respectively ($P < 0.05$). No significant difference was observed between the control and 10 g.a.i ha^{-1} 7 DAT with the MDA content being the highest at 40 g.a.i ha^{-1}.

3.2. Proline content

Proline content did not show any significant alteration in the leaves of the plants treated with 10 g.a.i ha^{-1} imidacloprid as compared to control at all the growth stages. However, the presence of imidacloprid at higher concentrations (30 and 40 g.a.i.ha^{-1}) enhanced proline levels 63.82%, 107.82% 3 DAT, 60.2% and 102.52% at 7 DAT, and 32.4% and 57.88% 15

DAT respectively in comparison with that of control plants (fig 2). The maximum enhancement was found with 40 g.a.i ha^{-1} imidacloprid at 15 DAT.

3.3. Antioxidant enzyme activity

No significant difference in the SOD activity was observed among the control, 10 and 20 g .a.i ha^{-1} imidacloprid treatments at 3 DAT. However, it increased significantly with all the insecticide treatments as observed at 7 and 15 DAT, compared with the control (fig 3). The gain was maximum at 40 g.a.i ha^{-1} imidacloprid at 7 DAT followed by 3 DAT and 15 DAT.

The CAT activity also showed a similar variation trend (fig 4), as did SOD with respect to age of the plant, thus having its maxima at 7 DAT. It increased marginally at the lower concentrations of imidacloprid (10 and 20 g.a.i. ha^{-1}) with respect to control at all the observed growth stages, but started declining sharply at higher doses of chemical 30 and 40 g.a.i. ha^{-1}. The CAT activity was the highest at 20 g.a.i ha^{-1} during the whole experimental duration. Like the SOD activity, GR activity increased considerably under the lower doses of Imidacloprid treatments, and attains the maximum with (40 g.a.i. ha^{-1}) 7 DAT, followed by 3 DAT and 15 DAT respectively (fig 5).

The activity of APX was also raised with increasing concentration of imidacloprid, gaining the most with highest dose of chemical (40 g.a.i. ha^{-1}) at 7 DAT, followed by 3 DAT and 15 DAT respectively (fig 6). The APX activity at 3 DAT was 32.58%, 69.66%, 128.08% and 178.6% higher in the 10, 20, 30 and 40 g.a.i ha^{-1} imidacloprid treatments than in the control, respectively. No significant difference was observed between the control and 10 and 20 g.a.i

ha^{-1} imidacloprid treatments at 7 DAT and between the control and 10 g.a.i ha^{-1} imidacloprid treatments at 15 DAT.

In the present study, as compared to control a dose dependent reduction in Asc and DHA by 56.65 and 31.19 %, respectively, at 15DAT was observed under the highest concentration of (40 g a.i ha^{-1}) imidacloprid used (fig. 7A and 7b). A dose dependent decrease was also observed in GSH content by 61.39 % under the highest (40 g a.i ha^{-1}) imidacloprid concentration as compared to its control at 15 DAT (fig 8A). But on the other hand as compared to its respective control, an increase in GSSG by 93.2 % at 3 DAT under 40 g a.i ha^{-1} insecticidal treatment was observed (fig 8B).

3.4. Photosynthetic pigments

The photosynthetic pigment chlorophyll showed marginal increase 1.6% and 4.8%; 5.4% and 10.4%; and 4.2% and 13.2% over their respective controls at lower concentration of imidacloprid treatment at 3 DAT, 7 DAT and 15 DAT respectively, touching maximum with 20 g.a.i.ha^{-1} at 7 DAT followed by 3 and 15 DAT respectively. However, it showed a consistent decreasing trend at higher concentrations (30 and 40 g.a.i ha^{-1}) of imidacloprid treatment, in a dose dependent manner over their respective controls at all the growth stages (fig 9A).

Like chlorophyll content, the carotenoid content also increased at lower concentrations of the insecticide treatment over their respective controls at all the observed growth stages, showing maximum enhancement at 20 g.a.i ha^{-1} at 7 DAT followed by 3 and 15 days respectively, and then decreased considerably at higher concentrations at all the observed growth stages with

maximum decrease at 40 g.a.i ha^{-1} at 15 DAT followed by 3 and 7 DAT respectively (fig 9B).

4. Discussion

Lipid peroxidation may be the first step of cellular membrane damage caused due to insecticide stress, and has been extensively used as a marker of oxidative stress (Hazarika et al. 2003). Increase in lipid peroxidation rate is regarded as a general response to many stresses like heavy metals (Vanaja et al. 2000), and salt stress (Hernandez et al. 2000). The increased concentration of MDA in *Brassica juncea* L. plants particularly at higher concentrations of imidacloprid (30 and 40 g.a.i ha^{-1}) suggests that these are highly susceptible to insecticide stress. Fig 1 showed age and dose dependent increase in lipid peroxidation rate in imidacloprid-treated plants than that of the untreated plants. Compared to control, the average values of MDA content increased by 57.7 % at day 15 after imidacloprid treatment under 40 g.a.i ha^{-1}. Exposure of *Glycine max*. L. to insecticide deltamethrin or other pesticides led to increase in lipid peroxidation in leaves and roots (Bashir et al. 2007b; Song et al. 2007; Parween et al. 2012). The increased lipid peroxidation in the present study suggested that ROS induced damage may be one of the main toxic effects of imidacloprid.

Plants respond to a variety of stresses by accumulating certain specific metabolites; the most conspicuous of them being amino acid- proline (Jaleel et al. 2007). Proline may affect the solubility of various proteins, thus protecting them against denaturation under stressful conditions and in plant cells acts as an osmoregulator, a soluble nitrogen sink, signal of senescence and an indicator of plant resistance to stress (Bashir et al. 2007a). Compared to the control, no significant change in the proline content in plants treated with 10 and 20 g.a.i

ha^{-1} imidacloprid in present study, suggests little or even no oxidative injury occurring in plants (fig 2). It might be due to the presence of chloropyridene side chain in imidacloprid that structurally resembles nicotinamide and nicotinic acid (niacin), known as systemic plant resistance inducers and possess antioxidant activity (Ogata et al. 2002). By contrast, the proline content increased significantly at higher doses of Imidacloprid used, indicating that the oxidative damage caused by lipid peroxidation was never fully prevented. The other reason behind this could be the over production of ROS at highest dose of imidacloprid (Zhang et al. 2011). Similar results in proline content was also reported by other workers under insecticide stress (Parween et al. 2011).

Plants have evolved complex antioxidant system to mitigate and repair the damage caused by ROS. In the present study, Imidacloprid-treated seedlings showed a significant enhancement in superoxide dismutase (SOD) activity at higher doses, especially at 7 DAT. The ability of plants to mitigate oxidative stress partially relies on the induction of SOD activity and subsequently on the upregulation of other downstream antioxidant enzymes (Alscher et al. 2002). SOD is likely to play a central role in the defence against toxic reactive oxygen species (ROS) and constitute the first line of defence with in a cell against ROS (Liang et al. 2003). SOD processing is known to be substrate inducible and an increase in the SOD activity may be attributed to the increased production of active oxygen species as substrate that lead to increased expression of genes encoding SOD (Abedi and Pakniyat 2010). Reactive oxygen species of O_2^- have been considered as the central components of signal transduction which triggers the defense genes responsible for oxidant enzymes, such as SOD (Arrora et al. 2002). Conversely, the increased enzyme activity contributes to the removal of O^{2-} (Liu et al. 2011). The relatively low activity at 15 DAT could be because old leaves generally contain lower

metabolic activities than younger leaf and this is why the old leaves are more prone to enhanced oxidative injury than the young leaves (Haddad et al. 2009). Our observations on SOD activity find support from several earlier findings concerning the effects of air pollutants (Hernandez et al. 2000), and salinity (Qureshi et al. 2007) on the antioxidant defence system in plants. It was also reported that SOD activity in rice and wheat exposed to 1,2,4-trichlorobenzene stress, wheat exposed to omethoate and in bitter gourd exposed to dimethoate were significantly increased compared with the control (Mishra et al. 2009; Zhang et al. 2011).

H_2O_2 produced from the action of SOD diffuses rapidly across the membranes and is very toxic to the chloroplasts, nucleic acids, protein and lipids (Gille and Singler 1995). Therefore, it is important that H_2O_2 be scavenged rapidly by the antioxidative defence system to water and oxygen which can be eliminated by CAT and a series of peroxidase (POD) (Guo et al. 2006). It seems to suggest that the cooperation between H_2O_2 scavenging enzymes and SOD plays a significant role in plant resistance to stressful environments. Significant increase in the activities of CAT and APX in the present investigation suggests their role in constant detoxification of H_2O_2 in *B. juncea* L. seedlings under Imidacloprid toxicity. CAT activity was highly sensitive to low concentrations of Imidacloprid treatments, and was thus stimulated by imidacloprid to scavenge H_2O_2 to alleviate lipid peroxidation. However, at higher levels of imidacloprid, ROS load might have exceeded the antioxidant defense capacity in *B. juncea* plants as is evident by low activities of CAT (fig 4). As a consequence, severe oxidative damage occurred as is evident from the marginal leaf necrosis at higher concentrations in present study. Similar results were found by (Zhang et al. 2011; Parween et al. 2012) in Wheat and *Vigna radiata* under omethoate and chloropyriphos stress

respectively. Streb et al. (1993) have reported similar changes in CAT activity in the Paraquat- treated Secale cereale. Decline in CAT activity is regarded as a general response to many stresses and is supposedly due to inhibition of enzyme synthesis or change in assembly of the enzyme subunits (Somashekaraiah et al. 1992).

GR is localized mainly in the chloroplast in which it represents about 80 % of total GR activities in leaf tissues. It can be also found in cytosol, glyoxysomes, and peroxisomes (Jimenez et al. 1997). Like APX, GR is one of the major components in the ascorbate–glutathione cycle, by which the efficient recycling of glutathione is ensured by GR. Therefore, it plays an essential role in the protection of chloroplasts against the oxidative damage by maintaining a high reduced/oxidized glutathione (GSH/GSSG) ratio (Noctor and Foyer 1998). The present study showed that the GR activity has increased with the increasing concentration of imidacloprid (fig 5). The reason behind this could be that the Asc-Glu cycle might be operating at a high rate in order to detoxify the ROS in these plants, or the reduced glutathione pool has to be maintained at high levels so that it does not become limiting for the synthesis of phytochelatins, the small peptides involved in the sequestrations of various metal ions in the vacuoles (Stolt et al. 2003; Parween et al. 2012) and in the inactivation of pesticides by conjugate formation. It has been observed that plants under environmental stimuli tend to have high activities of GR (Batish et al. 2006). Hence, a critical role is played by GR in protecting plants against oxidative stress.

The non-enzymatic cellular antioxidants, like ascorbate and glutathione, undergo changes during oxidative stress (Nakano and Asada 1981). In the present study, a dose dependent reduction in Asc and DHA as compared to their respective controls was observed. Similar results were reported by (Qureshi et al. 2007) and (Parween et al. 2012) under heavy metal

and insecticide stress respectively. Oxidative stress appears to cause decline in the ratio of Asc and dehydroascorbate (DHA) (Qureshi et al. 2007). Glutathione acts as an antioxidant and is believed to protect the cell against oxidative stress by maintaining cellular redox potentials. It may react directly with ROS, protect protein thiol groups and is believed to be involved in enzymatic detoxification of H_2O_2 (Kok De and Stulen 1993). The enhanced levels of GSSG in *B. juncea* due to imidacloprid toxicity suggest its involvement in the detoxification of reactive oxygen species and free radicals, directly (non-enzymatic) as well as through certain enzymes. It is believed that GSH (Wingate et al. 1988) or GSSG (Winglse and Karpinski 1996) or a change between GSH and GSSG (Foyer et al. 1997) may function as signal for activating stress-responsive gene expression under stress situations.

There was a significant reduction in total chlorophyll when concentration of imidacloprid was increased from 30- 40 g.a.i ha^{-1}. Several studies reported that the decline in chlorophyll content might be caused due to reduction in the synthesis of chlorophyll, possibly by increasing the enzyme activity of chlorophyllase (Raja et al. 2012). Carotenoid is an important component of defence mechanism of plants and protect chlorophyll and photosynthetic membrane from photooxidative damage (Mishra et al. 2008). Therefore, decline in carotenoid content could have serious consequence on chlorophyll, phycocyanin as well as thylakoid membrane (Prasad et al. 2005). The enhanced level of carotenoid at lower doses of imidacloprid (10 and 20 g.a.i ha^{-1}) (fig 9B) occurred to protect the plant against the photodynamic damage. Similar to this, increase in the level of carotenoid at low dose under dimethoate (Mishra et al. 2008), UV-B (Lingakumar and Kulandaivelu 1993) and chloropyriphos (Parween et al. 2011) exposure were observed. High doses of imidacloprid caused considerable reduction in carotenoid content and this condition results in serious

consequence on Chl and photosynthetic apparatus leading to reduced photosynthesis efficiency of the plant. Significant reduction in Carotenoid content was reported under insecticide stress (Mishra et al. 2008). In our study, the decrease in the photosynthetic pigments at 15 DAT as compared to the 3 DAT and 7 DAT can be because of senescence of leaves (Sestak 1981).

Conclusion

Mustard could tolerate imidacloprid stress at lower concentrations as is evident from the enhancement of photosynthetic pigments at lower concentrations of insecticide. The possible mechanisms involved might be attributed to higher antioxidant enzyme activity and lower lipid peroxidation and membrane permeability. However, our results suggest that at higher doses, a high lipid peroxidation rate indicates degradation of the cell-membrane system, which would disturb fundamental biochemical and physiological processes in plants and enhance the production of ROS. These biochemical results can be inferred as internal tolerance mechanisms and may help us to develop tactics for reducing the risk caused due to insecticide contamination in the crop production.

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References

Abedi T, Pakniyat H. 2010. Antioxidant enzyme changes in response to drought stress in ten cultivars of oilseed rape **(***Brassica napus* L.). Czech J. Genet. Plant Breed. 46:27–34.

Aebi H. 1984. Catalase in vitro. Methods Enzymol. 105:121-126.

Alscher RG, Erturk N, Heath LS. 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress. J. Exp. Bot. 53:1331–1341.

Anderson ME. 1985. Determination of glutathione and glutathione disulfide in biological samples. Methods Enzymol. 113:555–570.

Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. Plant Physiol. 24: 618–633.

Asada K. 1984. Chloroplasts: formation of active oxygen and its scavenging. Methods Enzymol. 105:422-435.

Bashir F, Siddiqi TO, Mahmooduzzaafar, Iqbal M. 2007a. Effect of different concentrations of mancozeb on the morphology and anatomy of *Lens culinaris* L. Ind. J. Environ. Sci. 11:71-74.

Bashir F, Mahmooduzzafar, Siddiqi TO, Iqbal M. 2007b. The antioxidative response system in *Glycine max* (L.) Merr. exposed to Deltamethrin, a synthetic pyrethroid insecticide. Environ. Pollut. 147:94-100.

Bates LS, Wadern RP, Teare ID. 1973. Rapid estimation of free proline for water stress determination. Plant Soil. 39:205-207.

Batish DR, Singh HP, Setia N, Kaur S, Kohli K. 2006. 2-Benzoxazolinone (BOA) induced oxidative stress, lipid peroxidation and changes in some antioxidant enzyme activities in mung bean (*Phaseolus aureus*). Plant Physiol. Biochem. 44:819–827.

Beauchamp CO, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44:276-287.

Conway HE, Kring TJ, McNew R. 2003. Effect of imidacloprid on wing formation in the cotton aphid (Homoptera: Aphididae). Fla. Entomol. 86:474-476.

De Vos CHR, Schat H, Vooijs R, Ernst WHO. 1989. Copper-induced damage to the permeability barrier in roots of *Silene cucubalus*. J. Plant Physiol. 135:164–169.

Foyer CH, Lopez Delgado H, Dat JF, Scott IM. 1997. Hydrogen peroxide and glutathione-associated mechanisms of acclamatory stress tolerance and signaling. Physiol. Plant. 100:241–254.

Ganguly S, Bhattacharya S, Mandi S, Tarafdar J. 2010. Biological detection and analysis of toxicity of organophosphate and azadirachtin-based insecticides in *Lathyrus sativus* L. Ecotoxicology. 19:85–95.

Gille G, Singler K. 1995. Oxidative stress in living cells. Folia Microbiol. 2:131–152.

Gonias ED, Oosterhuis DM, Bibi AC. 2008. Physiological response of cotton to the insecticide imidacloprid under high-temperature stress. J. Plant Growth Regul. 27: 77−82.

Guo Z, Ou W, Lu S, Zhong Q. 2006. Differential responses of antioxidative system to chilling and drought in four rice cultivars differing in sensitivity. Plant Physiol. Biochem. 44:828–836.

Haddad R, Morris K, Buchanan-Wullaston V. 2009. Molecular characterization of free radicals decomposing genes on plant developmental stages. In: Proceedings of world academy of science, engineering and technology, pp. 37.

Halliwell B. 1987. Oxidative damage, lipid peroxidation and antioxidant protection in chloroplasts. Chem. Phys. Lipids. 44:327–340.

Hazarika A, Sarkar SN, Hajare S, Kataria M, Malik JK. 2003. Influence of malathion pretreatment on the toxicity of anilofos in male rats: a biochemical interaction study. Toxicology. 185:1–8.

Hernandez JA, Jimenez ZA, Mullineaux PM, Sevilla F. 2000. Tolerance of pea (*Pisum sativum* L.) to long-term salt stress is associated with induction of antioxidant defences. Plant Cell Environ. 23:853-862.

Jaleel CA, Gopi R, Pannersalvam R. 2007. Alterations in lipid peroxidation electrolyte leakage, and proline metabolism in *Catharanthus roseus* under treatment with triadimefon, a systemic fungicide. Comptes Rendus Biologies. 330:905-912.

Jan S, Parween T, Siddiqi TO, Mahmooduzzafar. 2012. Antioxidant modulation in response to gamma induced oxidative stress in developing seedlings of *Psoralea corylifolia* L. J. Environ. Radioact. 113:142-149.

Jimenez A, Hernandez JA, del Rio LA, Sevilla F. 1997. Evidence for the presence of the ascorbate–glutathione cycle in mitochondria and peroxisomes of pea leaves. Plant Physiol. 114:275–284.

Khan SM, Kour G. 2007. Sub acute oral toxicity of chlorpyriphos and protective effect of green tea extract. Pesticide Biochem. Physiol. 89:118–123.

Kok De L, Stulen I. 1993. Role of glutathione in plants under oxidative stress. In: De Kok LJ, Stuten I, Rennenberg H, Brunold C, Rauser WE (Eds.), Sulfur nutrition and assimilation in higher plants: regulatory agricultural and environmental aspects, SPB Acedamic Publishing., The Hague, pp. 125–138.

Liang YC, Chen Q, Liu Q, Zhang WH, Ding RX. 2003. Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt-stressed barley (*Hordeum vulgare* L.). Plant Physiol. 160:1157– 1164.

Liu Y, Jiang H, Zhao Z, An L. 2011. Abscisic Acid is involved in brassinosteroids-induced chilling tolerance in the suspension cultured cells from *Chorispora bungeana*. Plant Physiol. 168:853-862.

Mishra V, Srivastava G, Prasad SM. 2009. Antioxidant response of bitter gourd (*Momordica charantia* L.) seedlings to interactive effect of dimethoate and UVB irradiation. Sci. Hortic. 120:373–378.

Mishra V, Srivastava G, Prasad SM, Abraham G. 2008. Growth, photosynthetic pigment and photosynthetic activity during seedling stage of cowpea (*Vigna unguiculata* L.) in response to UV-B and dimethoate. Pesticide Biochem. Physiol. 92:30-37.

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22:867–880.

Noctor G, Foyer CH. 1998. Ascorbate and Glutathione: Keeping active oxygen under control. Ann. Rev. Plant Physiol. 49:249-279.

Ogata S, Takeuchi M, Teradaira S, Yamamoto N, Iwata K, Okumura K, Taguchi H. 2002. Radical scavenging activities of niacin related compounds. Biosci. Biotechnol. Biochem. 66:641-645.

Parween T, Jan S, Mahmooduzzafar Fatma T. 2011. Alteration in nitrogen metabolism and plant growth during different developmental stages of green gram (*Vigna radiata* L.) In response to chloropyriphos. Acta Physiol. Plant. 33:2321-2328.

Parween T, Jan S, Mahmooduzzafar, Fatma T. 2012. Evaluation of oxidative stress in *Vigna radiata* L. in response to chlorpyrifos. Int. J. Environ. Sci. Technol. 9:605–612.

Pinto E, Sigaud-Kutner TCS, Leitao MAS, Okamoto OK, Morse D, Colepicolo P. 2003. Heavy metal-induced oxidative stress in algae. J Phycol. 39:1008–1018.

Prasad SM, Kumar D, Zeeshan M. 2005. Growth, photosynthesis, active oxygen species andantioxidants responses of paddy field cyanobacterium *Plectonema boryanum* to endosulfan stress. J. Gen. Appl. Microbiol. 51:115–123.

Qureshi MI, Abdin MZ, Qadir S, Iqbal M. 2007. Lead-induced oxidative stress and metabolic alterations in *Cassia angustifolia* Vahl. Plant Biol. 51:121–128.

Raja W, Rathaur P, Suchit AJ, Pramod WR. 2012. Endosulfan induced changes in growth rate, pigment composition and photosynthetic activity of mosquito fern *Azolla microphylla*. J Stress Physiol. Biochem. 8: 98-109.

Rehman F, Khan FA, Anis SB. 2013. Assessment of aphid infestation levels in some cultivars of mustard with varying defensive traits. Arch. Phytopathol. Plant. Protect. http://dx.doi.org/10.1080/03235408.2013.860724.

Sestak Z. 1981. Leaf ontogeny and photosynthesis. In: Johnson CB (Eds.), Physiological processes limiting plant productivity, Butterworths., London, pp. 147-158.

Sgherri CLM, Loggini B, Puliga S, Navari-Izzo F. 1994. Antioxidant system in *Sporobolus stapfianus*: changes in response to desiccation and rehydration. Phytochem. 33:561–565.

Somashekaraiah SV, Padmaji K, Prasad ARK. 1992. Phytotoxicity of cadmium ions on germinating seedlings of mung bean (*Phaseolus vulgaris*): involvement of lipid peroxidation in chlorophyll degradation. Physiol Plant. 85:85-89.

Song NH, Yin X, Chen GF, Yang H. 2007. Biological responses of wheat (*Triticum aestivum*) plants to the herbicide chlorotoluron in soils. Chemosphere. 69:1779–1787.

Stolt JP, Sneller FEC, Bryngelsson T, Lundborg T, Schat H. 2003. Phytochelatin and cadmium accumulation in wheat. Environ. Exp. Bot. 49:21-28.

Streb P, Michael-Knauf A, Feicrabend J. 1993. Preferential photoinactivation of catalase and photoinhibition of photosystem II are common early symptoms under various osmotic and chemical stress conditions. Physiol. Plant. 88:590-598.

Vanaja M, Charyawa NVN, Rao KVN. 2000. Effect of cadmium on carbohydrate, nucleic acids, and phenolic content in *Stigeoclonium tenue* Kutz. Indian J. Plant Physiol. 5:253-256.

Winglse G, Karpinski S. 1996. Differential redox regulation by glutathione and glutathione reductase and Cu–Zn SOD gene expression in *Pinus sylvestris* L. needles. Planta. 198:151-157.

Zhang B, Guixin C, Changzhou W, Jun Y, Zhiqiang L, Yongchao L. 2011. The growth and antioxidant defense responses of wheat seedlings to omethoate stress. Pesticide Biochem. Physiol. 100:273–279.

Figure Captions

Fig-1 Change in MDA content (nmol g^{-1} FW) at various growth stages of *Brassica juncea* L. Cv. alankar exposed to different concentrations of imidacloprid. Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4)

Fig-2 Change in proline content (μg g^{-1} FW) at various growth stages of *Brassica juncea* L. Cv. alankar exposed to different concentrations of imidacloprid. Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4)

Fig-3 Change in SOD activity (Units mg^{-1} protein min^{-1}) at various growth stages of *Brassica juncea* L. Cv. alankar exposed to different concentrations of imidacloprid. Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4)

Fig-4 Change in CAT activity (Units mg^{-1} protein min^{-1}) at various growth stages of *Brassica juncea* L. Cv. alankar exposed to different concentrations of imidacloprid. Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4)

Fig-5 Change in Apx activity (Units mg^{-1} protein min^{-1}) at various growth stages of *Brassica juncea* L. Cv. alankar exposed to different concentrations of imidacloprid. Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4)

Fig-6 Change in GR activity (Units mg^{-1} protein min^{-1}) at various growth stages of *Brassica juncea* L. Cv. alankar exposed to different concentrations of imidacloprid. Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4)

Fig-7 Variation in ascorbate content at various growth stages of *Brassica juncea* L. treated with different concentration of imidacloprid. **(A)** Ascorbic acid (Asc) content (m mol g^{-1} FW) and **(B)** dehydroascorbate (DHA) content (m mol g^{-1} FW). Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4)

Fig- 8 Variation in glutathione content at various growth stages of *Brassica juncea* L. treated with different concentration of imidacloprid. **(A)** reduced GSH content (n mol g^{-1} FW) and **(B)** oxidised GSSG content (n mol g^{-1} FW). Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4).

Fig- 9 Effect of different concentrations of imidacloprid on photosynthetic pigments of *Brassica juncea* L. Cv. alankar at different growth stages. **(A)** Total chlorophyll content [mg g^{-1} FW]. **(B)** Carotenoid content [mg g^{-1} FW]. Values with different letters are significantly ($p<0.05$) different from each other (Duncan's multiple range test). The values represent Mean ± SE (n =4)