



Pesticide-induced oxidative stress and antioxidant responses in tomato (*Solanum lycopersicum*) seedlings

Shakirullah Khan Shakir¹ · Shahid Irfan¹ · Basreen Akhtar¹ · Shafiq ur Rehman¹ · Muhammad Khan Daud² · Nadia Taimur¹ · Azizullah Azizullah¹

Accepted: 12 February 2018 / Published online: 1 March 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Excessive use of pesticides can adversely affect the growth of non-target host plants in different ways. Pesticide-induced stress can affect non-target plants through elevated levels of reactive oxygen species (ROS) responsible for detrimental effects on cell metabolism, biochemical and other physiological activities. In response to oxidative stress, plant activates antioxidant defense system consisting of both enzymatic and non-enzymatic components. In the present investigation, three commonly used pesticides, emamectin benzoate, alpha-cypermethrin and imidacloprid, were assessed for causing oxidative stress in tomato. The oxidative damage induced by these pesticides at five different concentrations i.e. 1/4X, 1/2X, recommended application dose (X), 2X and 4X in the root and shoot tissues of tomato plant/seedlings were evaluated. Following pesticide exposure for 35 days, cell viability, cell injury, total soluble sugar (TSS) and total soluble proteins (TSP) were measured. Antioxidant activities were estimated by measuring activity levels of superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) peroxidase (POD), ascorbate peroxidase (APX) and proline. Hydrogen peroxide (H₂O₂) levels were analysed as ROS, lipid peroxidation was measured in term of thiobarbituric acid reactive substances (TBARS) as membrane damage caused by ROS was also assessed. Analysis of the data revealed that pesticides application at higher concentrations significantly elevated ROS levels and caused membrane damage by the formation of TBARS, increased cell injury and reduced cell viability both in root and shoot tissues compared with non-treated plants. Moreover, a gradual decrease in the levels of TSS and TSP was observed in plants subjected to increasing doses of pesticides. To cope with pesticide-induced oxidative stress, a significant increase in levels of antioxidants was observed in the plants exposed to higher doses of pesticides. Shoot tissues responded more drastically by producing higher levels of antioxidants as compared to root tissues indicating the direct exposure of shoots to foliar application of pesticides. Taken together, these results strongly suggested that the application of pesticides above the recommended dose can provoke the state of oxidative stress and can cause oxidative damages in non-target host plants.

Keywords Pesticide-induced stress · ROS · Oxidative stress · Anti-oxidants · TBARS · Proline

Introduction

In today's modern world, several toxic chemicals are spilled in agricultural fields as a consequence of modern agricultural practices, heavy industrialization and faster

urbanization. Due to increased rate of pest attack and unsuccessful trend in the production of pest resistant crop varieties, chemical pesticides are common tool to protect crops against pests (Yardim and Edwards 2003; Siddiqui and Ahmed 2006). During past few decades the use of pesticides has increased many folds. It has been estimated that 4.6 million tons of chemical pesticides are used worldwide annually, of which 85% are used in agriculture while the remaining 15% for other purposes (Idrovo 2000; Zhang et al. 2011). Among different categories of pesticides, global consumption of herbicides shares 47.5%, insecticides 29.5%, fungicides 17.5% and other pesticides contributes 5.5% (De et al. 2014). Like other parts of the world, large quantities of different pesticides are used in

✉ Azizullah Azizullah
azizswabi@hotmail.com

¹ Department of Botany, Kohat University of Science and Technology, Kohat 26000, Pakistan

² Department of Biotechnology and Genetic Engineering, Kohat University of Science and Technology, Kohat 26000, Pakistan

Pakistan. According to an estimate, about 173,000 tons of pesticides are used annually in Pakistan with the expenses of about Rs. 13,000 million and this quantity is continuously increasing with an annual rate of about 6% (FAO 2007). In agriculture, pesticides are used mainly to control pests and minimize crop losses, but their indiscriminate and intensive use has resulted in serious environmental hazards as well as aggravated the pesticide poisonings in other biota (Carvalho 2006; Haq et al. 2008; Akhtar et al. 2009). The toxicity of pesticides does not remain restricted to the target organisms only but can also affect non-target organisms including plants and animals as well as impair activities of soil microbial communities (Dobsikova 2003; Rachid et al. 2008). Since pesticides are poisons, their indiscriminate and unskilled use has resulted in several problems like damage to plants and animals, increased resistance of pests to pesticides, accumulation of residues in fruits and vegetables, severe biodiversity losses and decline in natural habitats (Baig et al. 2012). Nevertheless, the direct and indirect impacts of pesticide overdosing on ecosystems as well as human health are of great concern and remain largely under-reported.

In advanced countries, strict monitoring and regulation system for pesticides ensure the safe use and proper handling of pesticides. The control schemes further ensure their use on scientific basis that support their effectiveness against target pests and not posing significant hazard to the environment and human health (Glover-Amengor and Tetteh 2008). In contrast, the quantities of pesticide to be used effectively in developing countries mostly rely on manufacturer's recommendations. The recommendations are based on data derived from toxicity related tests, crop residue analyses, environmental fate testing and ecotoxicology testing under different agro-climatic and socio-cultural conditions. Although this information is useful, may not be appropriate under local conditions (Glover-Amengor and Tetteh 2008). Most of the farmers in developing countries lack formal training and information on the use and safe-handling of pesticides. In addition, the pesticide dealers are mostly untrained and usually advise pesticides unwisely in two or three times higher concentration than the recommended dose (Ecobichon 2001; Asogwa and Dongo 2009). As each crop is susceptible to attack by more than one type of pests, it is usually treated with several pesticides in combination (Tabashnik 1989). In countries like Pakistan, pesticides are often used indiscriminately and in excess amount (Khan 2014; Khan et al. 2012; Sheikh et al. 2013; Shakir et al. 2016). The pesticide dealers always recommend pesticides in excessive dose because they are more interested in earning their profit rather than guiding the farmers properly (Rehman 1994).

Over application of pesticides can interfere with various processes in plants like cell growth, photosynthesis, respiration, biosynthetic reactions, and molecular

composition in plants (DeLorenzo et al. 2001; Shakir et al. 2016). Studies revealed that pesticides usage can cause several adverse effects in non-target host plants like lowering of pollen performance (Cali and Candan 2009; Tort et al. 2005), impairment of reproductive processes in potato (Rio et al. 2012), disruption in photosynthesis and enzyme activities in cucumber (Xia et al. 2006), retarded germination and growth in soybean (Aksoy et al. 2013), disturbance of morphological and physiological parameters in maize (Kilic et al. 2015) and impaired growth and production of photosynthetic pigments in tomato (Shakir et al. 2016). These elusive adverse effects can add up and lead to economic losses when multiple crops are grown.

At the cellular level, pesticide-induced stress has been found to produce oxidative stress, contributing to the toxicity in the form of reactive oxygen species (ROS), viz. hydrogen peroxide (H_2O_2), superoxide ($O_2^{\cdot-}$), and hydroxyl radical (OH^{\cdot}). ROS is a collective term that describes the chemical species formed upon incomplete reduction of oxygen (D'Autréaux and Toledano 2007). Under normal growth conditions, the production of ROS in cells is very low, while stress conditions such as drought, salinity, chilling, heavy metals and pesticides, elevate the levels of ROS in plant cells. ROS molecules are highly toxic and can oxidize most of the lipids, proteins and nucleic acids subsequently causing death of the cells due to lipid peroxidation, membrane damage and inactivation of enzymes. Oxidative stress can also occur as damage to biological systems or by impairing antioxidant defense systems. Among various stress markers, lipid peroxidation is an important one and is indicated by the formation of thiobarbituric acid reactive substances (TBARSs). To cope with oxidative stress, plants develop a complex antioxidant defense system, consisting of both antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), catalase (CAT) and non-enzymatic antioxidants like ascorbic acid (AsA) and proline etc., that scavenge free radicals and peroxides (Prasad et al. 2005a; Gratão et al. 2008; Gill and Tuteja 2010). Increased stress tolerance in plants exposed to various stresses is often associated with higher levels of antioxidants, particularly through the enhancement of antioxidant enzyme activities (Gomes-Junior et al. 2006; Gratão et al. 2008; Fidalgo et al. 2011, 2013). Among key antioxidant enzymes, SOD catalyzes the dismutation of superoxide to molecular oxygen and hydrogen peroxide (H_2O_2) and constitutes the first line of antioxidant defense. CAT, APX and POD are implicated in the scavenging of H_2O_2 (Goel et al. 2003; Gajewska and Skłodowska 2008). Non-enzymatic antioxidants include a range of compounds such as ascorbate, carotenoids, phenolic compounds and several nitrogenous metabolites such as amino acids, especially proline, which possesses a powerful antioxidant

activity required to redress the deleterious effects of ROS (Gill and Tuteja 2010; Hayat et al. 2012). Changes in the activity of these enzymes indicate redox alterations related to oxidative stress. ROS, TBARS and antioxidants have been frequently used in plants to assess environmental stresses. Several other studies indicate pesticide -induced toxicity by causing oxidative stress i.e. ROS production and their scavenging mechanism in plants (Bolwell and Wojtaszek 1997; Banerjee et al. 2001; Foyer and Noctor 2005). The mechanism includes the stimulation of anti-oxidative enzyme activity as well as lowering the content of anti-oxidants (Srivastava et al. 2004) and disrupting the electron transport chain (Qadir et al. 2004).

Tomato (*Solanum lycopersicum*) is a major horticultural crop with an estimated global production of over 120 million metric tons (FAO 2007) and has a high economic value worldwide. In Pakistan, tomato is the second major vegetable crop widely grown in different parts of the country throughout the year (Mirza 2007). For example, in 2011 tomato was grown on an area of 39,918 hectares which gave an estimated annual production of 433,128 tones. In 2009–2010, Pakistan exported 5692 tons of tomato and earned 77 million PKR (Khokhar 2013). Due to the rapid increase in population, the demand for domestic consumption of tomato in Pakistan is increasing day by day. Tomato is consumed in a variety of ways, i.e. in the form of vegetable, salad, fruit, ketchup, and chatni etc. (Chohan and Ahmad 2008). In Pakistan, the yield of tomato is lower as compared to the developed countries mostly due to pest's attack (Shakoor et al. 2010). To overcome these losses, farmers extensively use pesticides in higher concentrations. As discussed above, farmers in Pakistan apply pesticides on crops in doses several times higher than the recommended one. Pesticides application in such higher doses can negatively impact growth and yield of tomato. In a previous study we found that over application of the pesticides commonly used in Pakistan (emamectin benzoate, alpha-cypermethrin and imidacloprid) adversely affected different growth parameters in tomato (Shakir et al. 2016). However, studies on the mechanism of toxicity of these pesticides in crops like tomato can hardly be found in literature. A number of studies have shown that application of different chemicals and pesticides can lead to the generation of ROS and oxidative stress in plants which further activates the antioxidant defence system in exposed plants (Bolwell and Wojtaszek 1997; Banerjee et al. 2001; Foyer and Noctor 2005). We hypothesized that these pesticides might cause oxidative stress in tomato which may adversely affect its growth. The present study was therefore designed to investigate the effects of different doses of these three commonly used pesticides, emamectin, alpha-cypermethrin and imidacloprid, on oxidative stress as well as antioxidants defence responses in tomato. The main objectives

of this study were: (1) to study the effect of emamectin, alpha-cypermethrin and imidacloprid on the generation of ROS (H_2O_2) and oxidative stress in term of membrane lipid peroxidation (TBARS assay) in tomato seedlings, (2) to evaluate antioxidant responses of tomato to the applied pesticides by measuring the activities of various enzymatic and non-enzymatic antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) peroxidase (POD), ascorbate peroxidase (APX) and proline) and (3) to assess the effect of resultant stress on cell injury and cell viability as well as on total soluble proteins and sugars in tomato. The effects of the used pesticides were studied in both shoots and roots of tomato. The findings of this study provide an insight into the phytotoxicity of these pesticides in non-target host plants, which can be helpful in formulating future strategies to minimize the adverse effects of pesticides on non-target host crops. Furthermore, these parameters in tomato can be used as endpoints to evaluate the ecotoxicological and deleterious effects of insecticides in the agroecosystem.

Materials and methods

Plant material

Tomato (*Solanum lycopersicum*) was selected as experimental plant. A known variety of tomato (BSS-30) was obtained from a certified dealer in Bannu city, Khyber Pakhtunkhwa, Pakistan.

Pesticide treatments

To examine the effects of three commonly used pesticides including emamectin benzoate, alpha-cypermethrin and imidacloprid on tomato plant, five different concentrations of each pesticide were prepared and sprayed over the foliage at weekly intervals for a period of 35 days. The selected pesticides belong to three different classes of pesticides, i.e. alpha-cypermethrin is a pyrethroids, imidacloprid is neonicotinoids and emamectin belongs to abamectin. These are broad spectrum insecticides, affecting the nerve impulses in the insects. Emamectin possesses systemic mode of action with avermectine as active ingredient which causes paralysis in the insects by activating chloride channels in nerves cells. Alpha-cypermethrin is a pyrethroid with non-systemic contact mode of action which modulates the sodium channels causing hyperexcitation in nerves accompanied with stomach action. The imidacloprid is a systemic insecticide containing neonicotinoids which mimics the agonist action of acetylcholine and cause hyperexcitation in the nervous system of exposed insects. The selection of these pesticides was based on literature which reveals these to be among the most

commonly used pesticides in Pakistan (Khooharo et al. 2008; Khan et al. 2012; Shakir et al. 2016). A general informal discussion with farmers and pesticide dealers in a tomato growing area confirmed the same. Since the dealers usually prescribe higher doses of pesticides than the dose recommended by manufacturer, the concentrations tested for each pesticide were: recommended dose (X), two times higher (2X), four times higher (4X), half (1/2X) and quarter (1/4X) of the recommended doses, i.e. five different concentrations were tested for each pesticide. For emamectin, the doses of 1/4X, 1/2X, X, 2X and 4X correspond to 10 mg/L, 20 mg/L, 40 mg/L, 80 mg/L and 160 mg/L, respectively, while for cypermethrin 30 mg/L, 60 mg/L, 125 mg/L, 250 mg/L and 500 mg/L, respectively. Similarly, 1/4X, 1/2X, X, 2X and 4X doses of imidacloprid were 125 mg/L, 250 mg/L, 500 mg/L, 1000 mg/L and 2000 mg/L, respectively.

Experimental procedure

Experiments were conducted in plastic pots containing sterilized sand. A total of ten seeds were placed with appropriate distance in each pot. Before sowing, seeds were surface sterilized with 80% (v/v) ethanol for 5 min and then rinsed with distilled water for three times. All glassware was autoclaved at 121 °C for 15 min before use. For germination, pots containing seeds were kept in a growth chamber under dark conditions at a temperature of 25 °C for 5 days. After the germination was completed, a photoperiod of 16/08 h light/dark period with a light intensity of 500 mol/m²/s was provided and 27 ± 2 °C temperature and 60% humidity was maintained throughout the experiment. Each treatment was sprayed with 5 mL of the respective solution after interval of every 7 days. Hoagland's solution was used for nutritional requirement of the experimental plants. Each experiment was conducted in three independent replicates. After 35 days, the experiment was discarded and all the biochemical determinations were performed.

Analysis of stress markers

Fresh samples (0.5 g) of aerial parts (shoot) and roots were ground with the help of a mortar and pestle using liquid nitrogen and homogenized in 50 mM phosphate buffer (pH 7.8) under chilled conditions. The homogenized mixture was filtered through four layers of muslin cloth and centrifuged at 12,000×g for 10 min at 4 °C. The samples were subjected to analyses of lipid peroxidation in term of TBARS, H₂O₂ as representative reactive oxygen species (ROS), activities of enzymatic antioxidants like superoxide dismutase (SOD; 1.15.1.1), guaiacol peroxidase (POD, EC 1.11.1.7), ascorbate peroxidase (APX; 1.11.1.11), catalase (CAT; 1.11.1.6) and glutathione reductase (GR; 1.6.4.2) and activities of the non-enzymatic antioxidants proline.

Moreover, total soluble protein (TSP), total soluble sugar (TSS), cell injury and cell viability were analysed as stress makers. The methods used to assess various stress biomarkers are as follows.

H₂O₂ determination

Analysis of H₂O₂ was carried out on fresh plant materials spectrophotometrically as described by Willekens et al. (1997). Absorbance at 390 nm was observed in a reaction mixture containing 1 mL of enzyme extract, 1 mL of 10 mM potassium phosphate buffer (pH 7.0) and 2 mL of 1 M KI. Amount of H₂O₂ produced was expressed as μM/g fresh weight (FW), on the basis of previously established calibration curve.

Determination of lipid peroxidation

The level of lipid peroxidation products was measured in terms of thiobarbituric acid reactive substances (TBARS) using the method of Hodges et al. (1999) with slight modification. Fresh root/shoot samples weighing 200 mg were ground in 3 ml of 0.25% (w/v) 2-thiobarbituric acid (TBA) in 10% tri-chloro acetic acid (TCA) using mortar and pestle. Homogenate was heated for 30 min at 95 °C and then quickly cooled in an ice bath and centrifuged at 10,000×g for 10 min. The absorbance of supernatant was recorded at 532 nm, and nonspecific turbidity was corrected by subtracting the absorbance of the same at 600 nm. The level of lipid peroxidation was calculated and expressed as TBARS.

Analysis of SOD activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by measuring its capacity of inhibiting the photochemical reduction of nitro blue tetrazolium (NBT) by following the method of Giannopolitis and Ries (1977) modified by Wang et al. (2010). A total of 3 mL of reaction mixture containing 50 mM of phosphate buffer (pH 7.8), 10 mM of Methionine, 1.17 mM of Riboflavin, 56 mM of NBT, and 25 μL of enzyme extract was placed under light conditions at 4000 lux for 10 min while the control sample was placed under dark as well as light conditions. The absorbance of solution was measured at 560 nm. One unit of SOD is defined as the enzyme activity that inhibited the photo reduction of NBT to blue Formazan by 50%. In control samples, a 25 μL distilled water was used in place of enzyme extract. The following formula was used to elucidate the levels of SOD in plant samples:

- $X = OD \text{ sample} - OD \text{ blank}$
- $SOD = X \times 10 \text{ (amount of buffer used)} / 0.5 \text{ (amount of plant sample in g)} / 1.0.025 \text{ enzyme extract} / 1.235$

Determination of POD activity

Guaiacol peroxidase (POD, EC 1.11.1.7) activity was assayed according to the method of Putter (1974) and Cakmak and Marschner (1992) with some modifications. The reaction mixture (3 ml) consisted of 100 μ L enzyme extract, 100 μ L guaiacol (1.5%, v/v), 100 μ L H₂O₂ (300 mM) and 2.7 ml of 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). Increase in the absorbance due to oxidation of guaiacol was measured through spectrophotometer (UV-2600) at 470 nm. Enzyme activity of POD at 25 \pm 2 $^{\circ}$ C was calculated with following formula.

$$\text{POD (nM/g FW per minute)} = (\text{OD} \times \epsilon) / (\text{W} \times \text{T}).$$

Where: OD = Optical density of samples at 470 nm, ϵ = 0.18 $\text{mM}^{-1} \text{cm}^{-1}$, W = weight of sample, T = time duration taken.

Analysis of CAT activity

Catalase (CAT, EC 1.11.1.6) activity was determined by the method of Cakmak and Marschner (1992). Simply, 0.5 g fresh leaf material was grounded in 10 mL of extraction buffer (0.5 M Na-phosphate, pH 7.3, 3 mM EDTA, 1% PVP, 1% Triton X 100) and centrifuged at 10,000 rpm for 20 min at 4 $^{\circ}$ C. The assay mixture (3 mL) was comprised of 100 μ L enzyme extract, 100 μ L H₂O₂ (300 mM) and 2.8 mL 50 mM phosphate buffer with 2 mM EDTA (pH 7.0). The CAT activity was determined by consumption of H₂O₂ (extinction coefficient of 39.4 mM/cm) at 240 nm for 30 s using the following formula:

$$\begin{aligned} \text{Catalase} = & \text{OD} \times \text{Volume of buffer (V)} \\ & \times \text{volume of enzyme (v)} \\ & \times \text{volume of sample in cavet (A)} / \epsilon \times \text{Weight} \end{aligned}$$

Where, V = 10 mL, v = 0.1 mL, A = 3 mL, ϵ = 39.4 $\text{mM}^{-1} \text{cm}^{-1}$, w = 0.5 g.

Analysis of GR activity

Glutathione reductase GR (EC 1.6.4.2) activity was determined by the method of Giannopolitis and Ries (1977) with slight modifications. Fresh leaf material (0.5 g) was ground in 2 mL of extraction buffer (0.1 M Na-phosphate, pH 7.0, 3 mM EDTA, 1% PVP, 1% Triton X 100) and centrifuged at 10,000 rpm for 10 min. GR activity was expressed as $\mu\text{M min}^{-1} \text{mg}^{-1}$ protein

Determination of APX activity

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured by the method of Nakano and Asada (1981). The

reaction mixture consisted of 100 μ L enzyme extract, 100 μ L ascorbate (7.5 mM), 100 μ L H₂O₂ (300 mM) and 2.7 mL 25 mM potassium phosphate buffer with 2 mM EDTA (pH 7.0). Absorbance was recorded at 290 nm for 1 min and oxidation of ascorbate was observed by the change in absorbance at 290 nm (ϵ 2.8 $\text{mM}^{-1} \text{cm}^{-1}$). For calculation of APX, the following formula was applied.

$$\text{APX} = \text{OD} \times (\text{V}) \times (\text{v}) / \epsilon \times \text{w}$$

Here, OD = optical density, V is volume of buffer for enzyme extract = 20 mL, v is volume of buffer in cuvette = 2.7 mL, ϵ = 2.8 $\text{mM}^{-1} \text{cm}^{-1}$, w is weight = 0.5 g.

Proline determination

Proline was extracted from plant samples by homogenization in 3% (w/v) sulphosalicylic acid and was quantified by the ninhydrin-based colorimetric assay as described by Bates et al. (1973). Simply, 0.5 g of fresh samples were homogenized in 5 ml of 3% sulphosalicylic acid using mortar and pestle and centrifuged at 4000 rpm for 30 min at room temperature. Supernatant of 1 ml was added in 1 ml of acid ninhydrin (1.25 g ninhydrin in 30 ml glacial acetic acid and 20 ml 6 M phosphoric acid) and 1 ml of glacial acetic acid and kept at 100 $^{\circ}$ C for an hour in oven. The reaction was completed in an ice bath and mixed with 2 ml toluene and placed at room temperature until the separation of two layers. The upper coloured layers were taken for absorbance at 520 nm. Proline content was calculated from a previously set standard curve and concentration in the treatment was calculated.

Determination of total soluble protein

The total soluble protein content was analysed according to Bradford (1976) modified by Theymoli and Sadasivam (1987), using Coomassie Brilliant Blue G-250 as dye and albumin as a standard. Furthermore, Bradford reagent (mixture of 13.3 g Cu-acetate, 1.8 mL glacial acetic acid and add distilled water to make 200 mL solution) was used for protein estimation. Fresh leaves (100 mg) were homogenized in a 1 mL phosphate buffer (pH 7.0) by using mortar and pestle. The crude homogenate was centrifuged for 15 min at 4000 rpm. In reaction mixture 2 mL distilled water, 20 μ L enzyme extract and 0.5 mL Bradford reagent were added. Distilled water was used as a blank and recorded the absorbance at wavelength of 595 nm by using a spectrophotometer (UV-2600) while bovine serum albumin (BSA) was used as a standard. Total protein content was calculated using the following formula.

$$\text{Total soluble protein (mg g}^{-1} \text{Fw)} = \text{C} \times \text{V} / \text{Vt} \times \text{W}$$

C = Absorbance coefficient value using equation of linear regression curve ($X = \text{OD} - 0.592/0.033$), V = Volume of

phosphate buffer, V_t = Volume of enzyme extract, W = Plant weight.

Total soluble sugars

To determine the content of total soluble sugars, slightly modified method of Shields and Burnett (1960) was followed. Delicate and fresh parts from shoots and roots (50 mg) were homogenized in liquid nitrogen and grinded to powder with the help of mortar and pestle. Then added a 3 ml of 90% ethanol and incubated it for 1 h at 60–70 °C. Extract was further mixed with 90% ethanol, and made the final volume 25 mL in a volumetric flask. Aliquot of 1 mL was mixed with 1 ml of 5% phenol and 5 mL sulphuric acid. Contents of total soluble sugar were measured through absorbance at 485 nm against the standard curve of glucose solution and expressed as mg g^{-1} FW.

Analysis of cell injury

For the determination of cell injury, membrane permeability in terms of electrolyte leakage was measured in fresh root and shoot, using electrical conductivity meter following the method of Lu et al. (2008). Pieces of fresh leaf and root were placed in test tubes, added 20 mL of distilled water and incubated overnight at 10 °C. The electrical conductivity of fresh plant samples (EC-I) and of samples autoclaved for 15 min at 120 °C (EC-II) was analysed. The cell injury of each sample was analysed through formula:

$$\text{Cell injury} = \frac{\text{EC-I}}{\text{EC-II}} \times 100$$

Analysis of cell viability

To determine cell viability, the technique of triphenyl tetrazolium chloride (TTC) reduction was used. Pieces of fresh leaves and roots (0.2 g) were incubated in 0.6% (v/v) TTC, phosphate buffer for 24 h at 32 °C. The samples were washed with distilled water and extracted with 95% ethanol at 65 °C for 4 h. Absorbance at 490 nm was measured and the cell viability was calculated as the absorbance/g of fresh weight.

Data analysis

The data are expressed as mean of three independent replicates and were analysed by using One-Way Analysis of Variance (ANOVA). Least significance difference (LSD) at 5% probability level was used to detect the significance of differences among treatment means. The difference was considered to be significant if p value was smaller than or equal to 0.05 ($p \leq 0.05$).

Results

Exposure to increased concentrations of pesticides induced ROS damage

Foliar application of the pesticides increased oxidative stress in tomato by enhancing the production of reactive oxygen species measured in terms of H_2O_2 levels. In comparison, ROS accumulated more in root than in shoot tissues. An increase of 42.5 and 53.8% was observed in tomato shoot tissue samples treated with 2X and 4X concentrations of imidacloprid, respectively (Fig. 1a). Similarly, an increase in H_2O_2 levels was recorded in root tissue samples exposed to increasing concentrations of pesticides. The recommended and 4X concentrations of emamectin caused an increase of 190.9 and 131.8%, respectively whilst 1/2X, recommended, 2X and 4X concentrations of imidacloprid resulted in 188.8, 155.5, 303 and 214.6% increase, respectively in H_2O_2 levels ($p \leq 0.05$) as compared with the control (Fig. 1b). Among the tested pesticides, least toxicity was observed in shoot tissues treated with cypermethrin. However, significant difference in H_2O_2 contents was observed in root tissues exposed to different concentrations of cypermethrin except 1/4X dose. The increase in contents of TBARS was also found in shoot and root subjected to pesticide stress. TBARS contents of shoot tissue significantly increased ($p \leq 0.05$), i.e. 37.2% only when exposed to 4X concentrations of cypermethrin. Similarly, in

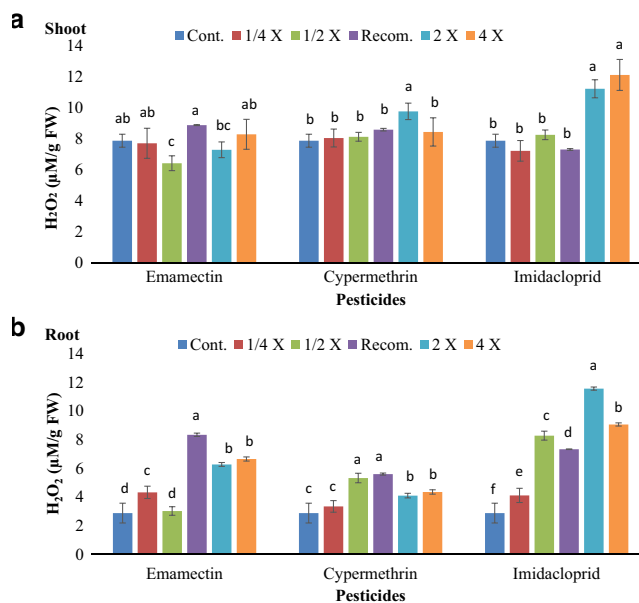


Fig. 1 Effect of the pesticides on shoot and root H_2O_2 levels in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Different alphabets indicate significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

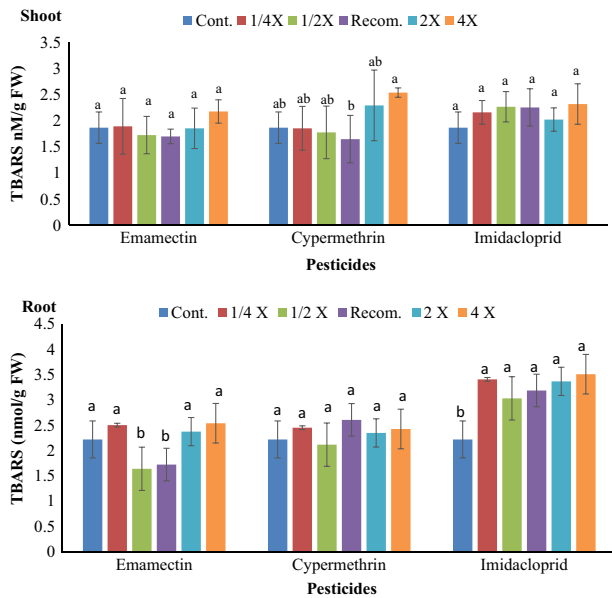


Fig. 2 Effect of the pesticides on shoot and root TBARS levels in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Different alphabets indicate significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

the case of root tissues all the treatments with different concentrations of imidacloprid significantly enhanced (53, 37, 43.8, 52 and 58.3%) membrane damage. A maximum increase (58.3%) of TBARS was observed in root tissues at 4X imidacloprid treatment (Fig. 2). These results indicated that exposure to higher doses of pesticides induced higher production of ROS which possibly caused increase in TBARS content and lipid peroxidation in tomato root and shoot tissue thereby disrupting cellular and enzymatic activities.

Pesticides application altered antioxidant enzyme activities

Pesticide stress led to significant alteration in antioxidant defence system both in shoot and root of tomato plants. A significant increase ($p \leq 0.05$) in the activities of antioxidant enzymes (SOD, POD, CAT, GR, APX and Proline) was observed in tomato seedlings when exposed to elevated levels of the applied pesticides as compared to the non-stressed control plants.

The obtained data revealed that the activity of SOD in treated tomato, both in shoot and root, significantly exceeded control levels ($p \leq 0.05$) and increased with increasing concentrations of pesticides (Fig. 3). In the case of shoot, all the applied concentrations of the three pesticides significantly enhanced the SOD levels except for 1/4X and 4X doses of cypermethrin. Interestingly, application of

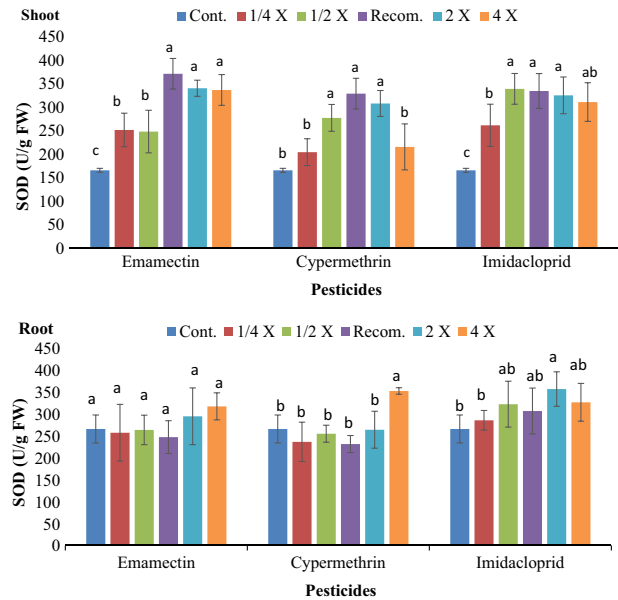


Fig. 3 Effect of the pesticides on shoot and root SOD levels in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Alphabet indicates significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

recommended concentration of emamectin in the shoot tissues resulted in the highest percentage increase (124%) in the enzyme activity as compared with the control. Moreover, 1/2X, recommended and 2X concentrations of cypermethrin also significantly elevated enzyme levels. Similarly, all tested concentrations of imidacloprid caused significant increase in the SOD activity in shoot tissues. However, exposure of roots to emamectin and cypermethrin exhibited reduction in enzyme activity at lower concentrations of the applied pesticides, followed by a significant increase at 4X pesticide overdose. Among treatments, 4X of cypermethrin and 2X of imidacloprid caused the maximum increase of 34 and 34.1%, respectively, in SOD activities, compared with the rest of the treatments ($p \leq 0.05$) (Fig. 3).

In the case of POD, shoot tissues exhibited a significant increase in its activity as compared with root tissue in response to pesticides stress. All the treatments of imidacloprid and two treatments of emamectin (2X and 4X) increased the POD level significantly ($p \leq 0.05$) in the shoot tissues. A maximum increase of 40.9% was observed for shoot samples treated with highest (4X) tested concentration of cypermethrin as compared with the control treatment (Fig. 4). In roots of tomato, exposure to 1/4X, recommended 2X and 4X concentrations of imidacloprid; 1/4X, recommended and 4X concentrations of emamectin; and 1/2X concentrations of cypermethrin significantly enhanced the POD activity, with the highest increase of 21.2% at 4X of imidacloprid. Among all the pesticide treatments,

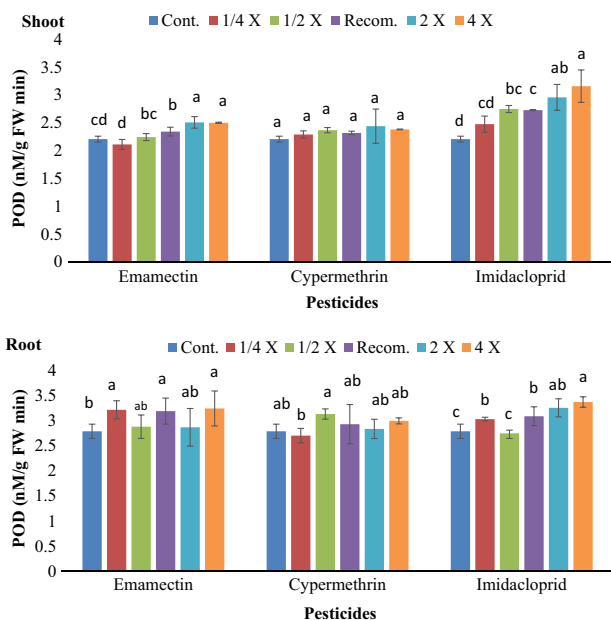


Fig. 4 Effect of the pesticides on shoot and root POD level in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Alphabet indicates significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

imidacloprid treated shoot samples exhibited an increase in the enzyme levels in a dose dependent manner ($p \leq 0.05$) (Fig. 4).

The activity of GR enzyme in the shoot and root tissues of tomato plant exposed to pesticides stress is shown in Fig. 5. Treatment with recommended, 2X and 4X doses of emamectin significantly enhanced the levels of GR both in shoot and root tissues. A gradual increase in the enzyme activity was measured in both tissues in a dose-dependent manner; except for treatment with 1/4X of emamectin which showed a significant decrease in GR activity as compared with the control. Cypermethrin resulted in a significant ($p \leq 0.05$) increase in the GR activity in root tissues at all the tested concentration whilst in shoot, a significant increase was observed only at 4X of this pesticide (Fig. 5). A remarkable increase in the enzyme levels was measured in tomato shoot and root tissue upon exposure to all the tested concentrations of imidacloprid. The significant increase in the enzyme activity in shoot samples followed a dose-dependent trend. The highest increase of 141.3% was observed in the shoot and 154.7% in root tissues subjected to 4X and 2X concentrations of imidacloprid, respectively (Fig. 5).

The activity of catalase enzyme in both shoot and root of seedlings subjected to pesticide stress significantly increased as compared to the control. Shoot tissue, exposed to 2X concentrations of emamectin and cypermethrin;

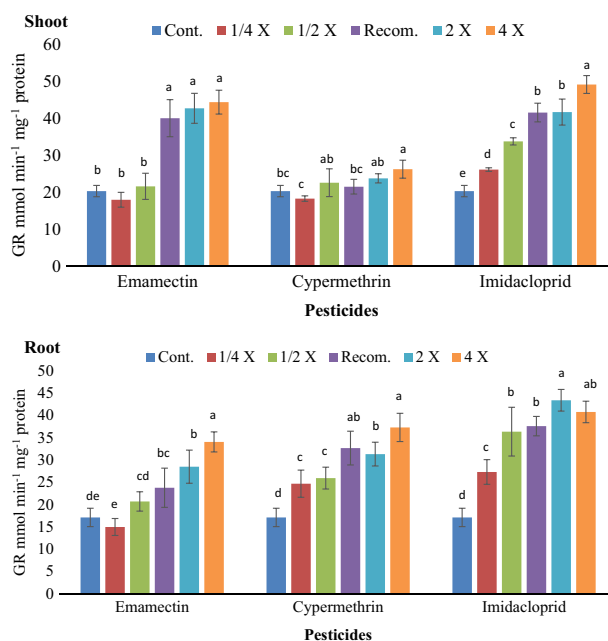


Fig. 5 Effect of the pesticides on GR activity in the shoot and root of tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Alphabet indicates significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

recommended, 2X and 4X concentrations of imidacloprid, showed very prominent and significant increase in the activity of this enzyme. Similarly, in the case of root, the highest increase of 42.3% was observed in samples treated with 4X concentration of imidacloprid. Collectively, catalase enzyme activity significantly increased ($p \leq 0.05$) in all the samples of roots treated with different concentrations (1/2X, recommended, 2X and 4X) of imidacloprid and cypermethrin. In the case of emamectin, only 2X dose significantly elevated catalase level in the root tissues (Fig. 6).

APX constitutes an important member of enzyme defence system that plays an important role in scavenging ROS to increase oxidative stress tolerance. Present results indicated that exposure to increasing doses of pesticides, significantly elevated APX levels both in root and shoot tissues of tomato ($p \leq 0.05$). The obtained data suggested that shoot tissues were more responsive than root tissues due to direct exposure to pesticide stress. Exposure of shoot tissue to different concentrations of imidacloprid, i.e. 1/4X, 1/2X, recommended, 2X and 4X, significantly raised APX levels (20.8, 19.2, 21, 20 and 21.9%, respectively) in a uniform manner. Root tissue samples exhibited an increase in the APX levels by 6.52, 19.72 and 23%, when treated with 4X concentrations of emamectin, cypermethrin and imidacloprid, respectively. Overall, a dose dependent trend of APX levels was observed in shoot tissues while no consistent trend in root tissues was found (Fig. 7).

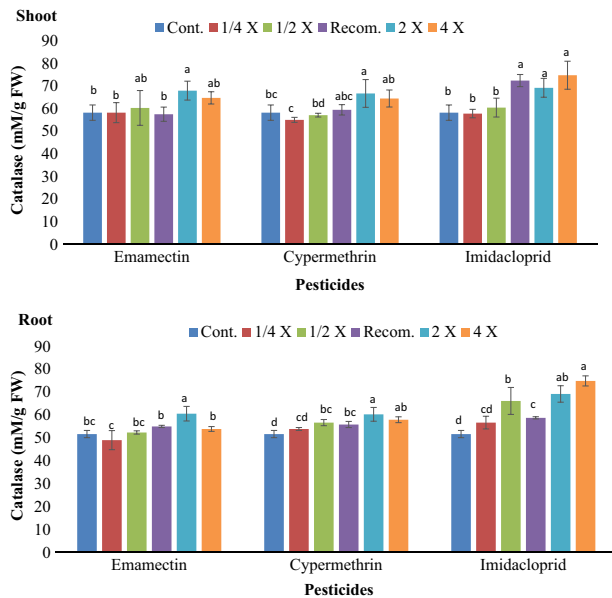


Fig. 6 Effect of the pesticides on shoot and root Catalases level in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Alphabet indicates significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

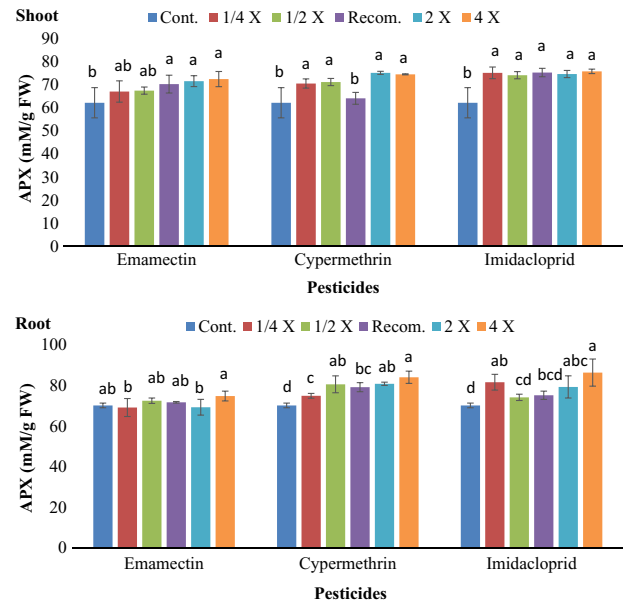


Fig. 7 Effect of the pesticides on shoot and root APX level in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Alphabet indicates significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

Pesticides exposure causes increase in Proline content in root and shoot tissues

Proline is considered as one of the important metabolites and accumulates in plant tissues in response to oxidative stress to protect plant tissues from damage by stress. In the current investigation, among the tested pesticides, imidacloprid significantly increased proline levels in the shoot tissues at all applied concentrations. Cellular proline content exhibited 65.8% increase at 1/4X pesticide concentration followed by steady increase of 132.3% (1/2X), 127.6% (X), 133.3% (2X) and 138.2% (4X) in the imidacloprid exposed shoots (Fig. 8). Similarly, 1/2X concentration of cypermethrin; recommended, 2X and 4X concentrations of both emamectin and cypermethrin significantly ($p \leq 0.05$) elevated the levels of proline in shoot tissue. No regular trend in proline accumulation was observed in root tissues treated with varying pesticide concentrations. Increasing pesticide concentrations significantly increased proline content of root tissue. Comparatively, proline accumulation was greater in roots than in shoots (Fig. 8).

Pesticides overdosing increases membrane permeability and decreases cell viability

Pesticides overdosing impaired membrane permeability as revealed by increase in electrolyte leakage (Fig. 9). The

highest increase was measured in shoot and root tissues treated with imidacloprid in comparison with the rest of the treatments. A marked increase of 55.3, 106.9, 113.9, 147 and 132.1% in root; 10.1, 52.6, 75, 112.4 and 106.9% in shoot tissues were observed at 1/4X, 1/2X, recommended, 2X and 4X concentrations of imidacloprid, respectively (Fig. 9). With the exception of 1/4X in roots, all the concentrations of imidacloprid caused significant increase ($p \leq 0.05$) in the electrolyte leakage values exhibiting similar trends in both tissues. In shoot, all the concentrations of cypermethrin significantly elevated ($p \leq 0.05$) electrolyte leakage indicating increased membrane permeability. In contrast, root tissues treated with cypermethrin showed no considerable differences from the untreated control. Treatment with 2X and 4X concentrations of emamectin resulted in significant increase in shoot electrolyte leakage and membrane peroxidation. On the other hand, recommended and 4X emamectin concentrations significantly enhanced membrane permeability in root tissue ($p \leq 0.05$). Overall analysis indicated that shoot tissues were more affected when exposed to higher doses of pesticides than roots of the tomato plant (Fig. 9).

The effects of pesticides on cell viability in shoot and root is presented in Fig. 10. Plant tissues, subjected to different doses of pesticides, showed a decreasing trend in the cell viability. Least cell viability was recorded in the samples treated with different concentrations of imidacloprid. All the

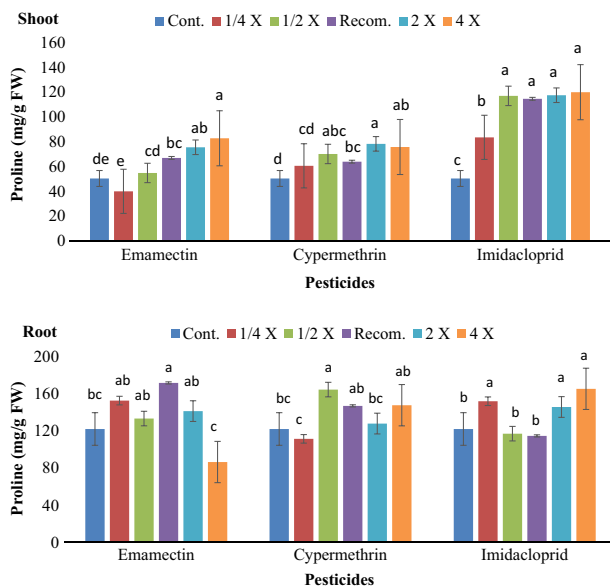


Fig. 8 Effect of the pesticides on shoot and root Proline contents in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Alphabet indicates significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

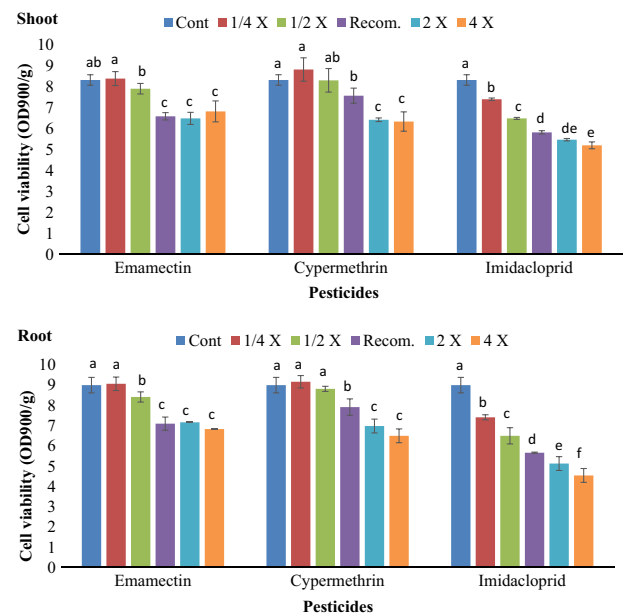


Fig. 10 Effect of the pesticides on shoot and root cell viability in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Alphabet indicates significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

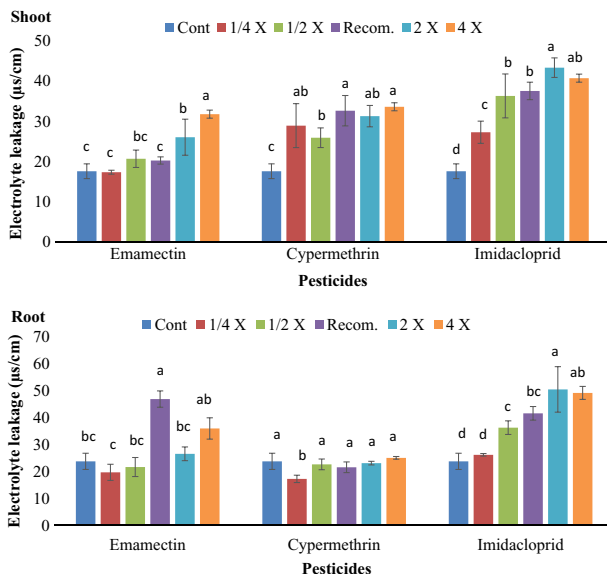


Fig. 9 Effect of the pesticides on shoot and root cell injury in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Alphabet indicates significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

applied concentrations caused a gradual decline in the viability of cells in shoot (−11, −22, −30.1, −34.3 and −37.5% below the control) and root tissues (−17.6, −27.8, −37.1, −43.1 and −49.6% below the control) (Fig. 10).

Similarly, recommended, 2X and 4X doses of cypermethrin and emamectin caused significant decline ($p \leq 0.05$) in cell viability both in shoot and root tissues. Treatment of shoot and root tissues with increasing concentrations of imidacloprid caused significant reduction in cell viability following a dose dependent pattern. Among all the treatments, minimum reduction in cell viability was recorded for samples exposed to 4X imidacloprid concentrations. Collectively, these data suggested that increased pesticide concentrations significantly decreased cell viability indicating potential toxicity of the pesticide overdosing (Fig. 10).

Pesticides causes a reduction in total soluble proteins and sugars

Total soluble protein is an important parameter for determination of phytotoxicity posed by environmental stresses. The evaluation of total soluble protein contents revealed significant toxic effects of pesticides treatments on tomato plant (Fig. 11). It was observed that application of higher doses of pesticides decreased the protein contents significantly ($p \leq 0.05$). Highest reduction in protein contents was recorded both in shoot and root tissues (72.4 and 76.6%, respectively) when plants were exposed to 4X concentration of imidacloprid (Fig. 11). Overall, imidacloprid and cypermethrin were most toxic and significantly decreased protein content of shoot and root tissues. Samples

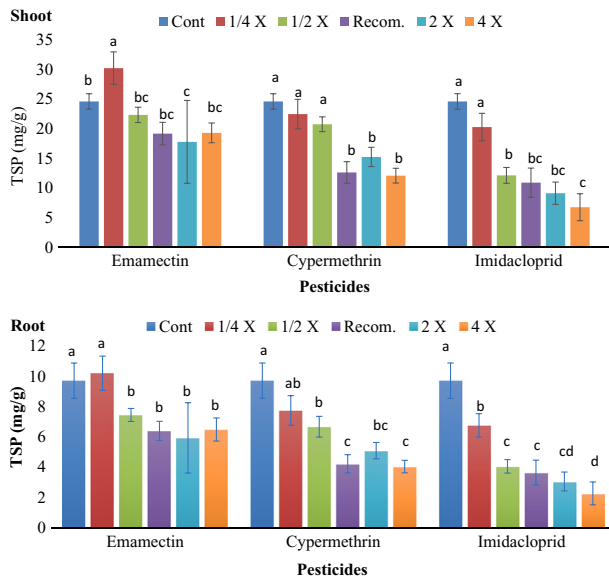


Fig. 11 Effect of the pesticides on shoot and root total soluble protein in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Different alphabets indicate significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

treated with emamectin also exhibited significant reduction in protein content, however, the effects were less severe as compared with plants exposed to higher concentrations of imidacloprid and cypermethrin (Fig. 11).

The level of TSS is an indicator of possible growth of metabolites and even a small decline in the production of TSS indicates stress condition. Present results revealed that application of all pesticides in the doses above the recommended one caused significant reduction ($p \leq 0.05$) in the TSS contents. The decline in TSS contents was more prominent in root samples rather than in shoots. Also, increasing concentrations of imidacloprid resulted in the highest significant decrease in total soluble sugar accumulation relative to cypermethrin and emamectin. The imidacloprid induced decrease in total soluble sugar followed a dose dependent trend (Fig. 12).

Discussion

Plants are affected by environmental stress and pollution in different ways that affect their overall growth. Damages occurs in plants in response to exposure to pollutants can be used as pollutants indicators in the environment as well as inflict the stress by analysing the changes in biomass, growth pattern, morphology, anatomy and physiology (Li et al. 2011; Balestri et al. 2014). Among the various

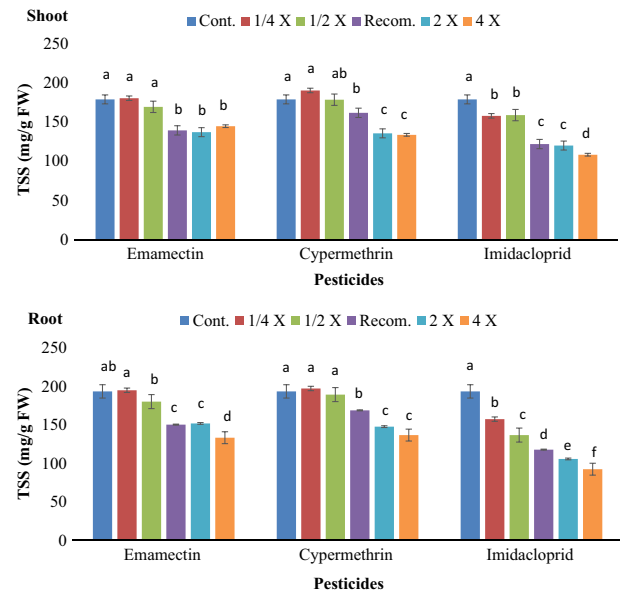


Fig. 12 Effect of the pesticides on shoot and root total soluble sugar level in tomato seedlings. Each bar represents the mean value of three independent replicates and the error bar shows standard deviation. Different alphabets indicate significant difference ($p \leq 0.05$) as revealed by one-way ANOVA with Least Significant Difference (LSD) as a post-hoc test used for each pesticide separately

environmental stresses, pest attack is a major constraint that decreases crops productivity, sometime up to 70% (Folnovic 2015). To protect plants from insect pests, weeds and diseases, use of chemical pesticides is the ultimate option for farmers. Although pesticide manufacturing companies recommend pesticides at a specific dose, pesticides dealers often prescribe over dosing to the farmers (Rehman 1994; Ecobichon 2001; Asogwa and Dongo 2009). These higher doses can probably harm the host crop by posing stress and overwhelmingly compromise plant cellular functions and viability. Plants exposed to abiotic stresses generate reactive oxygen species (ROS) which consequently cause peroxidation of lipid and membrane leakage (Prasad et al. 2005b). Usually oxidative stress causes oxidative damage to different macro-molecules in the cell and is considered as an important biomarker in evaluation of phytotoxicity caused by different stresses (Lushchak 2011).

To protect plants from the deleterious effect of oxidative stress and maintain a steady state level of ROS in the cell, enzymatic antioxidants such as superoxide dismutase (SOD), peroxidase (POD), glutathione reductase (GR), catalase (CAT), ascorbate peroxidase (APX) as well as non-enzymatic antioxidants like proline, ascorbic acid, glutathione, tocopherol and carotenoids are activated to scavenge free radicals and peroxides (Prasad et al. 2005c; Mittler et al. 2004). The role of SOD is to catalyse the superoxide into molecular oxygen and hydrogen peroxide

(H₂O₂) (Scandalios 1993). Similarly, CAT, APX and POD are implicated in the further scavenging of H₂O₂ (Goel et al. 2003). The detoxification of ROS, protection of membrane integrity and stabilization of the enzymes or proteins are assumed to be performed by the production of proline under stress condition (Liang et al. 2013).

In the present study, ROS levels significantly increased in seedlings exposed to different pesticides. The highest increase in H₂O₂ levels was observed in root tissues treated with imidacloprid. A possible explanation for the higher production of ROS by imidacloprid in roots relies on the systemic nature of this pesticide. Moreover, it can also be due to the higher applied dose of this pesticides than emamectin and cypermethrin (the applied doses were based on the recommended doses of each pesticide as described in Materials and methods section). These results are consistent with earlier findings that pesticides induced oxidative stress and lead to enhanced ROS levels in exposed plant tissues. Accumulation of ROS has been quantified in bitter melon leaves when exposed to increased concentrations of dimethoate pesticide (Mishra et al. 2009). Elstner (1990) suggested that under stressful conditions, the elevated level of lipid peroxidation might occur because of rapid generation of ROS, which ultimately affects the membrane lipids and consequently resulting in the formation of malondialdehyde (MDA). Exposure to endosulfan insecticide has resulted in increased ROS level even in some prokaryotic organisms like the cyanobacterium *Plectonema boryanum* (Prasad et al. 2005b).

The increased level of TBARS in the pesticides-treated tomato seedlings indicated high degree of membrane damage upon exposure to pesticide stress. In addition, the measured TBARS contents were higher in root than shoot tissues (Fig. 2). It could be attributed to the higher accumulation of ROS in root tissues (as discussed above) causing membrane damage due to lipid peroxidation and in turn generated TBARS. Ali et al. (2016) observed that application of bisphenol A (BPA) caused more oxidative damage (lipid peroxidation) in root tissues of *Oryza sativa* as revealed by elevated levels of TBARS contents. In a similar study, Parween et al. (2012a) reported that TBARS level increased in plants exposed to high concentrations of chlorpyrifos. Similarly, the application of deltamethrin and other pesticides increased the level of TBARS in both leaves and shoots of soybean plants (Bashir et al. 2007; Song et al. 2007). The elevated levels of lipid peroxidation in the present results indicates that ROS mediated plant damage may be one of the important phytotoxic impacts of pesticides toxicity.

Enzymatic and non-enzymatic antioxidants in plant cells act as signal for the activation of ROS scavenging system (Foyer et al. 1997). Thus, any alteration in antioxidant activities shows oxidative stress as well as stress tolerance

in stress exposed plants (Lee et al. 2001). Among the enzymatic antioxidants, SOD is an essential component of plant's antioxidative defence system and causes the dismutation of free radicals by the formation of H₂O₂. The present results revealed that the activity of SOD was significantly elevated in pesticide-treated seedlings. All the applied concentrations of tested pesticides enhanced the SOD levels in shoot tissues while root tissues showed no or little response to pesticides stress in term of SOD production (Fig. 3). The stimulation or inhibition of SOD activity predicts the extents of phytotoxicity at higher doses of pesticides in the treated seedlings. The little or no rise in the SOD activity of root system in response of pesticide stress speculate the inhibition of this enzyme due to higher oxidative damage in the root. According to Haddad et al. (2009), the application of chlorpyrifos accelerated the SOD activity in plants. Parween et al. (2012a) also reported enhanced level of SOD activity in *Vigna radiata* subjected to different levels of chlorpyrifos. Similarly, stimulation in the SOD activity was reported with the application of the insecticide deltamethrin in *Glycine max* L. (Bashir et al. 2007) and herbicide prometryne in wheat and rice (Jianga et al. 2010; Wu et al. 2010). It was also suggested that the activity of SOD was stimulated by scavenging of O₂⁻ to protect *Vigna radiata* plants from the toxicity of chlorpyrifos (Parween et al. 2012a). According to Nasrabadi et al. (2011), application of high concentration of chlorpyrifos and malathion stimulated the activity of SOD in tomato and brinjal seedlings.

The enzyme peroxidase (POD), an important antioxidant in plants, plays an important role in the growth and development of plants (Breda et al. 1993) and is used as indicator of oxidative stress. POD is involved in the breakdown of H₂O₂ as well as in some other physiological processes in plants (Bowler et al. 1992). Stimulation of the activity of POD is attributed to the resistance to stress and self-defence in the exposed plants. According to Aspinall and Paleg (1981), with the stimulation of POD activity the rate of respiration increases in plants when subjected to abiotic stresses. In the present study, elevated levels of POD were found in plants exposed to pesticides stress. Moreover, POD activity in root tissues was significantly inhibited while an increase was observed in the shoot tissues. A possible explanation for an increase in the POD activity in shoot tissue was probably because of direct exposure of shoot tissue to pesticides foliar application. Likewise, increased level of H₂O₂ in roots (as discussed above) could be due to the low activity of POD in roots as this enzyme is involved in breakdown of H₂O₂, or otherwise high accumulation of H₂O₂ in roots might have inactivated or lowered the activity of this enzyme. The higher response of POD to imidacloprid could be due to the higher dose of this pesticide. Karadge and Karne (1985) also demonstrated that POD

activity was stimulated in tomato leaves, when exposed to different concentrations of fungicides bavistin and calixin.

The activity of GR antioxidants in tomato seedlings was also raised with pesticides application (Fig. 5). It has been demonstrated earlier that GR activity raised in stress conditions as it acts as the precursor for the generation of phytochelatins to protect plants from oxidative damages (Seth et al. 2008; Sobrino-Plata et al. 2014). Activation of GR helps and contributes to the regeneration of ascorbate, which act as antioxidant in plants growing in stressful environments. It has been studied earlier that some pesticides like chlorpyrifos stimulated the activity of GR in in *Vigna radiata* L.

CAT is an important antioxidant enzyme and participates in the main defence system against accumulation and toxicity of ROS in plants by removing toxic H₂O₂ (Singh et al. 2006; Jin et al. 2008). In this study, catalase activities in both roots and shoots of tomato were altered upon exposure to different concentrations of pesticides. Among the tested pesticides, tomato seedlings showed significant response to higher concentrations of imidacloprid in term of catalase activity. This higher response of CAT to imidacloprid compared to the other tested insecticides could be explained by the higher concentrations of imidacloprid used (as compared to other pesticides; for detail see materials and methods) and its systemic mode of action. Oxidative stress caused by pesticides application was found somehow correlated with increasing concentration of pesticides. Like our findings, Parween et al. (2012a) demonstrated that the foliar application of chlorpyrifos insecticide, in *Vigna radiata* L., enhanced CAT activity. Contrary to our results, a gradual decrease in the activity of CAT was noticed in *Glycine max* L. exposed to deltamethrin (Bashir et al. 2007) and in *Vigna radiata* L. subjected to herbicide prometryne (Jianga et al. 2010).

APX is considered as one of the POD but it uses ascorbate as electron donor during the first step of Asc–Glu cycle to eliminate H₂O₂ (Aebi 1984). The level of APX and SOD activities in chloroplasts should be balanced so that the generation of H₂O₂ by SOD is removed by APX (Asada 2006). In this study, a dose-dependent increase was observed in APX levels in both roots and shoots of tomato exposed to different concentrations of pesticides. Shoot tissues were more responsive to different concentrations of pesticides (Fig. 7). Increased activity of APX in shoot resulted in more H₂O₂ detoxification as compared to root tissues. Moreover, shoots were more exposed to pesticide stress because of their foliar application than that of root tissues. Morimura et al. (1996) suggested the protective role of APX in plants exposed to insecticide stress by its pronounced stimulation to detoxify H₂O₂. Same findings were reported for triazole fungicide (Jaleel et al. 2006; Gopi et al. 2007) and prometryne (Jianga et al. 2010) in plants.

Parween et al. (2012a) also reported enhanced APX activity in *Vigna radiata* L., exposed to chlorpyrifos.

Plants produce and accumulate metabolites such as proline when they are exposed to abiotic stresses (Ashraf and Foolad 2007; Kovacik et al. 2009). The major functions of proline in plants are to detoxify ROS, to protect the plant cells membrane integrity, to stabilize enzymes or proteins, to act as osmoprotectant and to increase stress tolerance (Mittler 2002; Boaretto et al. 2014; Cia et al. 2012). The generation of proline acts as a signal molecule, which can be essential for plant recovery after exposure to environmental stress (Szabados and Savouré 2009). In the present investigation, overdosing of pesticides caused an increase in the level of proline, especially in the exposed shoots (Fig. 8). Increased level of proline in plants treated with high concentrations of pesticides could be attributed to cellular dehydration in response to pesticides toxicity as suggested by Yildiztekin et al. (2015). In comparison to root, upper parts of seedlings were more directly exposed to pesticides, which can be a possible explanation for different responses of proline in root and shoot tissues. Zhang et al. (2011) also reported elevated level of proline in wheat plants in response to the pesticide omethoate. Similarly, Bashir et al. (2007) and Wu et al. (2010) observed an increase in the level of proline under deltamethrin stress in *Glycine max* L. and prometryne herbicide stress in rice, respectively. The accumulation of proline was regarded as a stress indicator in *Vigna radiata* L. exposed to chlorpyrifos (Parween et al. 2012a). The increased level of proline in plants under abiotic stresses reveals its protective role in plants (Gratao et al. 2012; Mishra et al. 2014; Ahmad et al. 2015).

Tomato seedlings exposed to pesticides showed high values of electrolyte leakage expressing that higher doses of pesticides increased cell injuries. Shoot tissues were more affected than root while imidacloprid application caused more severe electrolyte leakage (cell injury) than the other tested pesticides. These findings support our findings of various oxidative stress markers discussed above where shoots were shown to be more responsive than roots and imidacloprid caused more severe effects. Earlier studies also suggested an increase in the levels of cell injury in term of electrolyte leakage as well as decrease in the values of cell viability in plants upon exposure to abiotic stresses (Daud et al. 2016).

The contents of protein in tomato seedlings subjected to pesticide stress showed a remarkable decrease. Among the tested pesticides, imidacloprid and cypermethrin caused more detrimental effects on proteins. The decreased level of total soluble protein in response to pesticide toxicity could be explained by the fact that toxicant produced due pesticide application retarded the synthesis of protein by inducing alteration in the activity of cytochrome oxidase, blockage of alternative respiratory pathways and by the

formation of succinate (Berger and Cwick 1990; Siddiqui and Ahmed 2006). It has been investigated that total protein contents in plants are affected by several stressors including xenobiotics (Bayramov et al. 2010; Djebar et al. 2014). Parween et al. (2011) demonstrated that foliar application of chlorpyrifos insecticide on *Vigna radiata* L. decreased the contents of protein.

Above the recommended doses, pesticides application caused severe reduction in the sugar contents, especially in the root tissues. Since root tissues act as a storage sink for accumulation of total soluble sugar produced by photosynthesis, retardation in this process results in lower level of TSS. In response to stress, phenolic compounds are produced in plant that can retard the overall growth through decline in the biosynthetic pathway of chlorophyll formation or by stimulation of degradation pathway that lead to reduced chlorophyll formation and ultimately cause retardation in the photosynthesis and sugar formation as described by Yang et al. (2002). Findings of our study corroborate the findings of Parween et al. (2011) who demonstrated that application of chlorpyrifos at higher concentrations decreased the contents of soluble sugar in the seedlings of *Vigna radiata* L.

Conclusion

It is concluded that overdoses of pesticides induced phytotoxicity in tomato seedlings by causing oxidative stress. The application of pesticides caused increased production of ROS which in turn caused increased lipid peroxidation (as revealed by TBARS content) and electrolyte leakage and hence impairing cell viability. Pesticides at higher concentration caused a reduction in protein and sugar contents of tomato seedlings. To reduce the detrimental impacts of pesticide-induced oxidative stress, the activities of different antioxidants (SOD, POD, GR, CAT, APX and proline) were observed to increase in tomato seedlings. Among the tested pesticides, imidacloprid caused maximum oxidative stress as revealed by different oxidative stress markers used in this study. Shoot tissues were more responsive than root in term of antioxidant defence against pesticides-induced oxidative stress. The present results are important for understanding the response mechanism of plants to pesticides stress. Further studies at molecular level are recommended for further in depth understanding of pesticides-induced phytotoxicity in crops like tomato. Furthermore, studies are needed to evaluate the oxidative stress-inducing mechanisms of these pesticides in tomato so that their adverse effects in host crops can be minimized in future.

Acknowledgements We acknowledge all the staff at Department of Botany for their help and support during this study.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study did not involve any animal or human samples and did not need any ethical approval for animal or human samples use.

Informed consent This study did not involve any human or human samples.

References

- Aebi H (1984) Catalase in vitro. *Method Enzymol* 105:121–126
- Ahmad A, Hadi F, Ali N (2015) Effective Phytoextraction of cadmium (Cd) with increasing concentration of total phenolics and free proline in *Cannabis sativa* (L) plant under various treatments of fertilizers, plant growth regulators and sodium salt. *Int J Phytorem* 17:56–65
- Akhtar MW, Sengupta D, Chowdhury A (2009) Impact of pesticides use in agriculture: their benefits and hazards. *Int Toxicol* 2:1–12
- Aksoy O, Deveci A, Kizilirmak S, Akdeniz GB (2013) Phytotoxic effect of quizalofop-P-ethyl on soybean (*Glycine max* L.). *J Biol Environ Sci* 7:49–55
- Ali I, Liu B, Farooq MA, Islam F, Azizullah A, Yu C, Su W, Gan Y (2016) Toxicological effects of bisphenol A on growth and antioxidant defense system in *Oryza sativa* as revealed by ultra-structure analysis. *Ecotoxicol Environ Saf* 124:277–84
- Asada K (2006) Production and scavenging of reactive oxygen species in chloroplast and their functions. *Plant Physiol* 141:391–396
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59:206–216
- Asogwa EU, Dongo LN (2009) Problems associated with pesticide usage and application in Nigerian cocoa production: a review. *Afr J Agri Res* 4:675–683
- Aspinall D, Paleg LG (1981) Proline accumulation: physiological aspects. Paleg LG, Aspinall D *The biochemistry and physiology of drought resistance in plants*. Academic Press, New York, NY, 205–207
- Baig SA, Akhter NA, Ashfaq M, Asi MR, Ashfaq U (2012) Imidacloprid residues in vegetables, soil and water in the southern Punjab, Pakistan. *J Agri Tech* 8:903–916
- Balestri M, Barresi M, Campera M, Serra V, Ramanamanjato JB, Heistermann M (2014) Habitat degradation and seasonality affect physiological stress levels of eulemur collaris in littoral forest fragments. *PLoS One* 9(9):e107698. <https://doi.org/10.1371/journal.pone.0107698>
- Banerjee BD, Seth V, Ahmed RS (2001) Pesticide-induced oxidative stress perspectives and trends. *Rev Environ Health* 16:1–40
- Bashir F, Siddiqi TO, Mahmooduzzafar IqbalM (2007) The anti-oxidative response system in *Glycine max* (L.) Merr. Exposed to deltamethrin, a synthetic pyrethroid insecticide *Environ Pollut* 147:94–100
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water stress studies. *Plant Soil* 39:205–207
- Bayramov SM, Babayev HG, Khaligzade MN, Guliyev NM, Raines CA (2010) Effect of water stress on protein content of some calvin cycle enzymes in different wheat genotypes. *Proc ANAS (Biol Sci)* 65:106–111
- Berger S, Cwick K (1990) Selected aspect of adverse nutritional effect of pesticides. *Ernahrung* 14:411–415

- Boaretto LF, Carvalho G, Borgo L, Creste S, Landell MGA, Mazzafera P, Azevedo RA (2014) Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. *Plant Physiol Biochem* 74:165–175
- Bolwell GP, Wojtaszek P (1997) Mechanisms for the generation of reactive oxygen species in plant defence – a broad perspective. *Physiol Mol Plant Pathol* 51:347–366
- Bowler C, Van Montague M, Inzé D (1992) Superoxide dismutase and stress tolerance. *Annu Rev Plant Physiol Plant Mol Biol* 43:83–116
- Bradford MM (1976) A rapid and sensitive method for the quantization of microgram quantities of protein utilizing the principle of protein dye binding. *Anal Biochem* 72:248–259
- Breda C, Buffard D, Van Huystee RB, Esnault R (1993) Differential expression of two peanut peroxidase cDNA clones in peanut plants and cells in suspension culture in response to stress. *Plant Cell Rep* 12(b):268–272
- Cakmak I, Marschner H (1992) Magnesium deficiency and high light intensity enhance activities of superoxide dismutase, ascorbate peroxidase, and glutathione reductase in bean leaves. *Plant Physiol* 98:1222–1227
- Cali IO, Candan F (2009) The effect of fungicide application on pollen structure in tomato (*Lycopersicon esculentum* Mill.). *Plant J Appl Biol Sci* 3:37–40
- Cia MC, Guimarães ACR, Medici LO, Chabregas SM, Azevedo RA (2012) Antioxidant responses to water deficit by drought-tolerant and -sensitive sugarcane varieties. *Ann Appl Biol* 161:313–324
- Cao S, Xu Q, Cao Y, Qian K, An K, Zhu Y, Binzeng H, Zhao H, Kuai B (2005) Loss-of-function mutations in DET2 gene lead to an enhanced resistance to oxidative stress in Arabidopsis. *Physiol Plant* 123:57–66
- Cardone A (2015) Imidacloprid induces morphological and molecular damages on testis of lizard (*Podarcis sicula*). *Ecotoxicology* 24:94–105
- Carvalho FP (2006) Agriculture, pesticides, food security and food safety. *Environ Sci Policy* 9:68–692
- Chohan TZ, Ahmad S (2008) An assessment of tomato production practices in Danna Katchely, Azad Jammu Kashmir. *Pak J Life Soc Sci* 6:96–102
- Daud MK, Hassan S, Azizullah A, Jamil M, Rehan N, Irum R, Qaiser MK, Zhu (2016) Physiological, biochemical, and genotoxic effects of wastewater on maize seedlings. *Pol J Environ Stud* 25:563–571
- D'Autréaux B, Toledano MB (2007) ROS as signaling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 8:813–824
- De A, Bose R, Kumar A, Mozumdar S (2014) Targeted delivery of pesticides using biodegradable polymeric nanoparticles. *Pege XXIII*, 99. <https://doi.org/10.1007/978-81-322-1689->
- DeLorenzo ME, Scott GI, Ross PE (2001) Toxicity of pesticides to aquatic microorganisms: a review. *Environ Toxicol Chem* 20:84–98
- Djebar BN, Reda M, Noureddine Z, Houria B (2014) Differential response to treatment with herbicide chevalier induced oxidative stress in leaves of wheat. *Ann Biol Res* 5:1–7
- Dobsikova R (2003) Acute toxicity of carbofuran to selected species of aquatic and terrestrial organisms. *Plant Prot Sci* 39:103–108
- Ecobichon DJ (2001) Pesticide use in developing countries. *Toxicology* 160:27–33
- Edwards CA (1986) Agrochemicals as environmental pollutants. In: Van Hofsten B, Eckstrom G (eds) *Control of pesticide applications and residues in food. A guide and directory*. Swedish Science Press, Uppsala
- Elstner EF (1990) *Der Sauerstoff- Biochemie Biologie Medizin*. BI-Wissenschaftsverlag, Mannheim
- FAO (2007) Food and Agricultural Organization Stat, core production 2005. <http://faostat.fao.org/site/340/default.aspx>. Accessed 4 June 2017
- Fidalgo F, Freitas R, Ferreira R, Pessoa A, Teixeira J (2011) *Solanum nigrum* L: antioxidant defense system isoenzymes are regulated transcriptionally and post translationally in Cd-induced stress. *Environ Exp Bot* 72:312–319
- Fidalgo F, Azenha M, Silva FA, de Sousa A, Santiago A, Ferraz P, Teixeira J (2013) Copper-induced stress in *Solanum nigrum* L. and antioxidant defense system responses. *Food Energy Secur* 2:70–80
- Folnovic T (2015) Yield losses due to pests. <http://blog.agrivi.com/post/yield-losses-due-to-pests>. Accessed 29 June 2017
- 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
- Foyer CH, Noctor G (2005) Oxidant and antioxidant signaling in plants: a re-evaluation of the concept of oxidative stress in a physiological context. *Plant Cell Environ* 28:1056–1071
- Gajewska E, Skłodowska M (2008) Differential biochemical responses of wheat shoots and roots to nickel stress: antioxidative reactions and proline accumulation. *Plant Growth Regul* 54:179–188
- Giannopolitis CN, Ries SK (1977) “Superoxide dismutases: I. Occurrence in higher plants”. *Plant Physiol* 59:309
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress 519 tolerance in crop plants. *Plant Physiol Biochem* 48(12):909–930
- Glover-Amengor M, Tetteh FM (2008) Effect of pesticide application rate on yield of vegetables and soil microbial communities. *W Afr J Appl Ecol* 12:1–7
- Goel A, Goel A, Sheoran IS (2003) Changes in oxidative enzymes during artificial aging in cotton (*Gossypium hirsutum* L.) seeds. *J Plant Physiol* 160:1092–1100
- Gomes-Junior RA, Moldes CA, Delite FS, Gratão PL, Mazzafera P, Lea PJ, Azevedo RA (2006) Nickel elicits a fast antioxidant response in *Coffea arabica* cells. *Plant Physiol Biochem* 44:420–429
- Gopi R, Jaleel CA, Sairam R, Lakshmanan GMA, Gomithinayagam M, Pannersevelm R (2007) Differential effects of hexaconazole and paclobutrazol on biomass, electrolyte leakage, lipid peroxidation and antioxidant potential of *Daucus carota* L. *Colloids Surf B* 60:180–186
- Gratão P, Monteiro C, Antunes A, Peres L, Azevedo R (2008) Acquired tolerance of tomato (*Lycopersicon esculentum* cv. Micro-Tom) plants to cadmium-induced stress. *Ann Appl Biol* 153:321–333
- Gratão PL, Monteiro CC, Carvalho RF, Tezotto T, Piotto FA, Peres LEP, Azevedo RA (2012) Biochemical dissection of diageotropica and never ripe tomato mutants to Cd stressful conditions. *Plant Physiol Biochem* 56:79–96
- Haddad R, Morris K, Buchanan-Wollaston V (2009) Molecular characterization of free radicals decomposing genes on plant developmental stages. *Int J Biol Biomol Agric Food Biotechnol Eng* 3:77–83
- Haq QU, Ali T, Ahmad M, Nosheen F (2008) An analysis of pesticide usage by cotton growers: a case study of district Multan, Punjab-Pakistan. *Pak J Agric Sci* 45:133–137
- Hayat S, Hayat Q, Alyemini MN, Ahmad A (2012) Salicylic acid enhances the efficiency of nitrogen fixation and assimilation in *Cicer arietinum* plants grown under cadmium stress. *J Plant Interact* 9:35–42
- Hodges DM, DeLong JM, Fomey CF, Prange RK (1999) Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta* 207:604–611

- Idrovo A (2000) Surveillance of pesticide poisonings in Colombia. *Public Health* 2:36–46
- Jaleel CA, Gopi R, Alagu Lal Shmanan GM, Panneerselvam R (2006) TDM induced changes in the antioxidant metabolism and ajmalicine production in *Catharanthus roseus* L. *G Don Plant Sci* 7:271–276
- Jianga L, Maa L, Suia Y, Hna SQ, Wua ZY, Fenga YX, Yanga H (2010) Effect of manure compost on the herbicide prometryne bioavailability to wheat plants. *J Hazard Mater* 184:337–344
- Jin XF, Yang XE, Islam E, Liu D, Mahmood Q, Li H, Li J (2008) Ultrastructural changes, zinc hyperaccumulation and its relation with antioxidants in two ecotypes of *Sedum alfredii* Hance. *Plant Physiol Biochem* 46:997–1006
- Karadge BA, Karne AV (1985) Influence of systemic fungicides. Bavistin and calixin on *Lycopersicon esculentum* Mill. leaves. *Biovigyanam* 11:166–168
- Khan A, Ahmad L, Khan MZ (2012) Hemato-biochemical changes induced by pyrethroid insecticides in avian, fish and mammalian species. *Int J Agric Biol* 14:834–842
- Khan AF (2014) Hazards of pesticide spray on vegetables. *The Daily Dawn Pakistan*, Internet Edition, June 12, 2014
- Khokhar KM (2013) Present status and prospects of tomatoes in Pakistan. <http://www.agricorner.com/present-status-and-prospects-of-tomatoes-in-pakistan/>. Accessed 19 July 2017
- Khooharo AA, Memon RA, Mallah MU (2008) An empirical analysis of pesticide marketing in Pakistan. *Pak Ecol Soc Rev* 46:57–74
- Kilic S, Duran RE, Coskun Y (2015) Morphological and physiological responses of maize (*Zea mays* L.) seeds grown under increasing concentrations of chlorantraniliprole insecticide. *Pol J Environ Stud* 24:1069–1075
- Kovacik J, Klejduš B, Hedbavny J, Backor M (2009) Salicylic acid alleviates NaCl-induced changes in the metabolism of *Matricaria chamomilla* plants. *Ecotoxicology* 18:544–554
- Lee-Fook-Choy LH, Seeneevassen S (1998) Monitoring insecticide residues in vegetables and fruits at the market level. AMAS, Food and Agricultural Research Council, Reduit, Mauritius, p 95
- Lee DH, Kim YS, Lee CB (2001) The individual response of the antioxidant enzymes by salt stress in rice (*Oryza sativa* L.). *J Plant Physiol* 158:737–745
- Li FL, Bao WK, Wu N (2011) Morphological, anatomical and physiological responses of *Campylotropis polyantha* (Franch.) Schindl. seedlings to progressive water stress. *Sci Hortic-Amst* 127:436–443
- Liang X, Zhang L, Natarajan SK, Becker DF (2013) Proline mechanisms of stress survival. *Antioxid Redox Signal* 19:998–1011
- Lu S, Yuejing Z, Niu Y, Bingru Z (2008) Antioxidant responses of radiation-induced dwarf mutants of bermuda grass to drought stress. *J Am Soc Hortic Sci* 133:360–366
- Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. *Aquat Toxicol* 101:13–30
- Martínez-Domínguez G, Nieto-García AJ, Romero-Gonzalez R, Frenich AG (2015) Application of QuEChERS based method for the determination of pesticides in nutraceutical products (*Camellia sinensis*) by liquid chromatography coupled to triple quadrupole tandem mass spectrometry. *Food Chem* 177:182–190
- Mirza I (2007). Tomato paste plant to be set up at Killa Saifullah. <http://www.pakissan.com/english/news/newsDetail.php?newsid=15041> Accessed 31 Aug 2007
- Mishra V, Srivastava G, Prasad SM (2009) Antioxidant response of bitter melon (*Momordica charantia* L.) seedlings to interactive effect of dimethoate and UV-B irradiation. *Sci Hortic* 120:373–378
- Mishra B, Sangwan RS, Mishra S, Jadaun JS, Sabir F, Sangwan NS (2014) Effect of cadmium stress on inductive enzymatic and nonenzymatic responses of ROS and sugar metabolism in multiple shoot cultures of Ashwagandha (*Withania somnifera* Dunal). *Protoplasma* 251:1031–1045
- Mitra A, Chatterjee C, Mandal FB (2011) Synthetic chemical pesticides and their effects on birds. *Res J Environ Toxicol* 5:81–96
- Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. *Trend Plant Sci* 7:405–410
- Mittler R, Vanderauwera S, Gollery M, Van Breusegem F (2004) Reactive oxygen gene network of plants. *Trends Plant Sci* 9:490–498
- Morimura T, Ohya T, Ikawa T (1996) Presence of ascorbate peroxidizing enzymes in roots of *Brassica campestris* L. cv Komatsuna. *Plant Sci* 117:55–63
- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol* 22:867–880
- Nasrabadi M, Ghayal N, Dhumal KN (2011) Effect of chlorpyrifos and malathion on antioxidant enzymes in tomato and brinjal. *Int J Pharma Biol Sci* 2:202–209
- Parween T, Jan S, Mahmooduzzafar FatmaT (2011) Alteration in nitrogen metabolism and plant growth during different developmental stages of green gram (*Vigna radiata* L.) in response to chlorpyrifos. *Acta Physiol Plant* 33:2321–2328
- Parween T, Jan S, Mahmooduzzafar FatmaT (2012a) Evaluation of oxidative stress in *Vigna radiata* L. in response to chlorpyrifos. *Int J Environ Sci Technol* 9:605–612
- Prasad SM, Dwivedi R, Zeeshan M (2005a) Growth, photosynthetic electron transport, and antioxidant responses of young soybean seedlings to simultaneous exposure of nickel and UV-B stress. *Photosynthetica* 43:177–185
- Prasad SM, Kumar D, Zeeshan M (2005b) Growth, photosynthesis, active oxygen species and antioxidants responses of paddy field cyanobacterium *Plectonema boryanum* to endosulfan stress. *J Gen Appl Microbiol* 51:115–123
- Prasad SM, Srivastava G, Mishra V, Dwivedi R, Zeeshan M (2005c) Active oxygen species generation, oxidative damage and antioxidant defense system in *Pisum sativum* exposed to UV-B irradiation. *Physiol Mol Biol Plants* 11:303–311
- Putter J (1974) In: Bergmeyer (ed) *Method of enzymatic method analysis*, vol. 2. Academic Press, New York, NY
- Qadir S, Qureshi MI, Javed S, Abidin MZ (2004) Genotypic variation in phytoremediation potential of *Brassica juncea* cultivars exposed to Cd stress. *Plant Sci* 167:1171–1181
- Rachid R, Djebar-Berrebah H, Djebar MR (2008) Growth, chitin and respiratory metabolism of *Tetrahymena pyriformis* exposed to the insecticide Novaluron. *Am-Eurasia J Agric Environ Sci* 3:873–881
- Rehman K (1994) Pesticides causing environmental pollution. *Habib N A workshop on people and pesticides*. Khoj-Research and Publication Center, Lahore, Pakistan, 39–40
- Rio AD, Bamberg J, Centeno-Diaz R, Salas A, Roca W, Tay D (2012) Effects of the pesticide furadan on traits associated with reproduction in wild potato species. *Am J Plant Sci* 3:1608–1612
- Scandalios JG (1993) Oxygen stress and superoxides dismutase. *Plant Physiol* 101:7–12
- Seth CS, Chaturvedi PK, Misra V (2008) The role of phytochelatin and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L. *Ecotoxicol Environ Saf* 71:76–85
- Shakir SK, Kanwal M, Murad W, Zia ur Rehman, Shafiq ur Rehman, Daud MK, Azizullah A (2016) Effect of some commonly used pesticides on seed germination, biomass production and photosynthetic pigments in tomato (*Lycopersicon esculentum*). *Ecotoxicology* 25:329–41
- Shakoor A, Sabri MA, Afzal M, Bashir MH (2010) Role of plant morphological characters towards resistance of some cultivars of tomato against phytophagous Mites (Acari) under greenhouse conditions. *Pak J Life Soc Sci* 8:131–136

- Sheikh SA, Nizamani SM, Panhwar AA, Mirani BN (2013) Monitoring of pesticide residues in vegetables collected from markets of Sindh, Pakistan. *Food Sci Technol Lett* 4:41–45
- Shields R, Burnett W (1960) Determination of protein-bound carbohydrate in serum by a modified anthrone method. *Anal Chem* 32:885–886
- Siddiqui ZS, Ahmed S (2006) Combined effects of pesticide on growth and nutritive composition of soybean plants. *Pak J Bot* 38:721–733
- Singh S, Eapen S, D'Souza SF (2006) Cadmium accumulation and its influence on lipid peroxidation and antioxidative system in an aquatic plant *Bacopa monnieri* L. *Chemosphere* 62:233–246
- Sobrino-Plata J, Meyssen D, Cuypers A, Escobar C, Hernandez LE (2014) Glutathione is a key antioxidant metabolite to cope with mercury and cadmium stress. *Plant Soil* 377:369–381
- 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
- Spier JD, Davies FT, Che JR, Heinz KM, Bogran CE, Starman TW (2008) Do insecticides affect plant growth and development? *Greenhouse Grower*. <http://aggie-horticulture.tamu.edu/faculty/davies/research/abstracts/pdfs>. Accessed 10 Feb 2017
- Srivastava S, Tripathi RD, Dwivedi UN (2004) Synthesis of phytochelatins and modulation of antioxidants in response to cadmium stress in *Cuscuta reflexa*—an angiospermic parasite. *J Plant Physiol* 161:665–674. <https://doi.org/10.1078/0176-1617-01274>
- Szabados L, Savouré A (2009) Proline: a multifunctional amino acid. *Trends Plant Sci* 15:89–97
- Tabashnik BE (1989) Managing resistance with multiple pesticide tactics: theory, evidence, and recommendations. *J Econ Entomol* 82:1263–1269
- Theymoli B, Sadasivam S (1987) *Biochemical methods* 2nd edition. *Plants Foods Hum Nutr* 37:41–46
- Tort N, Oztork I, Guvensen A (2005) Effects of some fungicides on pollen morphology and anatomy of tomato (*Lycopersicon esculentum* mill.). *Pak J Bot* 37:23–30
- Wang ST, He XJ, An RD (2010) Responses of growth and antioxidant metabolism to nickel toxicity in *Luffa cylindrica* seedlings. *J Anim Plant Sci* 7:810–821
- Willekens H et al. (1997) Catalase is a sink for H₂O₂ and is indispensable for stress defense in C3 plants. *EMBO J* 16:4806–4816
- Wu GL, Cui EJ, Tao EL, Yang EH (2010) Fluroxypyr triggers oxidative damage by producing superoxide and hydrogen peroxide in rice (*Oryza sativa*). *Ecotoxicol* 19:24–132
- Xia XJ, Huang YY, Wang L, Huang LF, Yu YL, Zhou YH, Yu JK (2006) Pesticides-induced depression of photosynthesis was alleviated by 24-epibrassinolide pretreatment in *Cucumis sativus* L. *Pestic Biochem Physiol* 86:42–48
- Yang CM, Lee CN, Chou CH (2002) Effects of three allelopathic phenolics on chlorophyll accumulation of rice (*Oryza sativa*) seedlings: I. Inhibition of supply-orientation. *Bot Bull Acad Sin* 43:299–304
- Yardim EN, Edwards CA (2003) Effects of organic and synthetic fertilizer sources on pest and predatory insects associated with tomatoes. *Phytoparasitica* 4:324–329
- Yildiztekin M, Kaya C, Tuna AL, Ashraf M (2015) Oxidative stress and antioxidative mechanisms in tomato (*Solanum lycopersicum* L.) plants sprayed with different pesticides. *Pak J Bot* 47:717–721
- Zhang WJ, Jiang FB, Oul JF (2011) Global pesticide consumption and pollution: with China as a focus. *Proc Int Acad Ecol Environ Sci* 1:125