

OXIDATIVE STRESS AND ANTIOXIDATIVE MECHANISMS IN TOMATO (*SOLANUM LYCOPERSICUM* L.) PLANTS SPRAYED WITH DIFFERENT PESTICIDES

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Abstract

A glasshouse experiment was conducted to appraise the influence of exogenously applied pesticides such as abamectin, thiamethoxam, pyriproxyfen and acetamiprid on oxidative defence system and some key physiological attributes in tomato (*Solanum lycopersicum* L.). Each of these pesticides was applied in three doses (recommended dose, twice and three times higher than the recommended dose). Higher doses of pesticides sprayed to the plants resulted in marked increase in leaf free proline content and electrolyte leakage, but in a decrease in shoot dry matter, *chl a*, *chl b* and *chl a+b* in tomato plants as compared to those plants not sprayed with pesticides. These reductions were greater in tomato plants sprayed with highest doses of thiamethoxam (144 mg L⁻¹), whereas the reverse was true for proline content and electrolyte leakage. The foliar application of pesticides at the highest levels caused enhanced accumulation of malondialdehyde (MDA) in most cases, and these being greater in treatment of foliar application of thiamethoxam at the highest level. The highest doses of pesticides promoted the activities of catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) in most cases. The results clearly indicate that application of pesticides at higher doses than recommended doses provoked both oxidative and antioxidative systems in tomato plants.

Keywords: Pesticides, Tomato, Oxidative stress, Enzyme activities, Membrane permeability.

Introduction

There are two major groups of stress factors such as natural and anthropogenic, the latter include pesticides, air pollutants and heavy metals (Lichtenthaler, 1996). Undoubtedly, pesticides have a crucial role in pest management and control of plant diseases so as to attain maximum crop yield. However, its excessive use particularly in terms of their application at high doses may increase especially in developing countries. So, it is important to optimize their use for safeguarding the consumers and producers as well as reducing environmental hazards (Dhaliwal & Singh, 2000; Parween *et al.*, 2011).

Pesticide use is very common in vegetable production in glasshouse conditions to protect pest-induced damage. However, the use of pesticide at high rates may cause toxicity problem, which can deleteriously affect plant growth and development. Foliar applications of pesticides may negatively affect leaf photosynthesis by partially blocking stomatal pores which allow exchange of gases. Therefore, reduction in photosynthetic activity can delay time of fruit harvesting or affect adversely fruit quality, as well as visible damage due to chemical toxicity (Xia *et al.*, 2006). The pesticides used in the present experiment such as thiamethoxam and acetamiprid belong to the neonicotinoid insecticide group. They were introduced worldwide about a decade ago as they have lower toxicity and high activity against pests and insects (Kuhara *et al.*, 2002; Fitzgerald, 2004). Abamectin is a macrocyclic lactone and is a major fermentation

component of avermectins, natural products produced from the soil bacterium *Streptomyces avermitilis*. It shows insecticidal, acaricidal and nematocidal activities (Putter *et al.*, 1981). Pyriproxyfen is known as an insect growth regulator (IGR). It is an analogue of a juvenile hormone (JHA). It is being widely used as an insecticide for the control of different pests (Liu, 2003; Liu & Stansly, 2004).

Plants have a well-developed anti-oxidative machinery to prevent cellular membranes from toxic effects caused by reactive oxygen species (Ren *et al.*, 1999; Zaefyzadeh *et al.*, 2009). It is reported that reactive-oxygen species (ROS) are responsible for various stress-induced damages to cellular structures. It is widely accepted that chemical toxicity results in oxidative stress due to the production of ROS (Banerjee *et al.*, 1999; Nicholas *et al.*, 2001; Chen *et al.*, 2010; Faize *et al.*, 2011). Under stress conditions plants may alter the activities of ROS scavenging enzymes, such as superoxide dismutase (SOD; EC1.15.1.1) and catalase (CAT; EC1.11.1.6) (Tuluze & Celik, 2006). To date, there seems to be no knowledge available in the literature on functioning of antioxidative defense machinery to pesticides, such as abamectin, thiamethoxam, pyriproxyfen and acetamiprid. Thus, we hypothesized that application of different pesticides at high doses could cause oxidative stress and hence regulate antioxidative defense system in tomato plants. Therefore, in the present study we examined the effects of pesticides sprayed at higher doses on key plant growth attributes, production of ROS and activities of antioxidant enzymes in the leaves of tomato plants.

Materials and Methods

Plant culture and treatments: A glasshouse experiment was conducted from the beginning of February to the middle of March, 2013 with tomato (*Lycopersicon esculentum* Mill.) cv. 'Hazera 5656 F1'. Before use in experimentation, all seed samples were sterilized using 1% v/v solution of sodium hypochlorite. Seeds at a rate of three were sown into each pot filled with 8 kg of a medium containing perlite and peat in 1:1 ratio. After germination, the seedlings were thinned to maintain one per plastic pot. The plants were subjected natural atmospheric conditions. A heater used to control temperature with day temperature (20-30°C) and night time temperature greater than 10°C. To minimize evaporation, the pots were placed under a black plastic sheet. Nutrient solution contained the following nutrients: P (31 mg L⁻¹), N (270 mg L⁻¹; NO₃ form), Ca (200 mg L⁻¹), K (234 mg L⁻¹), Mg (48 mg L⁻¹), S (64 mg L⁻¹), Mn (0.5 mg L⁻¹), Fe (2.8 mg L⁻¹), B (0.5 mg L⁻¹), Zn (0.05 mg L⁻¹), Cu (0.02 mg L⁻¹), and Mo (0.01 mg L⁻¹). The pH of the nutrient growth medium was adjusted to 5.5 with potassium hydroxide (0.01 M).

The experiment was laid out in a randomized complete block design with three repeats and each repeat consisted of 5 plants, i.e., overall 15 plants per treatment. The quantity of water added to each pot was within the range 200 mL to 750 mL depending plant size/volume. Four different pesticides were sprayed at three different doses (recommended dose (RD), two-fold and four-fold higher than RD) to the leaves of tomato plants three weeks after germination and plants were grown for further two weeks to appraise the effects of treatments. The pesticides used in the experiment were abamectin (4.5, 9.0 or 13.5 mg L⁻¹), thiamethoxam (36, 72 or 144 mg L⁻¹), pyriproxyfen (50, 100 or 200 mg L⁻¹), and acetamiprid (60, 120 or 240 mg L⁻¹) as Agrimec, Actara, Admiral and Mospilan, respectively, which are common trade names. The pesticide treatment solutions contained the surfactant 0.01% Tween-20 (C₅₈H₁₁₄O₂₆). Control plants were sprayed with same amount of distilled water and 0.01% Tween-20 (C₅₈H₁₁₄O₂₆). Fifty ml of treatment solution were sprayed to each pot.

At the end of the experiment, data for dry weights, chlorophyll content, electrolyte leakage, activities of antioxidant enzymes, and malondialdehyde (MDA) levels were recorded.

Chlorophyll determination: Chlorophyll content was extracted from fully expanded youngest leaves using 90% acetone solution and after recording the absorbance of the supernatant at appropriate wavelengths the chlorophyll concentrations calculated following Strain & Svec (1966).

Leaf free proline content: Five hundred mg of fresh leaf sample was grinded pieces with sulfosalicylic acid of 3% and filtered. Two ml were taken from filtered leaf sample into which 2 ml acetic acid and 2 ml ninhydrin reagent were added. Ninhydrin reagent was

prepared using ninhydrin, acetic acid and orthophosphoric acid. The samples that were placed in tubes were kept in a water bath (100°C) for one hour and then in ice. Four ml toluene were added over cooling samples and read in 520 nm by spectrophotometer. Afterwards, calculation was done comparing with proline standards Bates *et al.*, 1973).

Electrolyte leakage: The protocol described by Dionisio-Sese & Tobita (1998) was employed to assess electrolyte leakage. Pre-weighed (0.2 g) fresh leaf material in very small pieces placed in 10 ml ddH₂O water was incubated in a water bath for 2 h at 25°C and the electrical conductivity (EC₁) measured with an EC meter. For getting released electrolytes, all samples were then subjected to 121°C in an autoclave for 20 min. The value of EC₂ was determined after cooling the mixture when the temperature was 25°C.

Antioxidant enzymes and malondialdehyde : For determining the activities of key antioxidative enzymes, fresh leaf material (500 mg) was ground well Na-P buffer (50 mM) containing 1% soluble polyvinyl pyrrolidone. Superoxide dismutase (SOD) was determined following Beauchamp & Fridovich (1971), catalase following Kraus and Fletcher (1994), and POD according to Chance and Maehly (1955) method. The Bradford (1976) protocol was employed for estimating total soluble proteins. Leaf malondialdehyde (MDA) was analyzed following Cakmak & Horst (1991) with some modifications as suggested by Weisany *et al.* (2012).

Statistical analysis: The data for all attributes were subjected to the statistical package SAS version 9.1 (SAS Institute Inc., NC, USA) to work out analysis of variance using and significant differences among mean values were assessed using LSD test at $p \leq 5\%$.

Results

Dry matter and total chlorophyll content: Recommended doses (RD) of all applied pesticides did not change the dry matter and total chlorophyll content as compared with unsprayed control plants. However, these parameters decreased with increasing all pesticide levels. These reductions were highest in plants sprayed with high doses of thiamethoxam (144 mg L⁻¹) (Tables 1 and 2).

Electrolyte leakage and free proline content: Electrolyte leakage (EL) did not significantly change in plants exposed to recommended doses of pesticides, but increased doses of pesticides impaired membrane permeability by increasing EL (Table 2). The highest dose of thiamethoxam (144 mg L⁻¹) caused a marked effect on EL as compared to the other treatments. Leaf free proline content increased in the leaves of tomato plants sprayed with increasing doses of pesticides. Proline content was highest in the leaves of plants sprayed with the highest dose of thiamethoxam, which caused maximal electrolyte leakage.

Table 1. Chlorophyll a, chlorophyll b and total chlorophyll (mg/kg Fw) of tomato plants sprayed with various levels of pesticides.

Treatments	Chl a	Chl b	Chl a+b
Control	1080 a	740 abc	1820a
A1	1030 ab	790 a	1820 a
A2	1000 ab	680 ab	1680 a
A3	1020 ab	760 bcd	1780 ab
T1	1040 ab	690 abcd	1730 ab
T2	1000 ab	690 abcd	1690 ab
T3	980 ab	600 d	1580 b
P1	1050ab	720abc	1770 a
P2	1070 a	670 bcd	1730 ab
P3	980ab	640 cd	1620 b
AC1	1020ab	760 abc	1780 a
AC2	1040 ab	650 cd	1690 ab
AC3	1010ab	710 abc	1720 ab

Note: values followed by different letters, in the same column, are significantly different at $p \leq 0.05$

A1, A2 and A3: 4.5, 9.0 and 13.5 mg Abamectin L⁻¹; T1, T2 and T3: 36, 72 and 144 mg Thiamethoxam L⁻¹; P1, P2 and P3: 50, 100 and 200 mg Pyriproxyfen L⁻¹ and AC1, AC2 and AC3: 60, 120 and 240 mg Acetamiprid L⁻¹, respectively

Activities of key antioxidative enzymes and levels of MDA: Both control (unsprayed) plants and those exposed to recommended doses of pesticides had statistically similar values for all analyzed enzymes such as SOD, CAT and POD, and reactive oxygen species (ROS) including malondialdehyde (MDA) (Table 3). Increased doses of pesticides resulted in increased activities of enzymes mentioned above and increased MDA levels. Values of these parameters reached the highest with high doses of each pesticide tested. As mentioned above, the high doses of thiamethoxam resulted in considerably higher enzyme activities and MDA levels as compared to those plants sprayed with other pesticides.

Discussion

Pesticides are widely used in a broad range in the agricultural practice. However, excessive use of them may lead to pollute environment, so causing the toxicity effects on vegetables and crops (Mishra *et al.*, 2008; Parween *et al.*, 2011). For example, the toxic effects of insecticide, dimethoate, on growth and photosynthetic pigments of *Glycine max* L. (Panduranga *et al.*, 2005) and *Vigna unguiculata* L. (Mishra *et al.*, 2008) seedlings have already been investigated. Similarly, higher doses of imidacloprid applied to rice plants significantly reduced the germination and growth of rice (Stevens *et al.*, 2008). Furthermore, the present results are similar to those of Tiyaqi *et al.* (2004) for chickpea and Mishra *et al.* (2008, 2009) for *Vigna unguiculata* L. and *Momordica charantia*, respectively. Higher concentration of pesticides induced reduction in plant growth may be due to reduced activity of hydroxyl phenyl pyruvate dioxygenase which is required for meristematic growth and development which has been

Table 2. Proline (nmol/g FW) and electrolyte leakage (EL: %) and shoot dry matter (g/plant) of tomato plants sprayed with various levels of pesticides.

Treatments	Proline	EL	Shoot dry matter
Control	59 g	12.2j	103 a
A1	244 c	12.8 hj	105 a
A2	278 b	15.6 f	85 c
A3	341 a	22.3 b	80 d
T1	159 f	13.3 h	102 a
T2	188 e	16.2 f	92 b
T3	349 a	24.4 a	68 f
P1	219 d	13.2 h	104 a
P2	239 c	17.4 e	92 b
P3	339 a	21.3 c	74 e
AC1	209 d	14.1 g	103 a
AC2	209 d	17.6 e	96 b
AC3	245 c	19.3 d	84 c

Note: values followed by different letters, in the same column, are significantly different at $p \leq 0.05$

A1, A2 and A3: 4.5, 9.0 and 13.5 mg Abamectin L⁻¹; T1, T2 and T3: 36, 72 and 144 mg Thiamethoxam L⁻¹; P1, P2 and P3: 50, 100 and 200 mg Pyriproxyfen L⁻¹ and AC1, AC2 and AC3: 60, 120 and 240 mg Acetamiprid L⁻¹, respectively

confirmed by the findings of Luscombe *et al.* (1995) and Parween *et al.* (2011) while treating sugarcane and maize with isoxaflutole, and *Vigna radiata* with chlorpyrifos, respectively.

Table 3. Superoxide dismutase (SOD: Unit mg protein⁻¹), catalase (CAT: Unit protein⁻¹), peroxidase (POD: unit protein⁻¹) and malondialdehyde (MDA: nmol g⁻¹ FW) levels of tomato plants sprayed with various levels of pesticides.

Treatments	SOD	CAT	POD	MDA
Control	17.8 g	3.4e	6.8 d	1.7 e
A1	17.7 g	3.4e	6.9 d	1.8 e
A2	20.4 de	4.5 d	8.5 c	5.2 d
A3	24.4 bc	6.5 b	12.4 a	7.8 b
T1	18.5 fg	3.5e	6.4 d	1.7 e
T2	19.2 f	5.6 c	8.8 c	6.2 c
T3	29.0 a	8.9 a	12.4 a	8.9 a
P1	17.7 g	3.6e	6.9 d	1.9 e
P2	21.8 e	4.4 d	8.9 c	5.1d
P3	22.4d	5.6 c	9.7 b	7.9 b
AC1	19.2 f	3.4e	6.7 d	1.7 e
AC2	23.1 cd	4.4 d	7.2 d	4.5 d
AC3	25.2 b	5.5 c	9.8 b	6.8 c

Note: values followed by different letters, in the same column, are significantly different at $p \leq 0.05$

A1, A2 and A3: 4.5, 9.0 and 13.5 mg Abamectin L⁻¹; T1, T2 and T3: 36, 72 and 144 mg Thiamethoxam L⁻¹; P1, P2 and P3: 50, 100 and 200 mg Pyriproxyfen L⁻¹ and AC1, AC2 and AC3: 60, 120 and 240 mg Acetamiprid L⁻¹, respectively

Improvement in proline content in the leaves of plants treated with pesticides at the high rates could be due to a major response to cellular dehydration caused by pesticide toxicity (Table 1) induced reduction in fresh shoot weight of different pesticide-treated plants. These results are similar to those of Zhang *et al.* (2011) in which application of pesticide Omethoate significantly increased proline contents in wheat plants. Similarly, Du *et al.* (2006) reported that proline content increased in rice plants treated with pesticide 1, 2, 4-trichlorobenzene. The present results suggest a positive link between pesticide toxicity and increased tissue proline and reveal an adaptive role of proline in mitigating the damaging effects of pesticide toxicity.

One of the markers used in oxidative stress tolerance in plants is ROS (Ivanov & Kerchev, 2007; Foyer *et al.*, 1997; Noctor *et al.*, 2002; Tausz *et al.*, 2004). Generally, various stress factors causing the production of ROS may also result in reducing the CAT activity. For example, reduced activity of catalase was observed in spinach plants fumigated with SO₂ (Tanaka *et al.*, 1982). Activities of catalases and peroxidases are widely known to be responsible for the enzymatic suppression of hydrogen peroxide (Willekens *et al.*, 1995). Tang *et al.* (2006) reported that foliar application of methamidophos increased the activities of superoxide dismutase (SOD) and catalase (CAT) in Chinese cabbage as compared to unsprayed control plants. Moreover, the activities of SOD, POD and CAT increased significantly in wheat and rice plants by applications of 1,2,4-trichlorobenzene and in bitter melon by application of dimethoate (Mishra *et al.*, 2009; Zhang *et al.*, 2008; Zhang *et al.*, 2009). However, phenanthrene stress slightly increased the activities of SOD, POD and CAT in fresh tea plants (Mei *et al.*, 2009). The linkage between accumulation of lipid peroxidation and antioxidant defense activity is still a complex phenomenon which needs to be elucidated. The results of several studies reveal that increased activity of antioxidant defense, SOD activity in particular, is well correlated with reduced lipid peroxidation or MDA content, which has been reported in methamidophos-stressed Chinese cabbage (Tang *et al.*, 2006), in salt-stressed plants (Liang *et al.*, 2003; Zhu *et al.*, 2004), and in freezing stressed wheat plants (Liang *et al.*, 2008). However, there are some contrasting findings. For example, increased activities of SOD and POD were found to be associated with increased MDA content in mulberry plants grown under salinity regimes (Sudhakar *et al.*, 2001). The results of the present study revealed that increased MDA content (Table 3) in tomato plants treated with higher levels of all pesticides tested along with reduced shoot dry matter (Table 2) and higher MDA content caused increased electrolyte leakage. It has been well known that MDA accumulation causes the oxidation of unsaturated fatty acids in plant membranes thereby impairing membrane permeability (Djebali *et al.*, 2005; Ben *et al.*, 2005).

Conclusion

The present results clearly depict that the pesticides' application to tomato plants at higher concentration caused suppression in growth and chlorophyll content, and increased electrolyte leakage and proline. The deleterious effects of the pesticides were also shown by an increase in malondialdehyde (MDA). Therefore, the

present results reveal that the toxicity of pesticides at higher dosage to tomato seedlings took place partly by increased electrolyte leakage and MDA content.

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