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THE POTENTIAL EFFECT OF ORGANOPHOSPHORUS INSECTICIDES ON PEROXIDASE ENZYME ACTIVITY IN TOMATO PLANTS (*Solanum lycopersicum* L.) GROWN IN AL-HASSA, SAUDI ARABIA

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ABSTRACT

In this study, the effect of three organophosphate insecticides on the peroxidase activity in growing tomato plants under greenhouse conditions was investigated. The peroxidase activity was significantly increased under the influence of insecticide treatments with increasing concentrations of treatments, 2.0 fold of the recommended doses of the common uses organophosphorus insecticides (methidion, acephate and parathion) in Al-Hassa province. However, the significant interactions between insecticide and doses were observed at 20 days after spraying and then decreased after 40 days in treated plants compared with untreated plants. The most affecting insecticides were parathion and acephate, while methidion was the lowest. However, the parathion caused a highest peroxidase activity that reflected as intensity in electrophoresis gel and in concentration of peroxidase. The stress of organophosphate-insecticide in tomato plants was evaluated by the results of specific peroxidase activity in the treated tomato plants. The uses of electrophoresis and spectrophotometer approaches to measure the peroxidase allowed to assess different features of the enzymatic activity. These results definite that; in addition to a great number of peroxidase enzyme isoforms and each isoform is activated depending on the chemical structure and properties of the insecticide. Thus the misuse of pesticides affects normal plant growth, which could result in reduction of yield or quality.

Keywords: Peroxidase activity, organophosphorus insecticides, electrophoresis, enzyme isoform, tomato.

INTRODUCTION

Tomato plant (*Solanum lycopersium* L.) is among the highly cultivated vegetable crops worldwide. It was considered one of the main greenhouse crops. In 2012, more than 500 thousand tons of tomatoes were produced in Saudi Arabia. Most of that production (60%) was grown on soil in greenhouses (MOA, 2012). Tomato usually infests by many destructive insects and that has contributed to excessive use of insecticides on tomato plant. Pesticides which are used in the modern agricultural practices for pest control have some bad toxic effects on the cultivated plant especially at rate higher than the recommended one, (Pandy *et al.* 1994)

Practically to control of resistant pests, the highest rates are applied in the field, which, in many cases, a better control were reported, but the yields were reduced, (Calderón-Limón *et al*. 2002). However the uses of chemical pesticides for plant protection in controlling pest and diseases have been massively increased, and the increasing in the application rates could be resulted in negative plant productivity. García-Hernández *et al.* (2001) reported that, in Baja California Sur and other states of Mexico, the growth and yield of plants were affected by the uses of higher doses of organophosphate insecticides. Calderón-Limón *et al*. (2002), stated that, the higher application rate of organophosphate insecticides infrequently recorded with better control of resistant pepper weevil on hot pepper plants, but the yields not increased and even reduced, and the peroxidase activity of hot pepper, was increased at the highest insecticide application rates. Moreover, not only the highest doses of organophosphorus pesticides can stimulate the oxidative and anti-oxidative systems in tomato plants, but also the exogenously applied pesticides like; abamectin, thiamethoxam, pyriproxyfen and acetamiprid can influence on oxidative defense system in tomato, (Yildiztekin *et al*. 2015). Also reported with imidacloprid insecticides in potatoes, (Chauhan *et al*. 2013). Whereas, Maximum stimulation in the activities of antioxidant enzyme is a common phenomenon, with increasing abiotic stress, which is an adaptation for stress tolerance. The enhanced activities of peroxidase as antioxidant enzyme protect the plants from many cellular injuries and damages. In absence of antioxidant enzyme the plants are unable to survive under any type of stress, they became either susceptible or sensitive to stress and culminate into death. Because the peroxidase as antioxidant enzyme cause lipid peroxidation of the membranes, destabilize it and affect its permeability (Fang and Kao, 2000). On the other hand, the insecticides may alert the plant nutrient content. Whereas, Ebel *et. al.* (2000) found that in tomato and cucumber plant treated with imidacloprid the content of Mg and B decreased and K and Mn increased in both plants. The use of organ-chlorine resulted in soil contamination worldwide, and also could cause some injury to the treated plant in the early growth stage of sweet corn, waxy corn, cowpea, and cucumber, (Somtrakoon and Pratumma, 2011). Recently in Saudi Arabia Hajjar *et al*. (2014) reported the negative effect of methomyl and imidacloprid on the grown of new shoots of tomato plant grown in Al-Hassa, KSA, when those insecticides were applied at doses higher than recommended rate.

Furthermore, Teisseire and Vernet (2001) proposed the involvement of peroxidase in the tolerance of fungicide when they studied the effect of fungicide folpet on duckweed (*Lemna minor*) and stimulated activities of peroxidase, also Zhang *et al*. (2007) concluded that, the increasing in application of fungicides like carbendazim to the *Cucumis sativus* plants could improve their tolerance. Peroxidase activity is assumed to fulfill different functions in defense mechanisms, *e.g.* (a) generation of active oxygen species (AOS) (b) suberin synthesis (c) lignin synthesis (d) oxidation of phenols to their derivatives toxic for pathogens, (Bowles, 1990). The peroxidase enzyme composition and activity changes with the physiological status of the organism, therefore it has been used as biochemical indicator (Gaspar *et al*. 1991). Peroxidases effects in many physiological and biochemical processes; such as cell growth, expansion, differentiation, development, auxin catabolism, and lignification, (Sitbon *et al*. 1999; Lin and Kao, 1999), in addition to abiotic stress responses (Medina *et al.* 1999). Thus, the peroxidase activity in plant increase as responses to internal or external stimuli, and the negative correlation between the levels of peroxidase activity and levels of plant growth were also reported, (Fang and Kao, 2000).

This investigation aimed to study in the details, the effects of increasing the recommended application rate of commonly used organophosphate insecticides in Al-Hassa on the peroxidase activity as physiological stress marker in growing tomato plant under greenhouse condition.

MATERIALS AND METHODS

Tomato Treatments

The experiments were conducted in 2016/2017 in glasshouse at the King Faisal University, Al-Hassa, KSA. Day/night, temperatures are controlled at 25 + 2°C, Relative humidity never was below 40%.

Local tomato seedlings were obtained from a commercial nursery (Al-Hassa, Saudi Arabia) and transplanted into pots 4.5 inch. Plants in the pots were grown in peat moss (75% by vol.) with the rest consisting of native fine sand, perlite, and vermiculite. The plants were well-irrigated for a 2-week period to recover from transplant. Plants were fertilized with the irrigation water every four days 4 weeks with 20 mL each of 20–8.3–8.8 (N–P–K) nutrient solution at a concentration of 100 mg/L (ppm) of nitrogen. Four weeks after transplanting, ninety plants were selected for uniformity. The experiment was conducted as factorial design with two factors (type of insecticides and application doses). Three of the most common organophosphorus insecticides in Al-Hassa area were used in the experiments methidion, acephate and parathion. Treatments of each insecticide were conducted with three application rates (the recommended, doubled and half of the recommended rate) and each with 5 plants replications. Five seedlings were served as untreated controls. The insecticide dilution of each treatment was thoroughly sprayed onto each seedling to run-off. Plants were watered regularly in order to minimize plant stress, as environmental conditions, such as low air speed or high relative humidity, and cultural stresses may predispose plants to phytotoxicity, (Davidson *et al.* 1991). In addition, all spray applications were performed in the morning so that any phytotoxic effects were due directly to the insecticides. Plants were sprayed every 10 days for 40 days with the treatments during the flowering stage. Application started when 100 % of plants were in flowering stage.

Peroxidase Activity Assay

One day after the last spraying, two leaves were taken from each plant. All leaves had a size between 5 to 6 cm long and they were collected from the upper third of the plant. These samples were prepared according to García-Hernández *et al*. (2005); plenty wash with distilled water and then homogenize in a mortar (in ice water bath) with acetate buffer 50 mM (pH 5.1). Then the homogenate centrifuge at 30000 x g for 15 min., and the supernatant was used for assays of peroxidase activity. This activity was assayed by the method of Boehriner (1973) performed with a spectrophotometer, Smart Spec™ Plus (BioRad-USA).

Protein Pattern of Tomato Plant Leafs Affected by Pesticide Inducer

The protein concentration in the leaf extracts were estimated by the method of Bradford (1976), and carried out with a, spectrophotometer (Smart Spec™ Plus, BioRad-USA) to adjust protein concentration in polyacrylamide gel electrophoresis (SDS-PAGE).

Proteins were analyzed from the intercellular fluid of treated tomato leaves, from homogenates of leaves after intercellular fluid had been extracted. Proteins were extracted from 20 and 40 days-old plants.

The intercellular fluid of the leaves was extracted according to the method of Dewit and Spikman (1982), with some modifications. A known weight (10 g) of leaves was vacuum infiltrated under cool conditions (5 - 10ºC) for 30 min in a 250 ml beaker filled. Then the leaves were dry and centrifuged for 15 min at 8000 rpm at 4ºC. The supernatant was pipetted into Eppendorf tubes and stared frozen at - 20ºC before use. To obtain extracts from leaves after the intercellular fluid had been removed, the leaves were homogenized in a mortar and pestle with 1 ml of cold citrate-phosphate buffer pH 3.0. The extracts were passed through polyester vial and then filtered through a Whatman No. 1 filter paper before being centrifuged at 15,000 rpm for 20 min at 4ºC. The supernatant was stored as before.

Denaturing PAGE analysis were used for each extract. Extracts were boiled for 5 min in buffer (125 mM Tris base pH 6.8 containing 0.4% (w/v) SDS, 10% (w/v) glycerol, 4% (v/v) 2-mercaptoethanol and 0.02% (w/v) bromphenol blue). Protein quantity was determined using the Coomassie spot test (Harlow and Lane, 1988), with bovine serum albumin as the standard; 4 mg ml^{-1} protein was used for each track. Samples from intercellular fluid, leaf homogenates and run on slab gels using 3.9% (w/v) acrylamide stacking gels and 12% (w/v) separating gels (Laemmli, 1970) and either. Marker tracks were run routinely for molecular weight calculations. The gels were stained with Coomassie blue R-250 (BioRad).

Statistical Analysis

The analysis of variance was performed in split-plot design to evaluate the effect of treatments and interaction between pesticides and doses. Regression analysis and the Scott Knott test at the 0.05 significance level were also carried out to compare in all treatments. Data were analyzed through the COSTAT 6.4 program of statistical analysis. The data was statistically analyzed by means of ANOVA, (Costat Statistical Software, 1986; Parvaiz *et al*. 2014).

RESULTS AND DISCUSSION

Peroxidase Activity

Activity of peroxidase was recorded in untreated and treated tomato plants at 20 and 40 days after spraying with three organophosphorus pesticides (methidion, acephate and Parathion).

Data presented in Table (1) showed that spraying by studied organophosphorus pesticides were associated with a remarkable stimulation in peroxidase activity in all doses during examined periods compared with untreated ones. The highest amount of increase in peroxidase activity was recorded at 20 days after spraying in treated plants compared with untreated plants. Then the activity of peroxidase decreased after 40 days of spraying. At the same time, a slight stimulation in peroxidase activity was noticed in the case of sprayed plants by 2.0 folds of recommendation dose at 20 and 40 days after spraying, respectively. Parathion pesticide was the superior in this concern (216 mg ml^{-1} pyrogallol), while the methidion pesticide was the lowest (180 mg ml^{-1} pyrogallol) after spraying plants by 2.0 fold of recommended dose, comparing with untreated tomato plants (178 mg ml^{-1} pyrogallol).

The statistical analysis for the specific peroxidase activity in Table (1) shows the highly significant differences between the applied insecticides, in addition to significant differences between doses of treatments with same insecticide. Furthermore, the effects in interaction among the two factors; the insecticide, and dose were highly significant in the response of peroxidase activity. However, the obtained results are in consistence with the hypothesis reported by García-Hernández *et al.* (2005), that "the stress by insecticides also influences the ant-oxidative enzymatic activity". Where, the response of peroxidase activity was more affected by Parathion as compared to the control, followed by acephate and methidion. However, the potential effects of dose on peroxidase activity increased by increasing the application rate of each tested insecticides. The results in Table (1) indicated that the lower concentration treatments caused very less stimulation in peroxidase activity and it was almost similar with control, while with higher dose (2.0 fold), the peroxidase activity was highly stimulated as compared to control.

In general, the manufacturer's recommended application rate doesn't create a negative effect on plants, and even some reports indicated that some specific insecticides at low doses act as growth stimulant, such as carbofuran insecticide at low dose of soil application acted as positive stimulant to water melon plant, (Foster and Brust, 1995).

Table 1. Variance analysis of the specific peroxidase activity (mg mL^{-1} pyrogallol) in function of insecticides and doses

Pesticide dose		After 20 days of treatment					After 40 days of treatment				
Methidion	**0.5 Fold**	174 ± 9					98 ± 9				
	1.0 Fold	174 ± 8					145 ± 7				
	2.0 Fold	180 ± 5					156 ± 9				
	Control	178 ± 7					65 ± 4				
Acephate	**0.5 Fold**	176 ± 7					131 ± 8				
	1.0 Fold	198 ± 8					149 ± 7				
	2.0 Fold	209 ± 8					165 ± 9				
	Control	178 ± 7					65 ± 4				
Parathion	**0.5 Fold**	165 ± 6					78 ± 6				
	1.0 Fold	185 ± 4					96 ± 8				
	2.0 Fold	216 ± 4					100 ± 8				
	Control	178 ± 7					65 ± 4				
		Df	**SS**	**MS**	**F**	**P**	**Df**	**SS**	**MS**	**F**	**P**
Pesticide		2	1189	594	69.29	***	2	11745	587	10067.0	***
Doses		3	4524	1508	89.60	***	3	30622	102	2200.0	***
Pesticide × Doses		6	2068	344	20.48	***	6	5220.5	870	187.56	***
Error		18	303	16.8			18	83.5	4.639		
Total		29	8084				29	47671			

- Each value represents the mean of three replicates ± Standard Division.
*- ***; Highly significant at a P ≤ 0.05.*
- Df: Degree of freedom; SS: Sum of squares;
MS: Mean of squires; F: Calculated F; P: Significance level.

The enzyme peroxidase is an important antioxidant enzyme which plays a pivotal role in plant growth and development, (Breda *et al.* 1993). A close correlation exists between the enhanced activity of peroxidase (POD) and the concentration of phenolic substances, (Dickinson *et al*. 1982).The increase in POD has been linked with resistance to stress and self-defense. Under stress conditions the rate of respiration increases with stimulation in peroxidase activity (Aspinall and Poleg, 1981).

Determination of the Elicited Protein as Response to Tested Organophosphorus Pesticides Inducers after 20 and 40-days

Protein pattern content was determined in tomato plants treated with studied organophosphorus pesticides as non-biotic inducers. However, in Table (1) the peroxidase enzyme activities in treated tomato plants were significantly increased with duplication of the recommended rate, in contrast to untreated ones. New proteins were elicited interior tomato plants as a result of spraying with the studied pesticides as abiotic inducers and were varied in their number (Table 2). The variability analysis among inducers appeared protein bands, the highest bands (7 protein fractions) appeared with parathion and (6 protein fractions) with acephate at 0.5, 1, and 2 fold of the recommended dose and at both intervals 20, and 40 days post treatment. However, methidion appeared in 7 protein fractions at 1 and 2 fold of the recommended dose, but with 0.5 fold of recommended dose appeared only in (4 protein fractions) after both 20 and 40 days post treatment and it were resemble to untreated plants which gave (4 protein fractions). The molecular weight of each polypeptide was determined related to protein marker. The prominent polypeptide bands in all inducers (monomorphic or common polypeptide) were (135, 100, 75 and 48) kDa. These bands may be related to tomato plant (Table 2 and Fig. 1). The most prominent alteration (polymorphic bands) among the 3 studied inducers (63, 35 and 25) kDa. These bands may be related to systemic acquired resistance (SAR) of plant against a biotic stress of tested pesticides.

The performance of the polyacrylamide gel electrophoresis coincides with the results of peroxidase activity presented in Table (1). It was observed that for each insecticide treatment, the highest dose caused the most intense concentration though out each tested period (20 and 40 days, respectively). All the treatments (acephate, methidion and parathion) showed the same bands similar in the control with 135, 100, 75 and 48 KDa. The differences among these treatments are explained by presences of some bands as influenced of the tested pesticides as a biotic stress. The data of protein fractions of tomato plants treated with pesticides as a biotic chemical inducers using SDS-PAGE presented in (Table 2), and illustrated by (Fig. 1) shows the differences between the protein patterns of control and that comes as result of interaction between insecticide type, duration of post treatment time (20 &40 day), in addition to the effect insecticide doses. Thus, protein patterns of treated tomato plant were closely related in four protein (135, 100, 75 and 48 KDa), which represent also in untreated plants. Treatment of tested insecticides as a biotic stress resulted in changes of proteins originally presented with untreated plant. Peroxidase is one enzyme with numbers of isoforms, the physiological status and the stress of developing conditions in a plant could be involved in the apparition of each isoform, (Lobarzawsky *et al*. 1991). The potential variation in peroxidase activity can

Table 2. Protein fractions of tomato plants treated with pesticides as a biotic chemical inducers using SDS-PAGE.

Fraction paned molecular weight (KDa)	Untreated plant (Control)		Pesticides (Chemical inducers)																		Polymorphism
			Methidion						Acephate						Parathion						
			0.5 Fold		1.0 Fold		2.0 Fold		0.5 Fold		1.0 Fold		2.0 Fold		0.5 Fold		1.0 Fold		2.0 Fold		
	20 D	40 D	20 D	40 D	20 D	40 D	20 D	40 D	20 D	40 D	20 D	40 D	20 D	40 D	20 D	40 D	20 D	40 D	20 D	40 D	
180	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Monomorphic
135	++	+	++	+	++	+	++	++	++	+	++	+	+++	++	++	+	++	+	+++	++	Monomorphic
100	++	+	++	+	++	+	++	++	++	+	++	+	+++	++	++	+	++	+	+++	++	Monomorphic
75	+	+	+	+	++	+	++	++	+	+	+	+	+	+	+	+	+	+	+	+	Monomorphic
63	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Polymorphic
48	++	+	++	+	++	+	++	++	++	+	++	+	+++	++	++	+	++	+	+++	++	Monomorphic
35	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	Polymorphic
25	-	-	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	Polymorphic
Total polypeptide	4	4	4	4	7	7	7	7	6	6	6	6	6	6	7	7	7	7	7	7	

Monomorphic: Common polypeptide; Polymorphic: Specific polypeptide; - = Absence of band; + = presence of band & Number of (+) signs indicate for band density; 20 D: 20 day after spray; 40 D: 40 day after spray.

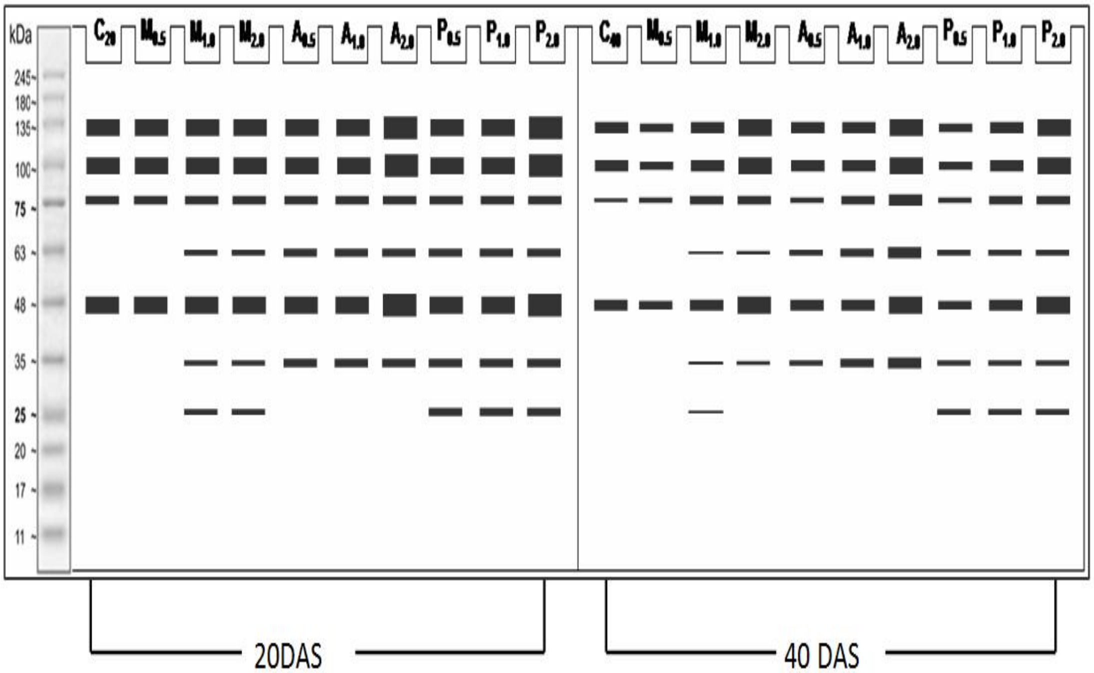

Fig. 1. A simulation of polyacrylamide gel electrophoresis (SDS-Page) of total soluble protein extracts from tomato plants leaves sprayed during 40 days with different treatments of organophosphorus insecticides.

Where: DAS: Days after spray; M: Prism Ultra Protein Ladder (25-180 kDa); P: parathion; A: acephate; M: methidion; 1) 0.5 fold, 2) 1.0 fold, 3) 2.0 fold; C_{20}: Control after 20 days of spraying; C_{40}: Control after 40 days of spraying;. Fold: Concentration of recommended dose.

be reflected in the growth and yield of plants, and play important role in some stages of the metabolism like the auxins catabolism, and lignin formation, (Fang and Kao, 2000). However, peroxidases have been directly or indirectly connected to some physiological processes such as abscission, dormancy, apical dominance, resistance to pest, auxins catabolism, and lignin formation, (Lobarzawsky *et al*. 1991; Fang and Kao, 2000). In this context, these results were in corresponding with what previously reported that some organophosphates insecticides, at higher levels doses of treatments caused decreasing in seed germination, survival rate, and alteration in meiotic behavior in the same plant species and cultivars, (Devadas *et al*. 1986; Lakshmi *et al.* 1988).

CONCLUSIONS

The changes in the activities of peroxidase as antioxidant enzyme under the influence of pesticide treatments were very clearly indicated in this study, whereas, the peroxidase activity was significantly increased under the influence of insecticide treatments with increasing concentrations of treatments. The most affecting insecticides were Parathion and Acephate, while Methidion pesticide was the lowest. The results of electrophoresis and spectrophotometer definite that, a great number of peroxidase enzyme isoforms and each isoform is activated depending on the chemical structure and properties of the insecticide.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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