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INVESTIGATIONS ON THE EFFECTS OF COMMONLY USED PESTICIDES ON TOMATO PLANT GROWTH

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ABSTRACT

Acetamiprid (ABA), imidacloprid (IM), abamectin (ABA), thiomethoxam (THM) and abamectin+chlorantraniliprole (ABAC) were applied on Hazera 5656 F1 (*Lycopersicum esculentum* Mill.) tomato variety under greenhouse conditions in Köyceğiz region of Muğla. MDA, proline and H_2O_2 contents as well as SOD, POD and CAT activities raised with increasing pesticide doses. On the other hand, increasing the dose of pesticides, decreased DM %, total chlorophyll and carotenoid contents. The plants sprayed with ABAC-3 showed 56 % proline content as compared to the control plants. ABA-3 treated samples showed highest increase in superoxide dismutase (SOD) activities while least decrease was shown by THM-1 treated samples. The highest doses of pesticides increased catalase (CAT) and peroxidase (POD) activities in most cases. The study concluded that use of high amounts of pesticides adversely affects the physiological and biochemical properties of tomato plants.

KEYWORDS:
Lycopersicum esculentum Mill., pesticides, antioxidative enzymes, proline

INTRODUCTION

Millions of humans are under the hunger line. Scientific and technological developments towards agricultural production have encouraged intensive farming, and thus the use of pesticides [1]. Due to the demands of yield maximization, environmental concerns over negative externality of agricultural production have been increasing [2]. Depending on their physicochemical properties, pesticide causes environmental problems. Some of them evaporate and cause permanent accumulation of toxic substances in the atmosphere, while others are broken down by photochemical means into toxic or non-toxic substances [3]. Moreover, use of uncontrolled pesticides can lead to physiological and metabolic changes in plants, that can lead to the loss of their quality and quantity, even death [4]. Also, pesticides cause the formation of reactive oxygen species (ROS) for instance hydrogen peroxide (H_2O_2), superoxide (O^{2-}) and hydroxyl (OH) radicals that are harmful to human health [5].

In Turkey, an average of 41.775 tons of pesticide was used annually between 2006 and 2016. In 2016, 50.054 tons pesticides were used in Turkey [6] that reflects an increase than the average amount.

Insecticides such as acetamiprid and thiamethoxam belong to the neonicotinoid group. They possesses lower toxicity and higher activity against harmful insects [7, 8]. Abamectin is a macrocyclic lactone, an important fermentation component of avermectins. Abamectin is used against insects and mites [9]. Imidacloprid belongs to a new pesticide class i.e. neonicotinoid [10]. Abamectin + Chlorantraniliprole has been reported to control *Tuta absoluta* pests [11].

Pesticides must optimally be fatal to the projected pest, but not to the non-projected species, including human being. Unluckily, this is not the state and persisting pesticide remainders can be established in food commodities like tomatoes at high doses. Therefore, there is a need for the decrease of the quantities of pesticides used in the cultivation of tomato [12].

In this study, we investigated the extent of stress in the tomato plant caused by different insecticides in addition to the determination of proline and protein concentration, chlorophyll, H_2O_2, and antioxidative enzyme (SOD, POD, CAT) contents. The outputs of this study were delivered to the producers and consumers in the Muğla province of Turkey.

MATERIALS AND METHODS

The study was carried out on Hazera 5656 F1 tomato variety (*L. esculentum* Mill.) in the Köycegiz region of Mugla city. A total of sixty plastic pots (20 L, filled with peat and river sand: ratio 2:1) including five systemic insecticides, three different doses (recommended dose by producer, two times, four times) in four replications. The control group was irrigated only. Spraying of pesticides was started on the 10-15th days of planting and repeated four times after each 15-20th day.

The following insecticides were used in this study:

1. Acetamiprid (Sumitomo), pyridylmethylamine, $C_{10}H_{11}ClN_4$, for mice oral dosage LD_{50}:185 mg/kg; recommended dose: 30 mg in 100 L water.
2. Imidacloprid (Bayer), $C_9H_{10}ClN_5O_2$, for mice oral dosage LD_{50}: 450 mg/kg; recommended dose: 100 mg in 100 L water.
3. Abamectin (Syngenta), $C_{48}H_{72}O_{14}$, for mice oral dosage LD_{50}: 11 mg/kg; recommended dose: 25 mg in 100 L water.
4. Thiamethoxam (Syngenta), $C_8H_{10}ClN_5O_3S$; recommended dose: 100 mg in 100 L water.
5. Abamectin+Chlorantraniliprole (Syngenta), recommended dose: 90 mg in 100 L water.

Experimental conditions are given below (Table 1).

Fresh plant samples were stored at 70℃ for 48 hours and dried weight was calculated. Plant height and stem diameter measurements of all plants were made during harvesting. Chlorophyll content was extracted from fully expanded young leaves using 90 % acetone solution using Strain and Svec [13] method. Free proline was extracted and determined as described by Bates et al. [14] while hydrogen peroxide content was determined spectrophotometrically according to the Velikova et al. [15] procedure at 390 nm.

SOD was determined by Beauchamp and Fridovich [16], CAT by Kraus and Fletcher [17], and POD by Chance and Maehly [18] method. The Bradford [19] protocol was used to estimate total soluble proteins. Leaf MDA was analyzed following Cakmak and Horst [20] with some modifications as suggested by Weisany et al. [21].

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≤5%.

RESULTS

The amount of DM% of the leaves showed a decrease in all groups compared to the control group. The highest decrease was recorded in the ABAC 3 group (12.03%), while the least (21.80%) was observed in ABA 1 (Fig. 1, left).

Maximum plant height in the control group was 59 cm, while the lowest plant height was determined in ABA 3 group with 37 cm (Fig. 1, right).

The application of insecticides on tomato plants caused a decrease in the total chlorophyll amount when compared with the control. The highest decrease was observed in the application of IM 3 (58.21 %) while the least was found in ABA 1 (6.73 %). Leaf carotenoid contents also reflected similar behavior (Fig. 2, left).

Protein concentrations of leaf samples decreased in all applications. The highest decrease was found in ABA 1 i.e. 45.21 % (Fig. 2, right).

The results of statistical analysis on the lipid peroxidation (MDA), proline amount and H_2O_2 content of the tomato plant leaves are given in Table 2.

Amount of leaf MDA was increased in all applications compared to the control. The highest increase was observed in ABA 3 group (7.77 mmol g^{-1} FW) while the lowest was found in IM 1 (2.31 mmol g-1 FW).

TABLE 1
Concentrations, codes and trade names of the used insecticides

Concentration	Code name	Trade Name
Control	Control*	-
Abamectin (25 mL/100 L)	ABA 1	Agrimec
Abamectin (50 mL/100 L)	ABA 2	Agrimec
Abamectin (100 mL/100 L)	ABA 3	Agrimec
Acetamiprid (30 mL/100 L)	ACE 1	Mospilan
Acetamiprid (60 mL/100 L)	ACE 2	Mospilan
Acetamiprid (120 mL/100 L)	ACE 3	Mospilan
Thiamethoxam (100 mL/100 L)	THM 1	Actara
Thiamethoxam (200 mL/100 L)	THM 2	Actara
Thiamethoxam (400 mL/100 L)	THM 3	Actara
Abamectin+Chlorantraniliprole (90 mL/100 L)	ABAC 1	Voliam Targo
Abamectin+Chlorantraniliprole (180 mL/100 L)	ABAC 2	Voliam Targo
Abamectin+Chlorantraniliprole (360 mL/100 L)	ABAC 3	Voliam Targo
Imidacloprid (100 mL/100 L)	IM 1	Confidor
Imidacloprid (200 mL/100 L)	IM 2	Confidor
Imidacloprid (400 mL/100 L)	IM 3	Confidor

*irrigation water only

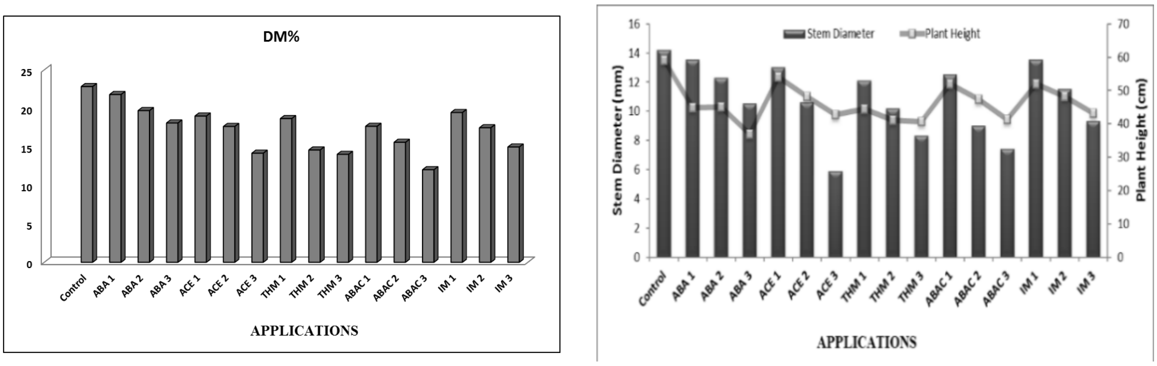

FIGURE 1
Effects of insecticide applications on dry matter content (DM%) of tomato plant leaves (left), plant heights (cm) and stem diameter (mm) (right).

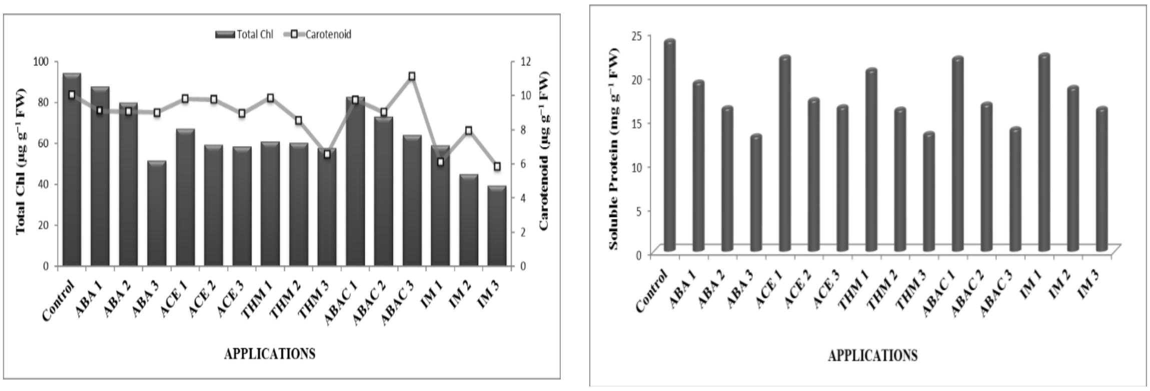

FIGURE 2
Effects of insecticides on the total chlorophyll, carotenoid (left) and protein content of tomato leaves (right).

TABLE 2
Effects of insecticide application on MDA, Proline and H_2O_2 in tomato leaves

Treatments	MDA (mmol g^{-1} FW)	Proline (unit mg protein^{-1})	H_2O_2 (unit protein^{-1})
Control	2,37±0,09g	27,80±0,16j	118,48±1,34i
ABA 1	2,72±0,19f	30,71±0,99hi	121,33±0,51i
ABA 2	4,74±0,07c	37,58±1,15de	136,96±0,81f
ABA 3	7,77±0,14a	43,28±1,32b	167,90±1,25b
ACE 1	2,87±0,11f	30,48±0,33hi	121,25±1,57i
ACE 2	4,09±0,09d	33,39±1,15fg	121,23±1,10i
ACE 3	6,21±0,1b	36,18±1,15e	134,16±2,41fg
THM 1	2,45±0,1g	28,73±0,49ij	129,98±2,28h
THM 2	3,30±0,1e	33,74±0,66f	142,74±1,03e
THM 3	4,74±0,04c	38,74±0,82d	155,71±0,07c
ABAC 1	2,49±0,06g	30,59±0,16hi	121,13±1,69i
ABAC 2	4,13±0,06d	41,19±1,65c	140,40±0,66e
ABAC 3	7,62±0,08a	49,33±0,99a	155,36±1,45c
IM 1	2,31±0,12g	24,89±0,33k	132,58±0,96gh
IM 2	3,13±0,06e	31,64±0,66gh	147,48±0,52d
IM 3	4,06±0,08d	33,04±0,33fg	174,51±1,18a

Note: The difference between the averages indicated by different letters in the same column is statistically significant ($p \leq 0.05$).

Highest proline content in the tomato plant leaves was observed in ABAC 3 while IM 1 group showed the least.

We found that the insecticide applications caused an increase of hydrogen peroxide in all the groups compared to the control (Table 2).

When the SOD amount of the leaves was examined, the greatest increase was seen in ABA 3 treatment (91.44%) and at least THM 1 (8.56%) when compared with control. Highest POD activity was observed in ABAC 3 (12.04 unit mg^{-1} protein) and lowest in THM 1 (3.03 unit mg^{-1} protein). When the specific CAT activity was examined, the highest activity was reflected by the ABA 3 (82.62 %) group while the lowest was found in IM 1 (7.34 %) (Table 3).

TABLE 3
Effects of insecticide application on antioxidant enzyme activities of tomato leaves

Treatments	SOD (unit mg^{-1} protein)	POD (unit mg^{-1} protein)	CAT (unit mg^{-1} protein)
Control	11,21±0,48d	3,03±0,15k	6,27±0,04h
ABA 1	13,69±0,03d	4,36±0,17fg	7,81±0,15e
ABA 2	16,54±0,16c	7,38±0,09cd	9,21±0,15c
ABA 3	21,46±1,50a	11,93±0,08a	11,45±0,10a
ACE 1	12,51±1,36d	3,73±0,02ghi	6,80±0,15g
ACE 2	17,97±1,49bc	6,74±0,17d	8,72±0,25d
ACE 3	18,44±1,79bc	7,43±0,11c	9,16±0,49c
THM 1	12,17±0,24d	3,28±0,04jk	7,28±0,18f
THM 2	16,88±1,58bc	5,10±0,05e	9,29±0,15c
THM 3	19,45±0,45ab	9,35±0,45b	11,22±0,23a
ABAC 1	13,12±0,95d	4,09±0,29fgh	6,84±0,03g
ABAC 2	17,78±0,60bc	7,54±0,10c	8,97±0,06cd
ABAC 3	21,18±0,23a	12,04±0,55a	10,79±0,21b
IM 1	12,22±0,62d	3,61±0,45hij	6,73±0,05g
IM 2	18,30±2,24bc	4,70±0,11ef	8,07±0,12e
IM 3	18,44±1,43bc	7,36±0,65cd	9,25±0,21c

Note: The difference between the averages indicated by different letters in the same column is statistically significant ($p \leq 0.05$).

DISCUSSION

Pesticides have positive effects, such as protecting plants against various disease agents, as well as some changes in plant metabolism caused by biotic stress when the recommended dose is exceeded [22]. In this potting experiment, we found that proportional reductions were observed due to increased concentrations of dry matter in the leaves when compared to controls at the end of the insecticide application to the tomato plants (Fig. 1, left). There are literature examples where the increasing dose amount resulted in the decrease of DM% of the plants under study eg. atrazine on the *Pisum sativum* L. [23], omethoate on wheat [24] and pyriproxyfen on maize [25].

Another parameter determined in this study was plant height and trunk diameter, which decreased with the increase in applied insecticide concentration (Fig. 1, right). Parween et al. [26] applied chlorpyrifos to *Vigna radiata* L. plants at different concentrations and found a decrease in the plant root and trunk lengths. The stated study agrees with the current study; and it appears that the use of agrochemicals at high concentrations has an inhibitory effect on plant development.

In the literature, it is reported that fungicides decrease photosynthetic pigment amounts and affect photosynthesis negatively [27]. In this study, when the insecticide application to the tomato plants was compared with the control, the total amount of chlorophyll and carotenoid in the leaves decreased remarkably (Fig. 2, left). In the literature, Chlorpyrifos and Imidacloprid pesticides were applied to the rice plant. The obtained data suggested that chlorophyll content affects the amount of proteins, plant root and trunk length [28].

When we study the results of our studies, it is seen that the protein content of plant leaf samples decreased in all insecticide applications compared to the control (Fig. 2, right). The soluble protein content of *Vigna radiata* L. leaves was decreased by 20.60 % in the 5th day leaves as reported [26]. On the other hand, Switch 62.5 Fludioxonil fungicide, an effective substance of WG, has been reported to increase the total protein content of leaves of *Vitis vinifera* L. by 48 % when compared to control [29]. In the literature given above, there are findings both ways.

As a result of this research, it was observed that there was a general increase in the MDA analysis results when the applications were compared with the control. It is thought that this causes insecticides to cause lipid peroxidation in plant tissue and cause membrane damage and impairment of membrane integrity (Table 2). Similarly, Omathoate spraying has caused an increased in the lipid peroxidation content in the wheat plants [24]. On the other hand, lipid peroxidation levels of *Pisum sativum* leaves decreased when 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) were applied [30]. The results of these investigations are in parallel with the lipid peroxidation results of our insecticide applied tomato leaves.

In this study, where we applied 5 different insecticides to tomato plants, leaf proline quantities also increased with increasing density (Table 2). The application of 1,2,4-trichlorobenzene (1,2,4-TCB) to rice plants has been reported to increase the proline content in plants [31]. Zhang et al. [24] has reported that the proline levels of wheat samples taken during the 5th and 7th days of insecticidal application increased in proportion to increasing doses of insecticide. These studies agrees to what we have observed in this study.

H_2O_2 in plants works as a signaling molecule that increases tolerance to various abiotic stresses at low concentrations, while organizing programmed cell deaths at high concentrations [32]. When the results of H_2O_2 in our study were evaluated, it was found that the insecticide applications were higher in all the groups compared to the control (Table 2). In a study, Mishra et al. [33] conducted UVB application of *Vigna unguiculata* L. plant, and found similar increase in the H_2O_2 concentrations of plant leaves under stress.

The antioxidative enzyme activities investigated in this study showed various increases according to the control depending on the increasing stress condition resulting from insecticide applications. One of the most important job of antioxidative enzymes is that it increase the amount of SOD that scavenges toxic oxygen radicals, thus prevent damage to the plant leaves. We found an increase in the SOD and POD of tomato plant leaves subjected to insecticides, especially in the case of ABA 3 and ABAC 3. CAT quantities of leaf samples were higher in ABA 3 and THM 3 groups. In general, we can state that the level of applied insecticide doses put the plants into stress (Table 3). We have found in our previous study on tomatoes [5] that the amount of SOD, POD and CAT were significantly increased when pesticide concentrations were increased. Moreover, dimethoate insecticide applied to bitter gourd plants and the application of 1,2,4-trichlorobenzene to wheat and rice plants significantly increased the SOD, POD and CAT activities [34-36]. In another study of Mancozeb on *Cassia angustifolia* Vahl. fungus, increasing of fungicide concentrations significantly increased the SOD activity while decreased the CAT activity [37]. Furthermore, the slight stimulant impacts on tomato growth caused by the lower doses of pesticides might be owing to the usage of some organic complex in pesticides by plants or it might be an output of tomato plants to exposure to low concentrations of toxic matters [38].

In conclusion, high concentrations of agrochemicals negatively affects the plant anatomy, physiology and biochemistry that further causes stress in the plants.

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