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Original Article

Changes Evaluation of Reserve Substances and Degradation Enzymes after Exposure of Tomato Plants (*Lycopersicon esculentum* Mill.) to Alpha - Cypermethrin, Chlorpyriphos and Pyrimicarb

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Abstract

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables, whose production and consumption increased quite rapidly. The impact of three xenobiotics such as alpha-cypermethrin, chlorpyriphos and pyrimicarb on reserve substances (proteins, starch and lipids) and degradation enzymes (protease and alpha-amylase) was investigated. The effect of the insecticides was observed by using four dilutions of the normal concentration used in agriculture (100%, 75%, 50% and 25%) for germinating seeds, and only the recommended concentration in agriculture for growing plants. The results suggest that the tested insecticides induced an accumulation of proteins in both treated seeds, and treated plants leaves and roots. Moreover, the protease activity was reduced in treated seeds and plants. Also a great accumulation of starch in presence of the insecticides was registered in treated seeds, and leafs and roots of treated plants, whereas this accumulation is accompanied with an inhibition of alpha-amylase activity. Concerning lipids, a significant increase was observed in treated samples compared to the control ones.

Keywords: insecticides, tomato, *Lycopersicon esculentum* Mill., reserve substances, degradation enzymes.

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetables in the world. In recent years, competition has intensified increasingly as world exports of tomato products from main suppliers. Processing tomatoes are attacked by various arthropods, plant diseases and nematodes which significantly reduce yield and quality of fruit [21].

In the Northern Morocco, the most important way to protect cultures is the use of the chemical pesticides. Many pesticide types are used, especially organochlorine pesticides, organophosphorus pesticides, carbamate pesticides and pyrethroids pesticides [8].

However, the use of these pesticides obtained by chemical synthesis represents the major cause of agricultural soil and groundwater contamination because of their persistence, biodisponibility and mobility [1]. In this way, the study of pesticide occurrence in agricultural soil of the Tangier region shows the presence of many pesticides types such as endosulfan isomers (alpha and beta), endosulfan sulfate, some DDT metabolites and alpha HCH [8].

In plant, the proteins are highly concentrated in seeds, reaching 40% of dry weight. The proteins function as reserves of amino-acids ensures growth plant after seed germination [23]. Proteases catalyse peptides and proteins hydrolysis at both intra- and

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extracellular levels, playing a very important role in the plant response to many vital processes. The proteases are generally classified in terms of their mechanism function, and amino acid. We differentiate then among serine protease, threonine protease, aspartate protease, cysteine protease and metalloproteases [24]. The starch is the essential polysaccharide of plant reserves; it is constituted by a chain of molecules of α-glucose, and present in two forms: amylose and amylopectine [23]. The plants degrade their starch-based reserves by using amylase, when monosaccharides and disaccharides are required for their growth and development. The α-amylase (α-1,4-glucan-4-glucanohydrolase, EC. 3.2.1.1) is a hydrolatic enzyme, catalysing the hydrolysis of α-1,4 glucosidic connections of polysaccharides internal chains. It's a key enzyme of the saccharidic metabolism [26]. The lipids are used in plants for energy storage, but they also have structural roles as the case of phospholipids. Some plants store food energy as oil particularly in seeds and fruits [23]. In this study, we evaluated the effects of three xenobiotics (alpha-cypermethrin, chlorpyriphos and pyrimicarb) on reserve substances (proteins, starch and lipids) and degradation enzymes (protease and alpha-amylase) in tomato (*Lycopersicon esculentum* Mill.).

2. Material and Method

2.1. Plant material and germination process

Tomato (*L. esculentum*) seeds obtained from the Vilmorin commercial seeds were surface-sterilized in 10% commercial bleach with stirring for 5 min, followed by extensive washing in sterile-distilled water. Batches of 50 tomato seeds were germinated in Petri dishes (diameter 9 cm) upon two layers of filter paper moistened with 6 ml of distilled water or insecticide solutions at the concentrations of 25%, 50%, 75% and 100% (100% represents the normal concentration used in agriculture), and maintained in a growth chamber in darkness at 25°C for 6 days. At various stages of tomato seed germination (3, 4 and 5 days), seeds of each replicate were collected for measurement. Germination time was determined as the time of rupture of seed coats, and the emergence of the radicle through the seed coat. For plants study, ten seeds were sown in each plastic pot for germination and growth. Seedlings were grown at 24/20°C (day/night) and 16 hours of light, and watered each day. After growth for 30 days, the seedlings were treated by the insecticides at concentration used in agriculture (100%). The tests were realised also on the 2^{nd}, 5^{th}, 8^{th}, 11^{th} and 14^{th} day after treatment.

2.2. Proteins measurement

Plantlets were homogenized in 0,1MTris-HCl, pH 7.2 and proteins were quantified according to Bradford [2], using bovine serum albumin as standard.

2.3. Starch measurement

The starch rate is measured in accordance with Valencia et al. [27], excepted some modifications.100 mg of each sample (leaves, roots, seeds) are crushed, filtered and solubilised in 2 ml of distilled water. 100 µl of this solution are added to 2.5 ml of lugol's solution. The absorbance is read at 580 nm, and the concentration of the starch is calculated with the standard curve by using starch solution as reference.

2.4. Lipids measurement

The extraction of lipids was done according to Van Handel [29], and quantification of lipids rate was evaluated by the methods of Zöllner and Kirsch [33]. The absorbance is read at 530 nm and the concentration of lipids is calculated with the standard curve by using cholesterol palmitate solution as reference.

2.5. Protease measurement

The protease activity was determined in accordance with Novillo et al. [20]. The reaction mixtures consisted of 0.05 ml of sample extract and 0.5 ml of 2% (w/v) azocasein. After 10 min of incubation at 30°C, the reaction was stopped by adding 20% (w/v) TCA. Precipitation and centrifugation (9000 g, 5 min) took place at 4°C. Then the absorbance at 440 nm was read. In the control, TCA was added to the culture medium prior to azocasein. One unit of protease activity was defined as the amount of enzyme that increased the absorbance by 0.1 at 440 nm per 1 h at 28°C.

2.6. Alpha - amylase measurements

The activity of α-amylase was evaluated according to Valencia et al. [27] with some modifications. The enzyme activity was assayed by measuring the reducing sugar released during the reaction, using starch as the substrate. Alpha amylases was extracted by homogenizing 100 mg of germinating seeds in 0.1M phosphate buffers, pH 7.0 and 0.9 ml of 100 mM sodium citrate buffer, pH 5.0. The extract was centrifuged at 9000 g for 20 min. The reaction mixture contained 0.1 ml of 0.5% soluble starch in 0.8 ml of 100 mM sodium citrate buffer, pH 5.0, and 0.1 ml of enzyme solution. After incubating for 15 min at 37°C, the suspension was stained with lugol and centrifuged at 4000 g during 15 min. The α-amylase activity was determined by

measuring the reducing sugar content at an absorbance of 580 nm.

2.7. Statistical analysis

Data were processed by using Statistica Software [34] for one-way analysis of variance (ANOVA) and the Tukey test for the Post-hoc tests.

A significance level of 0.05 was used for all statistical tests. Each value of the monitored parameters represents the average of 3 replications.

3. Results

3.1. Insecticides effect on proteins rate

Table 1 showed that the insecticides provoked an accumulation of protein in the treated growing seeds. This accumulation reaches for example 0.66 µg of proteins/mg of fresh weight (FW) for the seeds treated with alpha - cypermethrin at the concentration of 100%, vs. 0.14 µg/mg FW for the control.

Table 1. Effect of insecticides on proteins concentration (µg/mg FW) in tomato seedlings

	3rd day	4th day	5th day
Control	0.28± 0.02	0.14± 0.03	0.19 ± 0.01
α-cypermethrin			
25%	0.30 ± 0.02	0.51 ± 0.01***	0.3 ± 0.03**
50%	0.37 ± 0.03*	0.55± 0.05***	0.74 ± 0.07***
75%	0.31 ± 0.03	0.62 ± 0.04***	0.3 ± 0.02**
100%	0.45 ± 0.02***	0.63 ± 0.05***	0.34 ± 0.06*
Chlorpyriphos			
25%	0.39 ± 0.05*	0.16 ± 0.04	0.21 ± 0.01
50%	0.49 ± 0.03***	0.19 ± 0.02	0.35± 0.02**
75%	0.47 ± 0.01***	0.33 ± 0.05**	0.25 ± 0.02*
100%	0.49 ± 0.05**	0.34 ± 0.02**	0.32 ± 0.03**
Pyrimicarb			
25%	0.52 ± 0.09*	0.28 ± 0.03**	0.3± 0.05*
50%	0.53 ± 0.05**	0.38 ± 0.03***	0.38 ± 0.04**
75%	0.54 ± 0.03***	0.44 ± 0.01***	0.56 ± 0.1**
100%	0.31 ± 0.03	0.54 ± 0.06***	0.69 ± 0.01***

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

Table 2 illustrates that the proteins rate in treated leaves was higher when compared to the control, especially at the 2nd week of the test period. For example, it reached 0.55 µg/mg FW in pyrimicarb - treated leaves at 14th day, vs. 0.14 µg/mg FW for the control. Besides, a significant increase in treated root proteins rate was illustrated at the 2nd week of the test period too, and it's was for example c. 0.46 µg/mg FW in chlorpyriphos - treated samples at 14th day, compared to the control (0.35 µg/mg FW).

Table 2. Effect of insecticides on proteins concentration (µg/mg FW) in tomato plants leaves and roots

	Control	α-cypermethrin	Chloropyriphos	Pyrimicarb
Leaves				
2nd day	0.13 ± 0.04	0.17 ± 0.01	0.18 ± 0.01	0.13 ± 0.02
5th day	0.16 ± 0	0.17 ± 0.02	0.15 ± 0.03	0.53 ± 0.02***
8th day	0.1 ± 0.02	0.14 ± 0.02	0.2 ± 0.01**	0.23 ± 0.05*
11th day	0.24 ± 0.04	0.44 ± 0.03**	0.2 ± 0.04	0.38 ± 0.07*
14th day	0.27± 0.01	0.51 ± 0.09*	0.5 ± 0.06**	0.55 ± 0.05**
Roots				
2nd day	0.33 ± 0.01	0.3 ± 0.03	0.26 ± 0.09	0.33 ± 0.1
5th day	0.14 ± 0.06	0.19 ± 0.03	0.16 ± 0.05	0.19 ± 0.08
8th day	0.29 ± 0.03	0.29± 0.03	0.51 ± 0.08*	0.52 ± 0.01***
11th day	0.35± 0.02	0.61 ± 0.05**	0.32 ± 0.04	0.55 ± 0.07*
14th day	0.35± 0.05	0.71 ± 0.14*	0.46 ± 0.01*	0.66 ± 0.11*

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

3.2. Insecticides effect on starch rate

An accumulation of starch in treated seeds was observed especially at the 4th and 5th days of the test period for all insecticides concentrations. The starch rate in treated seeds was generally c. 1.5 µg/mg FW, compared to the control rate (c. 1.1 µg/mg FW). At the 3rd day, the difference between starch rate of treated and control samples

was generally insignificant, excepted for the seeds treated with alpha - cypermethrin at the concentration of 100%, which reached 1.47 µg/mg FW, vs. 1.16 µg/mg FW for the control ones (table 3). A similar observation was noted for the growing plants; the starch rate in treated leaves was very higher than the control ones, it's was varying between 1.67 µg/mg FW and 3.14 µg/mg FW, and 0.25 µg/mg FW and 0.44 µg/mg FW in the same order (table 4).

This profile was respected in starch measurement in roots; the starch rate in treated roots was more important than the control ones, it's was varying between 6.5 $\mu g.mg^{-1}$ FW and 16.01 µg/mg FW, and 0.92 µg/mg FW and 1.28 µg/mg FW respectively (table 4).

Table 3. Effect of insecticides on starch concentration (µg/mg FW) in tomato seedlings

	3rd day	4th day	5th day
Control	1.16 ± 0.07	1.01 ± 0.1	1.22 ± 0.04
α-cypermethrin			
25%	1.22 ± 0.09	1.45± 0.01***	1.66 ± 0.07***
50%	1.22 ± 0.05	1.65 ± 0.03***	1.66 ± 0.02***
75%	1.26 ± 0.04	1.6 ± 0.04***	1.59 ± 0.06***
100%	1.47 ± 0.02***	1.68 ± 0.04***	1.37 ± 0.04*
Chlorpyriphos			
25%	1.17 ± 0.03**	1.95 ± 0.08***	1.6 ± 0.07***
50%	1.32 ± 0.06**	1.49 ± 0.06***	2 .12 ± 0.05***
75%	1.37 ± 0.04***	1.72 ± 0.1***	2.06 ± 0.06***
100%	1.45 ± 0.06**	1.92 ± 0.02***	2.15 ± 0.09***
Pyrimicarb			
25%	1.05 ± 0.03	1.04 ± 0.1	1.53 ± 0.05***
50%	1.21 ± 0.07	1.36 ± 0.03*	1.37 ± 0.02**
75%	1.54 ± 0.03***	1.06 ± 0.04	1.32 ± 0.01*
100%	1.63 ± 0.04***	1.11 ± 0.15	1.44 ± 0.02**

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

Table 4. Effect of insecticides on starch concentration (µg/mg FW) of tomato plants leafs and roots

	Control	α-cypermethrin	Chloropyriphos	Pyrimicarb
Leaves				
2nd day	0.46 ± 0.04	1.67 ± 0.31***	2.48 ± 0.41**	1.4 ± 0.06***
5th day	0.44 ± 0.01	2.88 ± 0.42***	1.74 ± 0.15***	1.28 ± 0.27**
8th day	0.25 ± 0.01	2.85 ± 0.05***	1.56 ± 0.32**	1.19 ± 0.04***
11th day	0.46 ± 0.1	1.77 ± 0.21***	1.51 ± 0.24**	1.4 ± 0.15**
14th day	0.27 ± 0.04	3.14 ± 0.16***	2.04 ± 0.15***	3.03 ± 0.02***
Roots				
2nd day	1.02 ± 0.06	6.49 ± 0.65***	9.14 ± 1.06***	4.17 ± 0.08***
5th day	1.02 ± 0.05	9.71 ± 2.51***	9.39 ± 0.53***	10.99 ± 0.93***
8th day	1.28 ± 0.15	9.58 ± 0.32***	10.66 ± 0.69***	11.62 ± 2.07**
11th day	1.23 ± 0.18	15.35 ± 0.6***	12.02 ± 1.5***	13.65 ± 0.65***
14th day	0.91 ± 0.04	12.87 ± 0.95***	14.4 ± 0.73***	14.66 ± 1.24***

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

3.3. Insecticides effect of on lipids rate

The study of lipids rate in germinating seeds showed as illustrated in table 5 a significant increase since the 3rd day for all concentration used. The lipids rate fluctuates between 0.2 µg/mg FW and 0.3 µg/mg FW in comparison with the control (0.09 µg/mg FW). Concerning the growing plants, the lipids concentration was increased too after the insecticides application, and reached 0.3 µg/mg FW in treated leaves, far exceeding the control values (0.08 - 0.17 µg/mg FW; table 6). The lipids concentration increased in roots since the 2nd day.

During the second week, after alpha - cypermethrin treatment, lipid concentration increased more marked in roots, reaching 0.77 µg/mg FW in the 14th day, while in control only 0.27 µg/mg FW concentration was reported (table 6).

Table 5. Effect of insecticides on lipids concentration (µg/mg FW) in tomato seedlings.

	3rd day	4th day	5th day
Control	0.09 ± 0.005	0.09 ± 0.01	0.11 ± 0.02
α-cypermethrin			
25%	0.20 ± 0.01***	0.19 ± 0.005***	0.16 ± 0.005
50%	0.19 ± 0.01***	0.18 ± 0.01***	0.24 ± 0.03***
75%	0.2 ± 0.02***	0.18 ± 0.005***	0.21 ± 0.02**
100%	0.2 ± 0.01***	0.28 ± 0.005***	0.14 ± 0.005
Chlorpyriphos			
25%	0.2 ± 0.01***	0.22 ± 0.02***	0.21 ± 0.01***
50%	0.22 ± 0.01***	0.28 ± 0.02***	0.24 ± 0.02***
75%	0.29 ± 0.02***	0.23 ± 0.005***	0.24 ± 0.005***
100%	0.43 ± 0.03***	0.3 ± 0.03***	0.18 ± 0.005**
Pyrimicarb			
25%	0.24 ± 0***	0.23 ± 0.005***	0.22 ± 0.01***
50%	0.25 ± 0.04***	0.22 ± 0.02***	0.18 ± 0.01**
75%	0.22 ± 0.01***	0.22 ± 0.005***	0.19 ± 0.01***
100%	0.24 ± 0.05**	0.23 ± 0.01***	0.23 ± 0.005***

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

Table 6. Effect of insecticides on lipids concentration (µg/mg FW) of tomato plants leaves and roots

	Control	α-cypermethrin	Chloropyriphos	Pyrimicarb
Leaves				
2nd day	0.17 ± 0.01	0.26 ± 0.01**	0.16 ± 0.01	0.27 ± 0.02***
5th day	0.1 ± 0.01	0.3 ± 0.01**	0.24 ± 0.01***	0.3 ± 0.03***
8th day	0.08 ± 0.005	0.3 ± 0.01**	0.25 ± 0.005***	0.27 ± 0.01***
11th day	0.16 ± 0.005	0.18 ± 0.01	0.22 ± 0.005**	0.24 ± 0.01***
14th day	0.13 ± 0.01	0.15 ± 0.02	0.18 ± 0.02	0.2 ± 0.01*
Roots				
2nd day	0.44 ±0.03	0.68 ± 0.02***	0.6 ± 0.06**	0.55 ± 0.01*
5th day	0.26 ± 0.03	0.29 ± 0.01	0.55 ± 0.01***	0.68 ± 0.06***
8th day	0.35 ± 0.03	0.56 ± 0.05***	0.35 ± 0.01	0.58 ± 0.02***
11th day	0.28 ± 0.01	0.65 ± 0.05***	0.33 ± 0.03	0.72 ± 0.02***
14th day	0.27 ± 0.01	0.77 ± 0.03***	0.36 ± 0.01*	0.32 ± 0.04

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

3.4. Insecticides effect on protease activity

Data returnable in table 7 showed during all over the test period a great stimulation of protease activity in treated seedlings compared to the control. For example, the protease activity in seedlings treated with chlorpyriphos at the concentration of 100% was 4 times higher than control case; it was about 0.8 Unit of protease/min/g FW, vs. 0.2 Unit of protease/min/g FW for the control one.

Protease activity was increased in both leaves and roots in growing tomato plant at the first day after treatment.

The protease activity in the treated leaves was generally significantly greater than the control in all the test period (table 8).

Concerning treated roots, the increase in protease activity was especially marked during the first week after treatment, excepted for samples treated with pyrimicarb, which was higher in all test period, and it reached 1.57 Unit of protease/min/g FW at the 14th day, compared to the control (1.02 Unit of protease/min/g FW; table 8).

3.5. Insecticides effect of on α-amylase activity

Results of experiments carried out on α-amylase in seedlings are summarized in table 9. They indicated an inhibition of the enzyme activity in seeds germinated in presence of different insecticides concentrations. For example in seedlings treated with pyrimicarb, the α-amylase activity was 92% and 70% lower at the concentration of 100% in comparison with the control respectively at the 4th and the 5th days of the test period. Generally, the activity of α-amylase in the treated leaves and roots was significantly inhibited when compared to the control; this inhibition is observed after treatment by all insecticides, and practically in all days of the test period (table 10).

Table 7. Effect of insecticides on protease activity (Unit of protease/min/g FW) in tomato seedlings

	3rd day	4th day	5th day
Control	0.21 ± 0.005	0.22 ± 0.01	0.2 ± 0.01
α-cypermethrin			
25%	0.32 ± 0.1	0.69 ± 0.08***	0.52 ± 0.05***
50%	0.49 ± 0.04***	0.53 ± 0.07**	0.69 ± 0.06***
75%	0.53 ± 0.12*	0.66 ± 0.09**	0.42 ± 0.09*
100%	0.37 ± 0.08*	0.54 ± 0.07**	0.75 ± 0.05***
Chlorpyriphos			
25%	0.37 ± 0.02***	0.17 ± 0.02	0.23 ± 0.06
50%	0.39 ± 0.01***	0.57 ± 0.08**	0.57 ± 0.13**
75%	0.41± 0.02***	0.44 ± 0.03***	0.87 ± 0.1***
100%	0.3 ± 0.03**	0.56 ± 0.04***	0.8 ± 0.16**
Pyrimicarb			
25%	0.32 ± 0.03**	0.35 ± 0.02**	0.28 ± 0.01**
50%	0.43 ± 0.02***	0.4 ± 0.06**	0.42 ± 0.04**
75%	0.54 ± 0.02***	0.51 ± 0.01***	0.56 ± 0.02***
100%	0.38 ± 0.005***	0.71 ± 0.04***	0.67 ± 0.1**

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

Table 8. Effect of insecticides on protease activity (Unit of protease/min/g FW) of tomato plants leaves and roots

	Control	α-cypermethrin	Chloropyriphos	Pyrimicarb
Leaves				
2nd day	0.31 ± 0.01	0.81 ± 0.04***	0.84 ± 0.11**	0.37 ± 0.08
5th day	0.33 ± 0.04	1.78 ± 0.22***	0.78 ± 0.11**	0.44 ± 0.05
8th day	0.4 ± 0.07	0.62 ± 0.07*	1.4 ± 0.22**	1.3 ± 0.31**
11th day	0.44 ± 0.03	0.73 ± 0.05**	0.81 ± 0.03***	0.53 ± 0.02*
14th day	0.33 ± 0.02	1.43 ± 0.05***	0.64 ± 0.08**	1.37 ± 0.13***
Roots				
2nd day	0.94 ± 0.02	1.64 ± 0.18**	1.08 ± 0.03**	2.08 ± 0.16***
5th day	0.95 ± 0.02	1.44 ± 0.1**	1.63 ± 0.04***	1.05 ± 0.02*
8th day	1.01 ± 0.06	1.06 ± 0.04	1.19 ± 0.06	1.32 ± 0.11*
11th day	1.28 ± 0.04	1.16 ± 0.06	2.16 ± 0.25**	1.54 ± 0.04**
14th day	1.02 ± 0.04	1.03 ± 0.04	1.12 ± 0.12	1.57 ± 0.03***

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

Table 9. Effect of insecticides on α-amylase activity (Unit of protease/min/g FW) in tomato seedlings

	3rd day	4th day	5th day
Control	11.06 ± 1.1	15.43 ± 1.4	7.8 ± 0.42
α-cypermethrin			
25%	4.09 ± 0.6***	1.89 ± 0.17***	2.49 ± 0.43***
50%	6.03 ± 0.54**	6.07 ± 0.32***	2.71 ± 0.22***
75%	4.31 ± 1.2**	2.72 ± 0.27***	1.81 ± 0.11***
100%	4.2 ± 0.37***	3.77 ± 0.45***	1.44 ± 0.38***
Chlorpyriphos			
25%	4.63 ± 0.53**	5.93 ± 1.63***	4.49 ± 0.73**
50%	4.59 ± 1.01**	3.76 ± 0.24***	3.78 ± 0.25***
75%	9.15 ± 1	3.22 ± 0.71***	3.9 ± 0.52***
100%	3.5 ± 0.37**	2.83 ± 1.86***	2.54 ± 0.12***
Pyrimicarb			
25%	4.65 ± 0.92***	5.48 ± 0.35***	3.26 ± 0.18***
50%	1.06 ± 0.11***	1.45 ± 0.27***	2.28 ± 0.19***
75%	8.7 ± 0.92*	7.62 ± 0.36***	4.58 ± 0.41***
100%	4.11 ± 0.65***	1.03 ± 0.37***	2.48 ± 0.19***

*, ** and *** indicate significant difference at $P \leq 0.05$, 0.01 and 0.001 levels respectively.

Table 10. Effect of insecticides on α-amylase activity (Unit of protease/min/g FW) in tomato plants leaves and roots

	Control	α-cypermethrin	Chloropyriphos	Pyrimicarb
Leaves				
2nd day	16.86 ± 3.15	11.7 ± 1.3	4.43 ± 0.89**	3.08 ± 0.78**
5th day	17.18 ± 0.87	14.34 ± 0.22**	4.10 ± 1.5***	3.16 ± 0.9***
8th day	15.5 ± 3.26	4.12 ± 1.08**	3.84 ± 1.61**	6.44 ± 0.56**
11th day	18.47 ± 0.5	8 ± 0.51***	8.45 ± 1.51***	12.66 ± 0.57***
14th day	14.23 ± 3.09	5.68 ± 0.37**	2.19 ± 0.16**	7.83 ± 0.73*
Roots				
2nd day	28.63 ± 0.96	12.23 ± 1.12***	13.56 ± 0.51***	7.15 ± 0.43***
5th day	28.14 ± 4.21	8.23 ± 1.12**	14.3 ± 0.9*	7.74 ± 0.66***
8th day	15.88 ± 2.47	5.93 ± 0.15**	11.41 ± 1.53	7.27 ± 0.5**
11th day	36.23 ± 1.68	11.86 ± 1.02***	23.75 ± 1.56***	26.13 ± 2.5**
14th day	26.24 ± 2.26	12.4 ± 1.63**	24.55 ± 2.41	18.07 ± 2.6*

*, ** and *** indicate significant difference at P≤0.05, 0.01 and 0.001 levels respectively.

4. Discussion

The results obtained show that the insecticides induce an accumulation of total proteins in treated tomato seeds, and in shoots and roots of treated plants. Proteins are the primary effectors molecules of all living systems; therefore any adaptive response to environmental, physiological or pathological conditions will be eventually translated by alterations in protein activity, location and concentration [3, 25]. Wang and Zhou [29] studied the effect of chlorimuron-ethyl on wheat plants (*Triticum aestivum)*, and underlined that the soluble protein concentration in treated leaves (5, 70 and150 µg/kg of chlorimuron-ethyl) was higher than the control ones after 3 and 4 days of exposure; with increasing chlorim uron-ethyl concentrations, the soluble proteins concentration in leaves was also increased. Li et al. [14] demonstrated that soluble proteins concentration of *Triticum aestivum* seeds displayed increasing trend with the increase of the arsenic concentration. Other hand, Gianazza et al. [9] showed that there are obvious differences in proteins abundance between proteins patterns of whole plantlet extracts from *Lepidium sativum* grown under control conditions and after exposure to 200 mg/l of cadmium chloride.

In literature, it's proved that after exposure of plant to xenobiotics, there is an accumulation of many kinds of proteins, ie. (i) proteolytic proteins such as peptidase and protease [11], (ii) proteins involved in plants defence system such as peroxidase, catalase or superoxide dismutase [6, 14, 16], or (iii) proteins implied in the glutathione metabolism like glutathion-S-transferase, glutathion reductase and ascorbate peroxydase [7, 31, 32].

Concerning the protease activity, the results obtained show an increase of proteolytic activity as a response to the insecticide stress. This result is in agreement with literature reporting a stimulative effect of the proteases in the plants exposed to the xenobiotics. Lascano et al. [13] showed that the paraquat (dipyridylium) application to corn (*Zea mays*) induced a stimulation of the proteasic activity due to an accumulation of the ROS, whereas the same effect was observed after exposure of *Populus tremula* to cadmium [11]. The ROS accumulated after an oxydative stress, such as the anion superoxyde or the radical hydroxyl, interacts with proteins, deteriorating their structure. This interaction makes this complex a preferential target of the proteases, whose activity is stimulated in order to be able to eliminate them more rapidly [5].

Starch is quantitatively the most abundant storage material in all plant seeds [4]. The results obtained on this study showed a significant accumulation of starch in seeds, leaves and roots exposed to insecticides. Data from literature showed that generally when the plants are stressed by exposition to xenobiotics, the reserve substances rate and mobilisation are altered [17]. In this way, Kaushik and Inderjit [10] demonstrated that metosulfuron treated leaves of *Phaseolus aureus* had swollen chloroplasts with a large number of starch grains compared to the control, which could be attributed to reduced ability of leaf tissue to load sucrose into the phloem. In the same context, Kim et al. [12] showed that chlorsulfuron-treated canola (*Brassica napus*) leaves at 72h after treatment had swollen and disorganized chloroplasts, and starch granules were present in companion cells and mesophyll cells, unlike the control.

On the other hand, Mihoub et al. [17] demonstrated that heavy metals such as cadmium induce an accumulation of starch in *Pisum sativum,* due to the alteration of sugar mobilisation from the reserve tissues to growing tissues, and the perturbation of some enzymes responsible of their degradation.

The experiments undertaken to assess the effect of this three insecticides on amylase activity showed a very important decrease on amylase

activity in treated samples. We suggest that this inhibition of enzymes activity may be the essential cause of starch accumulation. Literature reported that amylase is inhibited by the action of many xenobiotics kinds such as pesticides and heavy metals. Chugh and Sawhney [4] demonstrate that the activities of total α-amylase and β-amylase were found to be significantly depressed by cadmium. Total amylolytic activity diminished progressively with increasing concentrations of the metal. In this context, Wilkinson [30] found that pesticides as alachlor and metolachlor inhibited GA synthesis in sorghum seedlings, inducing a decrease of α-amylase synthesis. In the same way, Mamdouh et al. [15] suggested that the decrease of α-amylase activity could result from a loss of endogenous GA. He observed that this reduction of GA level preceded the decrease of α-amylase activity. This phenomenon confirmed the regulatory role of GA on α-amylase.

Moreover, our results show generally an increase in lipids concentration in both germinating seeds and plants. The same results are reported in literature after exposure to many kinds of xenobiotics. Morinaka et al. [18] suggest that pyributicarb induce an accumulation of lipids in *Echinochloa oryzicola*, *Digitaria adscendens* and *Zea mays.* This accumulation is specifically observed in squalene rate, which is an intermediate of sterols and triterpenoids biosynthesis. Pyributicarb inhibits the metabolism of squalene at the first step by stopping the conversion from squalene to squalene epoxide catalyzed by squalene epoxidase; this action leads to a large increase in squalene rate. Another example is observed after the exposition of *Acer pseudoplantanus* to copper, which induce an increase in total lipids rate, including phospholipids, especially the phosphatidyl ethanol amine and the phosphatidyl cholines. It induces also glycolipids alterations such as an increase in digalactosyl diacylglycerol rate [22]. In addition, the exposure of *Brassica juncea* to cadmium provoked an augmentation of polar and neutral lipids [19].

5. Conclusions

It turns out that insecticides have a negative effect in both tomato seeds and growing plants. The insecticide influenced reserves substances such as starch, proteins and lipids, and also degradation enzymes like protease and α-amylase.

This effect was contrasted in an increase on total proteins concentration of treated seeds and plants, accompanied with an increase in protease activity too. Moreover, an increase in starch rate was observed in seeds and plants, due probably to the inhibition of α-amylase activity in treated samples. In other side, lipids concentration was affected too; a significant increase was notified in treated samples.

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