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Effect of three insecticides on tomato (*Solanum lycopersicum*) seedling germination and early plants growth

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Abstract

Tomato (*Solanum lycopersicum* L.) is one of the most important vegetables, whose production and consumption increased quite rapidly. The effect of three insecticides (alpha-cypermethrin, chlorpyriphos and pirimicarb) on seed germination and seedling growth of this species has been studied, based on morphological parameters monitored and by using four dilutions of the normal concentration used in agriculture (100%, 75%, 50%, 25%) for germinating seeds, and only the recommended concentration in agriculture for growing plants. The results show that the three insecticides induced a delay of germination and growth process. The germinated rate of seeds treated was lower compared to control, and the length of roots and shoots in treated seeds and plants was reduced.

Key words: Insecticides, Tomato, *Solanum lycopersicum*, Germination, Growth

1. Introduction

Tomato (*Solanum lycopersicum* L.) 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 (Oerke et al., 1994).

In the Northern Morocco, the most important way to protect cultures is the chemical pesticides use. Many pesticide types are used, especially organochlorine pesticides, organophosphorus pesticides, carbamate pesticides and pyrethroid pesticides (El Bakouri et al., 2008).

Synthetic pyrethroids are widely used as the broad-spectrum pest control agents in agricultural production because of their selective insecticidal activity, rapid biotransformation and excretion by the mammalian catabolic system and non-persistence in the environment (Ye et al., 2006). Moreover, the pyrethroid insecticides have a greater photostability and a relatively low toxicity when compared to the organochlorine and organophosphorus insecticides (Pang et al., 1994a, b). However, the risk of pesticide residues on the food consumed is present, due to the overuse and accumulation in food chain.

Organophosphorus insecticides (OP) constitute one of the most used pesticide classes employed for both agricultural and landscape pest control. Use of OP has increased considerably, due to their low toxicity and low persistence in environment and mammalian system, compared to organochlorine pesticides. The main mechanism of OP toxicity is related to irreversible binding to acetylcholinesterase (Kamath et al., 2008).

The carbamates correspond to N-substituted esters of carbamic acid and form three classes of carbamates: insecticide carbamate with a methyl group, herbicide carbamate with an aromatic or aliphatic compound, and fungicide carbamate with benzimidazol group (World Health Organization, 1986).

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 (Arias-Estevez et al., 2008). 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 (El Bakouri et al., 2008).

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Otherwise, seed dormancy and germination are complex adaptive traits in higher plants, seeing they are influenced by a numerous genes and environmental factors. The germination process starts with seed imbibition and ends with the protrusion of the embryonic axis (normally the radicle) through the enclosing tissues (Bewley and Black, 1985). This process is influenced by many environmental conditions such as salinity (Kashem et al., 2000), hydrous stress (Bradford et al., 1995), moisture and temperature (Bailly et al., 1996; Welbaum et al., 1998; Ravikumar et al., 2002).

In this study, we evaluated evolution of some morphological parameters such as germination and growth process of tomato (*S. lycopersicum*) in response to the application of three insecticides: alpha-cypermethrin (pyrethroid insecticide), chlorpyriphos (organophosphorus insecticide) and pirimicarb (carbamate insecticide), among the most used ones in the North of Morocco.

2. Materials and methods

2.1. Plant material and germination test

Tomato (*S. lycopersicum*) seeds were purchased from Vilmorin (La Ménitré, France) and surface sterilized in 10% (v/v) commercial bleach with stirring for 5 min, followed by extensive washing in sterile distilled water.

Treatment concentrations were prepared using control (distilled water) and 25%, 50%, 75% and 100% (v/v) of the original insecticide solutions (100% represents the normal concentration used in agriculture and will be diluted appropriately with sterile distilled water to give the final concentration). Batches of 50 tomato seeds were sown in 9 cm Petri dishes lined with two layers of filter paper, and 6 ml of each treatment solution was added. The filter papers were constantly moistened with the appropriate solution, and maintained in a growth chamber in darkness at 25°C for 6 days. Three repetitions were performed. At various stages of tomato seed germination (3, 4 and 5 days), seeds of each replicate were collected for measurement. Germination was determined as the time of seed coats rupture and radicle emergence.

2.2. Growing test

For growth study, ten seeds were germinated in each plastic pot (10 x 10 cm). Being watered each day, seedlings were grown together in the greenhouse (24/20°C day/night, 16/8h light/dark photoperiod). The plants used were maintained in greenhouse conditions for 30 days, and then were treated by various insecticides at the concentration used in agriculture (100%). The tests were realized on the 2^{nd}, 5^{th}, 8^{th}, 11^{th} and the 14^{th} day after treatment. Each value represents the average of 3 replications.

2.3. Parameters monitored

At the end of treatments, we have evaluated the germination rate of tomato seeds by counting the number of germinated ones in batches of 50 seeds. The root and shoot length of the germinated seeds were also measured. For the elongation of tomato growing seedlings, we scaled the length of shoots and roots. Each value represents the average of 3 repetitions.

2.4. Statistical analysis

Data were processed by using Statistica Software (Statistica, 1997) 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.

3. Results

The test period of germination was up to 5 days. The results of Table 1 showed that the germination rate of treated seeds was lower than the control ones. The germination rate of the control was around 97% in the whole test period. A significant decrease in tomato seeds germination rate was observed at different insecticide concentrations since the beginning of the test period; it was varying between 80% and 91% compared to control.

Concerning seedlings growth, different treatments showed a delay in the elongation process compared with control (Table 2). The shoot elongation rate increased concomitantly with time; but it was 25% and 50% reduced in presence of 25% and 100% treatment concentrations in the same order. A substantial inhibitory effect on shoot elongation was observed under insecticides treatment; It varies from 0.81 cm at the 3^{rd} day of treatment to 2.96 cm at the 5^{th} day for the control growing; however, it fluctuates from 0.16 cm to 1.64 cm respectively for the 100% Pirimicarb (3^{th} day) and 25% α-cypermethrin (5^{th} day).

A similar effect of insecticides was also observed in seedlings root elongation. At the 5^{th} day, it was highly reduced in presence of 25% α-cypermethrin (2.41 cm) than that of the control (3.60 cm) (Table 2).

Table 1. Insecticides effect on tomato seeds germination

	3rd day	4th day	5th day
Control	97% ± 1	97% ± 1	97% ± 1
α-cypermethrin			
25%	89% ± 1***	89% ± 1***	89% ± 1***
50%	80% ± 0***	80% ± 0***	80% ± 0***
75%	81% ± 2***	82% ± 2***	82% ± 2***
100%	88% ± 0***	88% ± 0***	88% ± 0***
Chlorpyriphos			
25%	89% ± 2**	91% ± 1**	91% ± 1**
50%	90% ± 2**	91% ± 3*	91% ± 3*
75%	87% ± 1***	90% ± 0***	90% ± 0***
100%	85% ± 3**	85% ± 3**	85% ± 3**
Pirimicarb			
25%	87% ± 1***	89% ± 3*	89% ± 3*
50%	88% ± 0***	88% ± 0***	88% ± 0***
75%	83% ± 2***	83% ± 2***	84% ± 0***
100%	84% ± 0***	84% ± 0***	84% ± 0***

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

Table 2. Insecticides effect on shoot and root elongation in tomato seedlings

	Shoot length (cm)			Root length (cm)		
	3rd day	4th day	5th day	3rd day	4th day	5th day
Control	0.81 ± 0.09	1.84 ± 0.09	2.96 ± 0.12	2.01 ± 0.14	2.81 ± 0.17	3.59 ± 0.07
α-cypermethrin						
25%	0.62 ± 0.02	1.19 ± 0.08**	1.64 ± 0.10***	1.31 ± 0.08***	2.02 ± 0.14***	2.41 ± 0.15***
50%	0.49 ± 0.08**	1.00 ± 0.25***	1.26 ± 0.33***	1.14 ± 0.08***	1.97 ± 0.16***	1.92 ± 0.21***
75%	0.50 ± 0.05**	0.90± 0.15***	1.30 ± 0.22***	1.26 ± 0.01***	1.57 ± 0.12***	2.05 ± 0.21***
100%	0.44 ± 0.07***	0.93 ± 0.17***	1.24 ± 0.08***	1.10 ± 0.03***	1.79 ± 0.05***	1.95 ± 0.14***
Chlorpyriphos						
25%	0.48 ± 0.04***	0.98 ± 0.12***	1.17 ± 0.11***	1.25 ± 0.11***	1.83 ± 0.07***	2.02 ± 0.07***
50%	0.47 ± 0.08***	0.81 ± 0.08***	0.99 ± 0.05***	1.25 ± 0.17***	1.70 ± 0.18***	1.75 ± 0.18***
75%	0.40 ± 0.02***	0.77 ± 0.04***	1.12 ± 0.03***	1.22 ± 0.04***	1.73 ± 0.04***	1.84 ± 0.05***
100%	0.23 ± 0.01***	0.47 ± 0.08***	0.63 ± 0.09***	0.59 ± 0.16***	1.13 ± 0.34***	1.20 ± 0.22***
Pirimicarb						
25%	0.28 ± 0.01***	0.58 ± 0.05***	1.00 ± 0.14***	0.74 ± 0.10***	1.42 ± 0.27***	1.97 ± 0.28***
50%	0.24 ± 0.01***	0.40 ± 0.01***	0.85 ± 0.22***	0.59 ± 0.01***	1.00 ± 0.14***	1.48 ± 0.20***
75%	0.20 ± 0.02***	0.47 ± 0.01***	0.89 ± 0.10***	0.48 ± 0.08***	1.09 ± 0.15***	1.58 ± 0.14***
100%	0.16 ± 0.01***	0.41 ± 0.06***	0.80 ± 0.08***	0.29 ± 0.02***	0.83 ± 0.09***	1.52 ± 0.26***

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

Furthermore, evaluation of length progression of growing seedlings shows a growth delay in the treated ones at the 2nd week of the test period. Shoot length of treated plantlets was around 19 cm, vs. 22.1 cm for the control ones (Table 3). Root length of treated plantlets was also lower and reaches 25 cm, vs. 26.7 cm in untreated seedlings (Table 4).

Table 3. Insecticides effect on shoot length (cm) in tomato seedlings.

	Control	α-cypermethrin	Chloropyriphos	Pirimicarb
2nd day	18.0 ± 1.0	17.9 ± 0.6	17.0 ± 2.3	16.6 ± 1.8
5th day	18.5 ± 0.5	18.1 ± 2.5	17.1 ± 0.2*	17.3 ± 1.7
8th day	19.2 ± 0.2	18.4 ± 0.3*	18.6 ± 1.0	18.1 ± 2.5
11th day	20.4 ± 0.4	18.9 ± 0.6*	18.8 ± 1.0	18.3 ± 0.7**
14th day	22.1 ± 0.6	19.6 ± 0.9*	19.8 ± 0.37**	18.7 ± 0.3**

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

Table 4. Insecticides effect on roots length (cm) in tomato seedlings.

	Control	α-cypermethrin	Chloropyriphos	Pirimicarb
2nd day	22.8 ± 0.7	22.6 ± 0.8	23.7 ± 0.8	23.2 ± 0.3
5th day	23.5 ± 0.6	23.3 ± 0.1	23.9 ± 0.1	23.7 ± 0.8
8th day	24.5 ± 0.7	23.9 ± 0.4	24.6 ± 0.1	24.2 ± 0.7
11th day	25.6 ± 0.3	24.1 ± 0.5**	24.8 ± 0.2*	24.3 ± 0.5*
14th day	26.7 ± 0.2	25.0 ± 0.5**	25.0 ± 0.4**	24.9 ± 0.4**

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

4. Conclusions and discussion

The percentage of germination may reflect the reaction rate of plant seeds to their living environment (Li et al., 2007). The results obtained in this study illustrate an inhibitory effect of germination after treatment by the tested insecticides at various concentrations. In literature, a declines in seed germination rate (more than 50 %) have been reported with other insecticides such as paraquat dichloride (l, l'-dimethyl-4, 4'-bipyridinium dichloride) at 1.0 mg/L in *Typha latifolia* (Moore et al., 1999). A similar effect was showed in *Triticum aestivum*, treated by 5–20 mg/kg of arsenic (Li et al., 2007).

The general symptom of plants under such stresses is growth inhibition (Yang et al., 2001; Wang et al., 2004; Wang and Yang, 2005). In our study, tomato seed-plantlets and seedlings growth was significantly delayed after insecticides application, affecting as well shoots as roots. In this way, many studies reported an inhibitory effect of growth after application of pesticides: (i) around 50%-decreasing of root growth in *Phaseolus vulgaris* and *Pisum sativum* after treatment with chlorsulfuron during the germination process (Fayez and Kristen, 1996), and (ii) low root growth in *Zea mays* seedlings in the presence of pesticides such as chlorsulfuron and metsulfuron-methyl (Fayez et al., 1994). In other way, this effect was also demonstrated with other xenobiotic types like heavy metals. Khatun et al. (2008) underlined a severe growth inhibition of shoots and roots in *Withania somnifera* because of copper contamination.

Many hypotheses explain this delay of growth in treated plants and seedling. Firstly, we can suggest that insecticides induce damages in the meristematic cells, given that Fayez and Kirsten (1996) showed an obvious influence of chlorosulfuron on the cellular structure of root caps of *Pisum sativum*, *Phaseolus vulgaris* and *Vicia faba*, and induce a reduction in root cell division, delaying the root growth (in Fayez and Kristen, 1996). Moreover, a similar effect was reported in literature after contamination by heavy metals. In this way, after exposition of Anatolian black pin (*Pinus nigra* spp. *pallasiana*) to different concentrations of lead, mitotic cell division was significantly decreased, and several mitotic anomalies such as c-mitosis, lagging chromosomes, multipolar anaphases and chromosome bridges were increased (Yücel et al., 2008).

In other side, the insecticides could affect the photosynthetic system by the inhibition of photosystem II and chain electron transport activities as reported for example by Mishra et al. (2008) in *Vigna unguiculata* when treated by dimethoate. Pesticides can also lead to a delay in photosynthetic pigments rates such as chlorophylls (Mishra et al., 2008).

Moreover, in literature the delay of plants growth was reported as an indicator of oxidative stress (Yang et al., 2001; Wang et al., 2004; Wang and Yang, 2005). Generally, the plants cells produce the ROS as a second messenger in some processes of growth and development (Schurmann, 2003; Borland et al., 2006; Grun et al., 2006). There is an interaction between activation and repression of ROS and phytohormones (McCarty and Chory, 2000; Delledome et al., 2003; Del Rio et al., 2006; Terman and Brunk, 2006; Shao et al., 2006, 2008). When the plant is under an oxidative stress, the excess of ROS production can lead to growth perturbations.

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