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ASSESSMENT OF THE INSECTICIDAL PROPERTY OF THE PEPPER *CAPSICUM ANNUUM* EXTRACTS ON THE LARVAL STAGES OF THE TOMATO LEAFMINER *TUTA ABSOLUTA* (LEPIDOPTERA: GELECHIIDAE)

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Abstract

The aqueous fruit extracts of the pepper *Capsicum annuum* L. (Solanales: Solanceae) were tested against the four larval stages of the tomato leafminer (TLM) *Tuta absoluta* Povolny (Lepidoptera: Gelechiidae). Random samplings were made from two greenhouses of Biskra, in the Southeast of Algeria. About 750 to 800 plants of the Sahara variety of tomatoes were planted in both greenhouses. In the laboratory, conditions with temperature, relative humidity (RH) and the photoperiod were 25° to 30°C, from 50% to 60% and 16L: 8D, respectively. A breeding of our species in the laboratory was made during six months of the study. For the toxicological assessments, three concentrations were chosen; 50 mg/L, 100 mg/L and 200 mg/L of the extracts. The results revealed that the extracts of *C. annuum* were very effective against the larvae of *T. absoluta*. After 24 h post-treatment, the mortality was less than 25% compared to that after 48h and 72h post-treatment which exceeded 50%. There was a non-significant difference in mortality rates between the four larval stages ($F_{3,}{}^{140} = 1.12$; $P = 0.344$). In contrast, highly significant differences existed between the time of exposure ($F_{2,}{}^{141} = 26.2$; $P = 0.001$) and the concentrations under study ($F_{3,}{}^{140} = 59.6$; $P = 0.001$).

Key words: Bio-insecticide, *Capsicum annuum,* Larval stages, Pepper, Tomato leafminer*, Tuta absoluta*

Introduction

The tomato is the culture which presents excellent qualities as festive as nourishing; it constitutes dietary source rich in vitamins and mineral salts (Kononkov and Vemulapalli 1988). At the world level, China is the first world producer of tomato with over 24% of the total world production in 2009. This production is mainly intended for the local consumption. The United States, which produces 10% of the world production occupies the 2nd position. They are followed by India, Turkey, Egypt and Italy. These six countries ensure more than 60% of the total world production. They are followed by 3 other producers who are Iran, Spain and Brazil (Heuse et al. 2013). The tomato production of Algeria in 2011 is 7.9 million qx for 18000 hectares of a surface (Heuse et al. 2013).

About one-third of the world agricultural production is destroyed by various devastating factors such as insects and fungal and/or viral diseases which cause enormous damages in the sowing's culture until their marketing (Thrupp 2000, Vega 2009). Whiteflies, aphids, leaf miners, acarids, thrips, noctuids, and bedbugs constitute the main devastating arthropods in greenhouses (Nicholls et al. 1998, Mascarin and Jaronski 2016). In recent years, the tomato leafminer (TLM) *Tuta absoluta*, a new but notorious pest of tomato, is reported to cause considerable losses both in greenhouses and fields (Chidege et al. 2016, Shashank et al.

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2015). Fight against TLM remains very difficult, because the insect exhibited a great resistance to many insecticides (Siquira et al. 2000, Haddi et al. 2012, Roditakis et al. 2013, Barakat et al. 2015). *T. absoluta* is a microlepidopteran insect native to South America (Torres et al. 2001, Brito et al. 2015, VMK 2017), which can attack all aerial parts such as leaves, flowers, stems and fruits of the tomato plant (Karadjova et al. 2013). This pest can cause up to 100% damage (Moreira et al. 2004, Niedmann and Meza-Basso 2006). It was first reported in Algeria in 2008 (Desneux et al. 2010).

The chemical fight against insects proved inconvenient to non-target fauna (Fradin and Day 2002). Alternative methods like biological and photochemical controls therefore have gained much attention to the scientists (Hanafy and El-Sayed 2013, Barakat et al. 2015). Numerous plants exhibit toxicological effects against many insect species, reducing their development and population growth (Gurjar et al. 2012, Shiberu and Getu 2016). We aimed by the present investigation at assessing the insecticidal properties of the aqueous fruit extracts of *C. annuum* against the larvae of *T. absoluta* under laboratory conditions.

Materials and Methods

Area study: Two study sites were chosen for sampling. The first one was El-Maleh site (Sidi Okba: 34°44'54.44"N 5°53'59.98" E), situated 10 km Southeast of Biskra (Algeria), which covered an area of 12.5 ha. Vegetable crops, plastic crops, olive trees and date palms are grown on this site. The second site was M'Ziraa (34 ° 49'54.89 "N 6 ° 16'59.68" E), located 80 km East of Biskra, extended over an area of 42 ha and dominated by market gardening and greenhouses cultures.

Sampling method and breeding of the insects: For the collection of insect specimens, one greenhouse from both sites was selected, in which 750 to 800 Sahara variety of the tomato plants were planted. Different stages of the larvae of the tomato leafminer (TLM) *Tuta absoluta* Povolny, 1917 (Lepidoptera: Gelechiidae) were applied to tomato leaves during October 2012 and March 2013, maintaining 20m is the distance between two consecutive plants. Infested leaves by the TLM were kept in cool plastic bags to prevent them from drying out. In the laboratory conditions with temperature (25° to 30°C), relative humidity (RH) 50% to 60% and the photoperiod (16L: 8D). Entomological forceps and needles were used to extract the larvae for placing under the binocular loupe for examination of the different stages. Adults were paired as soon as they emerged and placed in glass petri dishes each carrying a tender tomato leaf that will support egg-laying by the female moths.

Preparation of the aqueous extracts: Preliminary surveys showed that *C. annuum* fruit smell had repulsive effects on certain species of insects. Fine powder was prepared from dried fruits using a kitchen grinder. In three 1000 ml bottles, 0.05 g, 0.1 g and 0.2 g of the pepper powder were taken to prepare solutions of 50 mg/l, 100 mg/l and 200 mg/l concentrations, respectively with distilled water. The solution is mixed with a stirrer. The solution was left for 24 h in a place at ambient temperature and relative humidity, after which it was filtered off with a filter paper.

Toxicological tests: The aqueous extracts of *C. annuum* were sprayed onto petri dishes each containing 10 individuals from each larval stage of the insect (L1 to L4). The same number of larvae was placed in control Petri dishes for each of the concentrations of the extract. Three repetitions for each concentration were made. The mortality rate was calculated in terms of the average of the three determinations each of 10 individuals.

Statistical analysis: The variables measured corresponded to the larval mortality rates, which were corrected by the Abbott's (1925) formula. The collected data were first subjected to angular transformation according to the tables established by Bliss (Fisher and Yates 1938). Then the standardized data were subjected to one-way analysis of variance (ANOVA). To characterize the insecticidal effect of the *C. annuum* fruits used, the toxicological parameters and their confidence intervals were determined. The analyses were done using Statistics (version 8.0).

Results and Discussion

The exposure of the four larval stages (L1 to L4) of *T. absoluta* to three different concentrations of the aqueous fruit extracts of *C. annuum* showed a variable toxic effects at different times of exposure (24, 48 and 72 h)(Fig. 1). After 24 h post-treatment, the mortality was less than 25%, whereas for 48 h and 72 h post-treatments the mortality exceeded 50%. After 72 h exposure, however, the younger larvae were much more sensible compared to the older larvae, particularly L4 (Fig. 2).

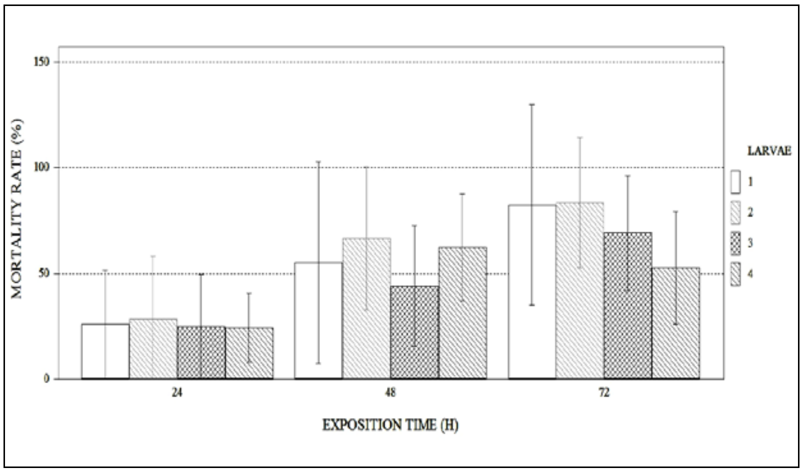

Fig. 1. Mortality of *T. absoluta* larvae against the time of exposure in hrs (1: larval stage 1, 2: larval stage 2, 3: larval stage 3, 4: larval stage 4).

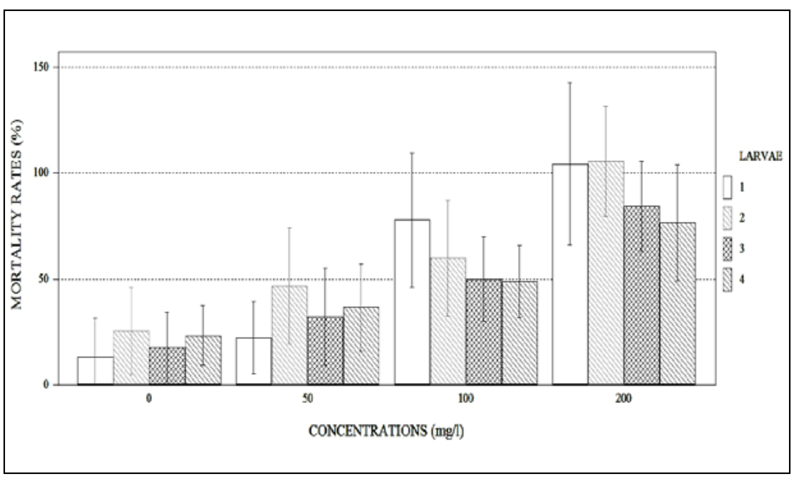

Fig. 2. Mortality of *T. absoluta* larvae against the concentrations applied (1: larval stage 1, 2: larval stage 2, 3: larval stage 3, 4: larval stage 4).

The analysis of variance (ANOVA) on the experimental data showed that there is not a significant difference between the larval stages come back to the mortality rate ($F_{3, 140}$ = 1.12; p = 0.344). But a highly significant differences were found to exist between the three exposure times ($F_{2, 141}$ = 26.2; p<0.001) and of the three concentrations used ($F_{3, 140}$ = 59.6; p<0.001).

The toxicological parameters revealed strong correlation values between concentrations, and mortality rates in the first order and also a high correlation between mortality rate and exposure time when R^2 values ranged between 0.755 and 0.949. The LD_{50} calculated to have an interval of 180.9 mg/l to 556.2 mg/l in the first and last larval stage consecutively. On the other hand, the calculated LD_{90} had an interval of 211 to 989 mg/l in the first and last larval stage consecutively (Table 1). It was therefore clear from the results that as larvae grew bigger they needed more treatment product and more exposure time for the effect to be increased.

Table1. Toxicological parameters of *C. annuum* fruit extracts against *T. absoluta* larval stages

Larval stages treated		Correlation equations	Confession of correlation	LLLD50<LD50< ULLD50 (mg/l)	LLLD90<LD90< ULLD90 (mg/l)
L1	24 h	y = 1.9932x - 0.3795	R² = 0.949	121.6<180.9<207.6	191.6<211.7<277.5
	48 h	y = 2.1593x - 0.3411	R² = 0.941		
	72 h	y = 3.7372x - 3.8982	R² = 0.841		
L2	24 h	y = 1.9932x - 0.6861	R² = 0.939	132.8<169.6<221.5	272.8<287.1<391.56
	48 h	y = 1.445x + 1.4982	R² = 0.897		
	72 h	y = 3.2721x - 1.753	R² = 0.756		
L3	24 h	y = 1.32x - 0.456	R² = 0.755	144.7<156.2<231.8	389.7<479.2<678.8
	48 h	y = 3.67x + 0.982	R² = 0.922		
	72 h	y = 1.71x - 0.553	R² = 0.834		
L4	24 h	y = 2.32x - 0.8823	R² = 0.839	404.7<556.2<891.9	709.7<989.6<1281.02
	48 h	y = 2.45x + 1.82	R² = 0.8978		
	72 h	y = 4.2721x + 0.753	R² = 0.852		

L1-L4 represents the larval stages of *T. absoluta*; $LLLD_{50}$: Lower limit of lethal concentrations 50, LD_{50}: lethal concentration 50, $ULLD_{50}$ upper limit of lethal concentration 50, $LLLD_{90}$: Lower limit of lethal concentrations 90, LD_{90}: lethal concentration 90, ULLD90 upper limit of lethal concentration 90.

Previous reports showed that the larvae of *T. absoluta* caused yield losses of up to 100% by attacking leaves, flowers, roots, and fruits (Barrientos et al. 1998, Chidege et al. 2016, VMK 2017). The results by Gacemi and Guenaoui (2012), who used Spinosad (*Azadirachtin indica*) and an insecticide *Emamectin benzoateun* against *T. absoluta*, showed excellent efficacy with 86.7% mortality on average and the persistence of the product was between 7 and 14 days. The present results are comparable to those showed by López et al. (2010) when they obtained a larval mortality rate of 90% on tomato leaves. The present results are encouraging in terms of good larvicidal activity of the aqueous fruit extracts of *C. annuum* against the larval stages of the TLM, in which up to 100% mortality after 72 h exposure at 200 mg/l was achieved. The early larval instars (L1 and L2) were sensitive to the treatments compared to the final larval instars (L3 and L4). These findings indicate that the pepper extracts can be used as a natural insecticide to fight against the notorious TLM *T. absoluta*.

The toxic effect of *C.annuum* extracts has been mentioned in several works. Madhumathy et al. (2007) proved its effectiveness against IV instar larvae *of Anopheles stephensi* and *Culex quinquefasciatus*. The treated larvae showed curling up, agitation, vigorous body movements which are the characteristic of neurotoxicity. Sukul et al. (1974) have tried the Nematicidal effect of *C.annuum*. It is recommended to illustrate the effect of primary and secondary metabolites of our fruits on parasitic and pest insects, because of its biodegradability in the marine and terrestrial ecosystem.

Conclusion

The extract used in this toxicological test is *C. annuum* against *T. absoluta* larvae. He presented an encouraging toxicological effect. The responses of the different larval stages were variable. The most precarious stages have been unsuccessful compared to late stages, which show some resistance. It is advisable to focus our research on the active principles of this extract to determine the active molecule responsible for this toxicity.

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