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Susceptibility of *Spodoptera littoralis* (Boisduval) larvaeto magnetism and some biorationale insecticides

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Abstract: The Egyptian cotton leafworm, *Spodoptera littoralis*, is a dangerous pest to many economic crops, including vegetable hosts such as tomato, cucumber and potato. In this study, extracts of tomato, cucumber and potato leaves were tested against the fourth-instar larvae of *S. littoralis*. Moreover, exposure to nanosilica, chlorfluazuron (IGR) or a magnetic field was also tested. The results explained that tomato leaf extract was the most toxic agent compared with cucumber and potato leaf extracts, nanosilica, and chlorfluazuron. When the LC_{50} of each extract was exposed to different periods of a magnetic field (10, 20 & 30 min), there was a positive correlation between the period of exposure and the toxicity. In addition, the binary mixture of each plant leaf extract and nanosilica or chlorflazuron (1:1), at the level of the LC_{25}, revealed a synergistic effect, which was the highest for tomato leaf extract and chlorfluazuron.

keywords: cotton leaf worm, susceptibility, magnetism, biorationale insecticides

1.Introduction

The Egyptian cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is considered one of the most destructive pests of most vegetables, crops and various fruits in Asia, Africa and Europe **(1)** and **(2)**. It infests about 112 host plants of different families in Middle East countries, as well as in Egypt **(3)** and **(4)**, leading to reducing the marketability of ornamentals and vegetables **(5)**.

The most common and effective control measure of *S. littoralis* has been achieved by chemical insecticides, but the long-term use of insecticides has helped in the development of resistance to this pest **(6),** and **(7).** In addition, the unwise and continuous use of insecticides to control agricultural pests leads to adverse effects on wildlife, beneficial insects, fish, animals, hazards to man, environmental pollution and toxic residues in foods **(8)** and **(9).**

To avoid the disadvantages of insecticides, the researchers have searched for eco-friendly and effective alternatives. Among them are the natural products of plant origin, magnetism and nano-materials that are receiving considerable attention **(10).** Although tomato, potato and cucumber leaves are by-products, they contain bioactive metabolites that can be used in the management of agricultural pests **(11)** and **(12).**

This study aims to evaluate the potential toxic effects of tomato, potato and cucumber leaf extract, chlorfluazuron as an insect growth regulator, nanosilica and a magnetic field against *S. littoralis* larvae.

2. Materials and methods

2.1. Insect culture

A laboratory strain of *S. littoralis* was initiated from the Division of Cotton Leafworm, Plant Protection Research Institute (PPRI), Dokki, Egypt. Larvae were reared in the laboratory on castor bean leaves (*Ricinus communis* L.) that were provided daily. Adults were fed on a 10% sugar solution and mated on the 3rd day of emergence in clean jars containing some leaves of tafla, *Nerium oleander* (L.) as an oviposition medium, **(13).** Insects were kept at constant conditions of 27± 2ºC, photoperiod of 14-h light and 10-h dark and 65 ± 5% RH.

2.2. Preparation of stock solutions of the plant extracts

Leaves of tomato (*Solanum lycopersicum* L.) (Solanaceae), cucumber (*Cucumis sativus* L.) (Cucurbitaceae) and potato (*Solanum tuberosum* L.) (Solanaceae) were collected from an organic farm, Mansoura city. They were authenticated by plant taxonomists in the PPRI. They were thoroughly washed with distilled water, shade-dried for one month at the room temperature, then separately ground into fine powder (~ 500 g each) using electric miller. The powder of each plant was soaked in a mixture of ethanol, acetone and petroleum ether, as solvent of equal proportion (1:1:1), in a tightly sealed flask for about one week. The flask was shaken with a shaker, and its content was filtered with Whatman no 1 filter paper. Then, the solvent was evaporated under reduced pressure. The dried crude extract was weighed and then diluted in distilled water plus 0.01% Tween-80 (Merck), as a nonionic surfactant, to provide a 1% stock solution (w/v). A series of dilutions were performed to obtain the desired concentrations and then kept at 4°C until use.

2.3. Preparation of a stock solution of chlorfluazuron and nanosilica

Chlorfluazuron (5% EC.) was purchased from Sumitomo Chemical Company, Egypt. It was prepared by dissolving chlorfluazuron plus 0.01% Tween-80 in distilled water to give a stock solution of 1000 ppm (l:l). Nanosilica was kindly offered by Zewail University, Egypt. A stock solution of 1000 ppm was also prepared by dissolving it in distilled water plus Tween-80 (l:l). A series of dilutions were performed to obtain the desired concentrations and then kept at 4°C until use.

2.4. Bioassays

Newly molted fourth-instar larvae of *S. littoralis* (33.75 mg each, < 11 day old) were chosen for the bioassays **(14).** Equal leaf discs of castor bean were dipped separately in four concentrations of each tested compound for 20 seconds, then left to dry. Ten larvae per each concentration were fed for 24 h on the treated leaves. Each concentration was replicated three times (a total of 30 larvae). A parallel control experiment was also conducted and repeated three times with 10 larvae each. The treated and untreated larvae were kept at 25 ± 2°C and 70% RH. After 24 h of treatment, the treated leaves were replaced daily by fresh untreated leaves for 7 days. The percentage of mortality was recorded daily and corrected using Abbott's formula **(15).** Then, data were subjected to probit analysis **(16)** to determine the LC_{50} and LC_{90}.

The toxicity index **(17),** at the level of LC_{50}, was determined as follows:

$$\text{Toxicity index} = \frac{LC_{50}\,\text{of the most toxic compound}}{LC_{50}\ \text{of each tested compound}}$$

2.5. Combined effects of plant extracts and magnetic field

The LC_{50} of each plant extract was estimated from the above-described bioassays. Then, 100 ml of each LC_{50} were separately exposed to the magnetic field with a power of 180 millitesla for 10, 20, and 30 min **(18).** The magnetic field was placed in the center of the magnetic apparatus (Patent 1663/ 2018, PPRI, ARC). Then, the extracts were filled in glass tubes and fixed in the center of the magnetic apparatus for the fixed time between the two poles of the apparatus **(18).** The LC_{50} of each plant extract at the three periods was used for dipping discs of castor bean leaves. Larvae fed on castor bean leaf discs dipped in distilled water, without exposure to magnetic field, considered as negative controls; while positive controls consisted of larvae treated with the LC_{50} of each plant extract without magnetism. Mortality percentage was recorded after 1, 3, 5 and 7 days after treatment.

2.6. Combined effects of plant extracts and chlorfluazuron and nanosilica

The interaction between each plant leaf extract of tomato, cucumber and potato as well as nanosilica and chlorfluazuron in relative to larval mortality, using the LC_{25} (at the ratio of 1:1), was differentiated according to the co-toxicity factor **(19)** as follows:

$$\text{Co} - \text{toxicity factor}\ (\%) = \frac{(\text{Observed mortality\%} - \text{Expected mortality \%})}{(\text{Expected mortality \%})}$$

Where:

- A positive factor of 20 or more is considered as synergism,

- A negative factor of 20 or more is considered as antagonism,
- Intermediate values (-20 and +20) are considered as an additive effect.

3. Results and Discussion

3.1. Post-treatment time-toxicity relationship against *S.littoralis*

Overall, the mortality percentage increased with the post-treatment time for all the tested concentrations (Table 1). The results showed that at 100, 250, 500, and 750 ppm, the total mortality percentages were 50, 76.66, 86.66 and 93.33%, respectively, with tomato leaf extract. These total percentages were 46.67, 60, 80, and 90%, respectively, with cucumber leaf extract, but they were 33.33%, 56.67%, 66.67% and 80%, respectively, with potato leaf extract. Nanosilica had the lowest effect with total mortality percentages of 13.33, 20, 30 and 46.67% at 10, 100, 1000 and 5000 ppm, respectively. The percentages were 56.67, 70, 83.33 and 96.67% at 50, 100, 200 and 300 ppm for chlorfluazuron, respectively.

3.2. Medium lethal concentration (LC_{50}) and 90% lethal concentration (LC_{90}) of tested biorational insecticides against *S. littoralis*

Results in **Table (2)** indicated that chlorfluazuron was the effective compound to larvae of *S. littoralis*, with LC_{50} of 44.53 and LC_{90} of 238.74 ppm. While the LC_{50} values of tomato leaf extract, cucumber leaf extract, potato leaf extract and nanosilica were 97.95, 130.97, 204.07 and 13449.66 ppm, respectively. The LC_{90} values were 579.05, 952.95, and 1769.78 and 33861867.6 ppm, respectively for the same compounds, respectively. Slope values were 1.76, 1.67, 1.49, 1.38 & 1.37 for chlorfluazuron, tomato leaf extract, cucumber leaf extract, nanosilica, and potato leaf extract, respectively. LC_{90}/LC_{50} values confirm the value of this criterion and reported 5.36, 5.91, 7.28, 8.67, and 2517.7 for chlorfluazuron, tomato leaf extract, cucumber leaf extract, potato leaf extract, and nanosilica, respectively. Hence, the highest slope value or the lowest ratio LC_{90}/LC_{50} means the steepest toxicity line. This means that tomato leaf extract was the most effective natural material and its mortality results approach the results of the synthetic insecticide chlorfluazuron. These results agree with **(20)**, who evaluated the efficiency of chlorfluazuron to control *S. littoralis.* Also**, EL Helaly *et al.*** (**21)** recorded the effectiveness of 500 ppm of nanosilica on some species of aphid. While, **Abd-Allah *et al.* (22)** tested the toxic effect of tomato leaf extract against *Tuta absoluta* and *S. littoralis*, and the total mortality rate reached 83.33% at 2000 ppm and 93.33% at 8000 ppm for the two pests, respectively. **Haga *et al.* (23)** attributed the higher toxicity of chlofluazuron to slower detoxification in the insect body.

3.3. Combined effects of plant extracts and magnetism

Data in **Table (3)** reveal that at 10, 20 & 30 min of exposure to the magnetic field, the mortality rate was 10, 23.33 & 46.67%, respectively with tomato leaf extract; 6.67, 20 & 40%, respectively with potato leaf extract and 3.33, 13.33 & 36.67%, respectively with cucumber leaf extract. **Hussein *et al.* (13)** and **Shehata *et al*. (18)** indicated that a magnetic field improved the properties of pesticides when applied to *S. littoralis*.

3.4. Combined effects of plant extracts and chlorfluazuron or nanosilica against *Spodoptera littoralis*

Table (4) shows that in binary combination (1:1), at the LC_{25} level, the highest co-toxicity factor (+ 80) was obtained in case of combining tomato leaf extract with chlorfluazuron. **El-Sheikh** (**24)** found that the combined effect of lufenuron + spinosad or emamectin benzoate was either additive or antagonistic on third and fifth instar larvae of *S. littoralis*.

In conclusion, the tested control agents are promising alternatives to conventional insecticides to control *S. littoralis* based on natural insecticides less damaging to local ecosystem. The tested agents are good candidates through integrated pest management approach to control *S. littoralis*, leading to decreasing the development of resistance.

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Table (1): Corrected mortality % of 4thinstar larvae of *S. littoralis* treated with certain bio-rational insecticides**.**

Treatments	Conc.(ppm)	Mortality %				Total mortality %
		One day	Three days	Five days	Seven days	
Tomato leaf extract	100	------	20	20	10	50
	250	3.33	30	33.33	10	76.66
	500	10	33.33	40	3.33	86.66
	750	10	26.67	33.33	23.33	93.33
Cucumber leaf extract	100	------	23.33	16.67	6.67	46.67
	250	10	30	10	10	60
	500	16.67	40	16.67	6.67	80
	750	23.33	40	20	6.67	90
Potato leaf extract	100	-----	10	13.33	10	33.33
	250	-----	16.67	30	10	56.67
	500	6.67	30	26.67	3.33	66.67
	750	10	30	30	10	80
Nanosilica	10	-----	-----	6.67	6.67	13.33
	100	-----	3.33	6.67	10	20
	1000	-----	6.67	10	13.33	30
	5000	3.33	10	16.67	16.67	46.67
Chlorfluazuron	50	-----	6.67	26.67	23.33	56.67
	100	-----	10	30	30	70
	200	3.33	13.33	30	36.67	83.33
	300	3.33	16.67	33.33	43.33	96.67

Table (2): Toxicity of certain biorational insecticides to *S. littorali*

Treatments	Conc. (ppm)	Corrected mortality%	LC_{50} (ppm)	LC_{90} (ppm)	Slope± S.D.	Toxicity index LC_{50}	LC_{90}/ LC_{50}	R	P
Tomato leaf extract	100	50	97.95	579.05	1.67± 0.22	45.47	5.91	0.997	0.836
	250	76.66							
	500	86.66							
	750	93.33							
Cucumber leaf extract	100	46.67	130.97	952.95	1.49± 0.21	33.99	7.28	0.974	0.217
	250	60							
	500	80							
	750	90							
Potato leaf extract	100	33.33	204.07	1769.78	1.37± 0.2	21.82	8.67	0.989	0.608
	250	56.67							
	500	66.67							
	750	80							
Nanosilica	10	13.33	13449.66	33861867.6	1.38± 0.07	21.82	2517.7	0.981	0.544
	100	20							
	1000	30							
	5000	46.67							
Chlorfluazuron	50	56.67	44.53	238.74	1.76± 0.25	100	5.36	0.946	0.093
	100	70							
	200	83.33							
	300	96.67							

Table (3): Mortality of certain plant extracts magnetized for different periods against larvae of S. littoralis

Treatments	LC_{50} (ppm)	Magnetization Time (min.)	Mortality after treatments %				Total Mortality %
			One day	Three days	Five days	Seven days	
Tomato leaf extract	97.95	10	-----	-----	3.33	6.67	10
		20	-----	6.67	10	6.67	23.33
		30	6.67	10	16.67	13.33	46.67
Potato leaf extract	204.07	10	-----	-----	3.33	3.33	6.67
		20	-----	3.33	10	6.67	20
		30	3.33	6.67	16.67	13.33	40
Cucumber leaf extract	130.97	10	-----	-----	-----	3.33	3.33
		20	-----	-----	3.33	10	13.33
		30	3.33	6.67	10	16.67	36.67

Table (4): Interaction of tomato, potato and cucumber leaf extracts combined with nanosilica or chlorfluazuron on *S.littoralis* larvae:

Treatments	Binary mixture	%mortality	Co-toxicityfactor%	Typeof interaction
Tomato + Nanosilica	$LC_{25} + LC_{25}$	66.67	33.34	Synergism
Potato leaf extract + Nanosilica	$LC_{25} + LC_{25}$	53.33	6.66	Synergism
Cucumber leaf extract + Nanosilica	$LC_{25} + LC_{25}$	60.00	20.00	Synergism
Tomato leaf extract + Chlorfluazuron	$LC_{25} + LC_{25}$	90.00	80.00	Synergism
Potato leaf extract + Chlorfluazuron	$LC_{25} + LC_{25}$	70.00	40.00	Synergism
Cucumber leaf extract + Chlorfluazuron	$LC_{25} + LC_{25}$	80.00	60.00	Synergism

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