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A comparative Study on The 2nd and 4th Instar Larvae of Cotton Leafworm, *Spodoptera Littoralis* Treated with Methomyl and Spinetoram in The Laboratory.

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Abstract

The effects of two insecticides, namely Methomayl and Spinetoram against the 2nd and 4th instars larvae of cotton leafworm *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) by LC_{50} concentrations were evaluated under laboratory conditions to evaluate the mortality percentage and biochemical effects. The results showed that larval mortality rate was positively correlated with the increase of concentrations of the tested insecticides, the methods of tested and time after exposure. The mortality rate in Methomayl was higher than Spinetoram. The two tested compounds caused significant decrease in the stimulation of acetylcholinesterase (AChE) and Total proteins, Total carbohydrates, GOT, GPT, Proteases, Amylase and Lipase activity and caused significant increase in the activity of GST, alpha esterase, Alkaline phosphatase.

Keywords: *Spodoptera littoralis, Cotton leaf worm, Methomyl, Spinetoram, Biochemical.*

Introduction

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval, 1833), a moth of the family Noctuidae (Lepidoptera) is a polyphagous insect pest that attacks a wide range of host plants, the larval stage is very destructive to several field crops such as cotton, vegetables, and some horticultural crops in both greenhouses and open fields, it is widely in Egypt Africa and Mediterranean Europe **(Ahmed *et al.*, 2019; Kandil *et al.*, 2003).** The control of *S.littoralis* depends mainly on using chemical insecticides **(Pavela and Vrchotová, 2013)** which causes a lot of serious problems such as resistance of insect pests to pesticides and pesticide residues in food that pose serious to human health and affects all living organisms **(Ayoub *et al.*, 2021; Moawad, 2021; Guedes *et al.*, 2016; Badalamenti *et al.*, 2021).**

The insecticide methomyl, an oxime carbamate, was first introduced in 1968 for broad spectrum control of several insect classes, including Lepidoptera, which infects various field crops, vegetables and fruits **(Kidd and James 1991).** Furthermore, Methomyl is classed as being highly toxic to humans, fish and aquatic invertebrates **(Farre *et al.*, 2002)**. Mode of Action of methomyl Similar to other carbamate insecticides, methomyl inhibits acetylcholinesterase (AChE), which is contained within synaptic junctions between neurons resulting in nerve and/or tissue failure and possibly death. **(Kuhr and Dorough 1976)**, Considered highly toxic to insects (larval and adult stages).

Spinetoram is a new semi-synthetic spinosyn developed by Dow AgroSciences (Indianapolis, U.S.A.) and registered under the Reduced Risk Pesticide Initiative by the U.S. Environmental Protection Agency (EPA) **(US.E.PA 1997; Powell & Giles 2008).** Spinetoram is produced from derivatives of the soil bacterium, Saccharopolyspora spinosa. It activates the nicotinic acetylcholine receptors **(Salgado 1998).** This compound is characterized by a high safety profile and relatively long persistence **(Yee *et al.*, 2007).**

The aim of this is study was directed to focus out the change in the activities of some important enzymes of the 2nd and 4th larvae of *Spodoptera littoralis* treated with Methomayl and Spinetoram insecticides i.e. (total protein, Total carbohydrate, protease, Lipase, amylase, acetylcholinesterase, glutathione-s-transferase (GST), phosphatases, Glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and α-Esterase).

Materials and methods

1. Mass Rearing:

A laboratory strain of *Spodoptera littoralis* (Boisd.) has been reared in the Syngenta Group Company Qaha, Al- Qalyubia Governorate (Egypt), reared on fresh leaves of castor bean, *Ricinus communis*, as described by **El-Defrawi *et al.*, (1964).** Under laboratory condition, the strain was reared at 25±2°C and 65±5% relative humidity without any chemical contamination. The insects

tested were reared for two generations before starting the experiments.

2. Tested compounds:

2.1. Common name: Spinetoram.

Trade name: **Rediant**12% EC obtained from Shams Chemicals Company.

Fermentation product of *Saccharopolyspora spinosa*, and an analogue of spinosad, a spinosyn.

2.2. Common name: Methomyl.

Trade name: Lannate 90% SP obtained from Land Green International.

Methomyl is an n-methyl carbamate insecticide.

3. Bioassay:

The experiment was conducted on insects in three different method: it is Feeding Method, Topical Application and Contact method against the 2nd& 4th instar larvae of the laboratory strain.

Five different concentrations of each tested pesticide were prepared from the stock solutions by diluting with water (v/v) in volumetric flasks to give the necessary concentrations which produce mortality between 20-80% of each pesticide. The mortality was corrected by Abbott formula **(Abbott, 1925).**

4. Methods of testing:

4.1. Feeding Method: The assess of the toxicity of the tested pesticides against the 2nd& 4th instar larvae of the laboratory strain and , was evaluated using leaf dipping technique according to **(Shepard 1958 ; Fahmy *et al.*, 1988)** as follows:

Fresh castor leaves were dipped in each concentration of the tested pesticide for 20 seconds and dried in the air before being offered to the larvae for feeding. Ten healthy larvae with three replicates were used for each concentration of the tested pesticide. The larvae were left to feed on treated leaves for 24 h. then the alive larvae were transferred to untreated leaves.

4.2. Topical Application: Topical application method was used to assess the activity of the tested pesticides against the 2nd & 4th instar larvae of the laboratory strain according to **Li *et al.*, (2007).** Larvae weighted 22 mg ± 2 were treated on the dorsal thoracic region with 1µl of each pesticides dilution using micro applicator (Burkad, United Kingdom). Three replicates for each concentration were used and each replicate containing 10 larvae. The larvae fed on castor leaves.

4.3. Contact method: *S. littoralis* larvae of both weight groups were exposed in closed 9 cm diameter Petri dishes for 90 min to pesticide residues which sprayed in petri dishes left to dry. 10 larvae were put in Petri dish with three replications per concentration in an experiment, following the exposure, Castor leaves were placed for the larvae to feed on. **(Simon Ascher and Nadia E. Nemny 1976).**

The mortality percentages were corrected using **Abbott's formula (1925)** as follows:

Mortality % = (% Observed mortality - % Control mortality / 100 - % Control mortality).

5. Biochemical studies:

5.1. Preparation of insects for analysis:

The insects were prepared following the method described by **Amin (1998).** They were homogenized in distilled water at a ratio of 50 mg per 1 ml. The homogenates were then centrifuged at 8000 r.p.m. for 15 minutes at 2ºC using a refrigerated centrifuge. The deposits were discarded, and the supernatants, referred to as enzyme extracts, can be stored for at least one week without significant loss of activity when kept at temperatures below 0ºC.

5.2. Preparation of samples for biochemical studies:

Insects were homogenized for biochemical analysis using a chilled glass Teflon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland). Following homogenization, the supernatants were stored in a deep freezer at -20ºC until they were needed for biochemical assays. Absorbance measurements of colored substances or metabolic compounds were conducted using a double beam ultraviolet/visible spectrophotometer (Spectronic 1201, Milton Roy Co., USA).

5.3. Determination of enzymes activities:

5.3.1. Determination of total proteins was carried out according to **Bradford (1976).**

5.3.2. Determination of total carbohydrates was carried out according to **Crompton and Birt (1967).**

5.3.3. Glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) were determined colorimetrically according to **Reitman and Frankle (1957).**

5.3.4. Determination of protease was done according to **Araman and Boctor (1972).**

5.3.5. Determination of Lipase was as described by ***Tsujita et al.*, (1989).**

5.3.6. Determination of amylase was done according to **Amin (1998).**

5.3.7. Determination of acetylcholinesterase (AChE) was done according to **Simpson (1964).**

5.3.8. Determination of glutathion s-transferase (GST) was carried out according to **Habig *et al.*, (1974).**

5.3.9. Determination of phosphatases was done according to **Powell and Smith (1954).**

5.3.10. Determination of α-Esterase was done according to **Van Asperen K. (I962).**

2.7. Statistical analysis:

The slope and LC_{50} values for each pesticide were calculated by probit analysis using Ldp line software according to **Finney (1971)**, and the Toxicity index (Ti) was calculated using the following equation **(Sun, 1950).**

$$\text{Toxicity index} = \frac{\text{LC50 of the most toxic compound}}{\text{LC50 of other compounds}} \times 100$$

Results and Discussion

1. Toxicological studies on the laboratory strains:

Effect of treatments with insecticides Methomyl and Spinetoram on 2nd& 4th instar larvae of *Spodoptera littoralis* were studied.

The larvae fed on treated leaves for 24 h. with Methomyl and Spinetoram in case of feeding method, larvae were treated on the dorsal thoracic region with 1 µl of each insecticides dilution using micro applicator in case of Topical Application method and larvae were exposed in closed 9 cm diameter Petri dishes for 90 min to insecticides residues which sprayed in petri dishes left to dry in case of contact method. The mortality percentage was recorded to calculate the LC_{25}, LC_{50}, LC_{90} and LC_{95} values.

Data in **Table (1),** show the mortality percentages values of Methomyl for the 2nd larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

The highest effect of the tested methods was feeding followed by topical application then Contact method.

Results are presented in **Table (1),** cleared that the mortality percentages with 50, 40, 30, 20 and10 ppm of Methomyl concentrations for the 2nd larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

The 50 ppm concentration of Methomyl after 3rd day of treatment was the most effective treatment against the 2nd larvae in case of feeding method, the mortality percentages reached to 96.67, 100 and 100 % after 1, 2 and 3 days of treatment, respectively. Also, the 50 ppm concentration of topical application was more effective than contact method after feeding method by 93.33, 93.33 and 93.33 % mortality for the 2nd larval instar after 1, 2 and 3 days of treatment, respectively. But, the 50 ppm concentration of Contact method treatment was the lowest effect for 2nd larvae of leafworm mortality percentage after 1, 2 and 3 days of treatment by 73.33, 86.67 and 90.00 %, respectively. While, the 10 ppm concentration of Methomyl in case of contact method was the lowest effective one which revealed to 23.33, 33.33 and 36.67 % after 1, 2 and 3 days of treatment, respectively.

Table 1. The Mortality percentages of the 2nd larval instar of *Spodoptera littoralis* treated with Methomyl in case of feeding, topical application and Contact method.

Method	Concentration (ppm)	Mortality percentages			Mean
		Period (day)			
		1	2	3	
feeding	50	96.67±3.33	100±0.00	100±0.00	98.89±1.11
	40	73.33±3.33	80.00±5.77	86.67±3.33	80.00±2.89
	30	56.67±3.33	66.67±3.33	70.00±0.00	64.44±2.42
	20	40.00±5.77	46.67±3.33	53.33±3.33	46.67±2.89
	10	23.33±3.33	33.33±3.33	36.67±3.33	31.11±2.61
Topical application	50	93.33±3.33	93.33±3.33	93.33±3.33^{aA}	93.33±1.67
	40	66.67±3.33	76.67±3.33	83.33±3.33^{bA}	75.56±2.94
	30	43.33±6.67	50.00±5.77	63.33±3.33^{cA}	52.22±4.01
	20	30.00±5.77	43.33±3.33	53.33±3.33^{dA}	42.22±4.01
	10	16.67±3.33	26.67±3.33	36.67±3.33^{eA}	26.67±3.33
Contact	50	73.33±3.33	86.67±3.33	90.00±0.00	83.33±2.89
	40	66.67±6.67	70.00±5.77	76.67±3.33	71.11±3.09
	30	50.00±5.77	56.67±3.33	60.00±5.77	55.56±2.94
	20	16.67±3.33	26.67±3.33	36.67±3.33	26.67±3.33
	10	6.67±3.33	20.00±5.77	23.33±3.33	16.67±3.33

Data in **Table (2),** showed the mortality percentages of Methomyl for the 4th larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method. Results are presented in **Table (2),** showed that the mortality percentages with 80, 70, 60, 50, 40 and 30 ppm of Methomyl concentrations for the 4th larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method. Also, the highest effect of the tested methods were feeding followed by topical application then Contact method. The 80 ppm concentration of Methomyl after 3rd day of treatment was the most effective treatment against the 4th larvae in case of feeding method, the mortality percentages reached to 73.33, 86.67 and 90.00 % after 1, 2 and 3 days of treatment, respectively. Also, the 80 ppm concentration of topical application was more effective than contact method after feeding method by 66.76, 76.67 and 83.33 % mortality for the 4th larval instar after 1, 2 and 3 days of treatment, respectively. But, the 80 ppm concentration of Contact method treatment was the lowest effect for 4th larvae of leafworm mortality percentage after 1, 2 and 3 days of treatment by 53.33, 60.00 and 76.67 %, respectively. While, the 30 ppm concentration of Methomyl in case of contact method was the lowest

effective one which revealed to 0.0, 6.67 and 13.33 % after 1, 2 and 3 days of treatment, respectively.

Table 2. The Mortality percentages values of methomyl for the 4th larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Method	Concentration (ppm)	Mortality percentages Period (day)			Mean
		1	2	3	
Feeding	80	73.33±3.33	86.67±3.33	90.00±0.00	83.33±2.89
	70	60.00±5.77	70.00±5.77	76.67±3.33	68.89±3.51
	60	50.00±0.00	56.67±3.33	60.00±5.77	55.56±2.42
	50	33.33±3.33	40.00±5.77	46.67±3.33	40.00±2.89
	40	23.33±3.33	26.67±3.33	36.67±3.33	28.89±2.61
	30	3.33±3.33	16.67±6.67	20.00±5.77	13.33±3.73
Topical application	80	66.67±3.33	76.67±3.3	83.33±3.33	75.56±2.9
	70	60.00±5.77	63.33±3.33	70.00±5.77	64.44±2.9
	60	43.33±3.33	46.67±3.33	53.33±3.33	47.78±2.22
	50	26.67±3.33	33.33±3.33	36.67±3.33	32.22±2.22
	40	16.67±3.33	20.00±5.77	26.67±3.33	21.11±2.61
	30	3.33±3.33	10.00±5.77	16.67±3.33	10.00±2.89
contact	80	53.33±3.33	60.00±5.77	76.67±3.33	63.33±4.08
	70	46.67±8.82	50.00±5.77	66.67±3.33	54.44±4.44
	60	33.33±3.33	40.00±5.77	50.00±5.77	41.11±3.51
	50	20.00±0.00	33.33±3.33	43.33±3.33	32.22±3.64
	40	6.67±3.33	13.33±3.33	23.33±3.33	14.44±2.94
	30	0±0	6.67±3.33	13.33±3.33	6.67±2.36

Data in **Table (3),** show the mortality percentages values of Spinetoram for the 2nd larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method. The highest effect of the tested methods was feeding followed by topical application then Contact method. Results are presented in **Table (3),** cleared that the mortality percentages with 50, 40, 30, 20 and10 ppm of Spinetoram concentrations for the 2nd larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

The 50 ppm concentration of Spinetoram after 3rd day of treatment was the most effective treatment against the 2nd larvae in case of feeding method, the mortality percentages reached to 33.33, 66.67 and 93.33 % after 1, 2 and 3 days of treatment, respectively. Also, the 50 ppm concentration of topical application was more effective than contact method after feeding method by 36.67, 63.33 and 86.67 % mortality for the 2nd larval instar after 1, 2 and 3 days of treatment, respectively. But, the 50 ppm concentration of Contact method treatment was the lowest effect for 2nd larvae of leafworm mortality percentage after 1, 2 and 3 days of treatment by 30.00, 53.33 and 70.00 %, respectively. While, the 10 ppm concentration of Methomyl in case of contact method was the lowest effective one which revealed to 3.33, 10.00 and 23.33 % after 1, 2 and 3 days of treatment, respectively.

Table 3. The Mortality percentages values of Spinetoram for the 2nd larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Method	Concentration (ppm)	Mortality percentages Period (day)			Mean
		1	2	3	
Feeding	50	33.33±3.33	66.67±3.33	93.33±3.33	64.44±8.84
	40	26.67±3.33	60.00±5.77	80.00±5.77	55.56±8.18
	30	16.67±3.33	36.67±3.33	70.00±10.00	41.11±8.41
	20	6.67±3.33	23.33±3.33	53.33±6.67	27.78±7.22
	10	3.33±3.33	20.00±5.77	36.67±3.33	20.00±5.27
Topical application	50	36.67±3.33	63.33±8.82	86.67±3.33	62.22±7.78
	40	30.00±0.00	56.67±6.67	73.33±6.67	53.33±6.87
	30	23.33±3.33	36.67±3.33	56.67±3.33	38.89±5.12
	20	16.67±6.67	23.33±3.33	40.00±5.77	26.67±4.41
	10	6.67±3.33	20.00±5.77	26.67±3.33	17.78±3.64
Contact	50	30.00±0.00	53.33±8.82	70.00±5.77	51.11±6.55
	40	23.33±3.33	40.00±5.77	63.33±6.67	42.22±6.41
	30	10.00±0.00	26.67±3.33	46.67±6.67	27.78±5.72
	20	6.67±3.33	16.67±3.33	30.00±5.77	17.78±4.01
	10	3.33±3.33	10.00±5.77	23.33±6.67	12.22±4.01

Data in **Table (4),** showed the mortality percentages of Spinetoram for the 4th larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Table 4. The Mortality percentages values of Spinetoram for the 4th larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Method	Concentration (ppm)	Mortality percentages Period (day)			Mean
		1	2	3	
Feeding	50	33.33±3.33	56.67±6.67	80.00±5.77	56.67±7.26
	40	26.67±3.33	43.33±3.33[b]	66.67±8.82	45.56±6.48
	30	20.00±0.00	36.67±3.33	50.00±5.77	35.56±4.75
	20	16.67±3.33	23.33±3.33	33.33±3.33	24.44±2.94
	10	10.00±5.77	16.67±3.33	26.67±3.33	17.78±3.24
Topical application	50	26.67±3.33	43.33±3.33	73.33±6.67	47.78±7.22
	40	20.00±5.77	36.67±6.67	60.00±5.77	38.89±6.55
	30	13.33±3.33	30.00±5.77	46.67±8.82	30.00±5.77
	20	6.67±3.33	16.67±3.33	30.00±5.77	17.78±4.01
	10	3.33±3.33	10.00±5.77	23.33±3.33	12.22±3.64
Contact	50	23.33±3.33	46.67±3.33	66.67±8.82	45.56±6.89
	40	20.00±0.00	33.33±3.33	50.00±5.77	34.44±4.75
	30	16.67±3.33	26.67±3.33	43.33±3.33	28.89±4.23
	20	6.67±3.33	16.67±3.33	26.67±3.33	16.67±3.33
	10	3.33±3.33	10.00±5.77	20.00±0.00	11.11±3.09

Results are presented in **Table (4),** showed that the mortality percentages with 50, 40, 30, 20 and 10 ppm of Spinetoram concentrations for the 4th larval instar of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Also, the highest effect of the tested methods were feeding followed by topical application then Contact method.

The 50 ppm concentration of Spinetoram after 3rd day of treatment was the most effective treatment against the 4th larvae in case of feeding method, the mortality percentages reached to 33.33, 56.67 and 80.00 % after 1, 2 and 3 days of treatment, respectively. Also, the 50 ppm concentration of topical application was more effective than contact method after feeding method by 26.67, 43.33 and 73.33 % mortality for the 4th larval instar after 1, 2 and 3 days of treatment, respectively. But, the 50 ppm concentration of Contact method treatment was the lowest effect for 4th larvae of leafworm mortality percentage after 1, 2 and 3 days of treatment by 23.33, 46.67 and 66.67 %, respectively. While, the 10 ppm concentration of Spinetoram in case of contact method was the lowest effective one which revealed to 3.33, 10.00 and 20.00 % after 1, 2 and 3 days of treatment, respectively.

Data in Table (5), and Fig. 1 showed that the LC_{25}, LC_{50}, LC_{90} and LC_{95} values of methomyl for the 2nd larval instar of *S. littoralis* in case of feeding, topical application and Contact method. The LC_{25} values were 12.54, 15.82 and 14.38 ppm for these treatments, respectively. Whereas the LC_{50} values were 21.59, 26.49 and 25.16 ppm, respectively. In addition the LC_{90} values were 60.67, 70.61 and 72.82 ppm, respectively and the LC_{95} values were 81.32, 93.23 and 98.41ppm, respectively.

The highest effect of the tested methods was feeding followed by topical application and Contact method.

Table 5. Lethal concentrations of methomyl against 2nd instar larvae of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Method	Day	LC_{25} (Low-Up.)	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Toxicity index	Slope ±SE
Feeding	1	12.54	21.59	60.67	81.31	100	2.8565 ±0.2665
Topical application	1	15.82	26.49	70.61	93.23	81.505	3.0103 ±0.2801
Contact	2	14.38	25.16	72.81	98.41	85.82	2.7772 ±0.2690

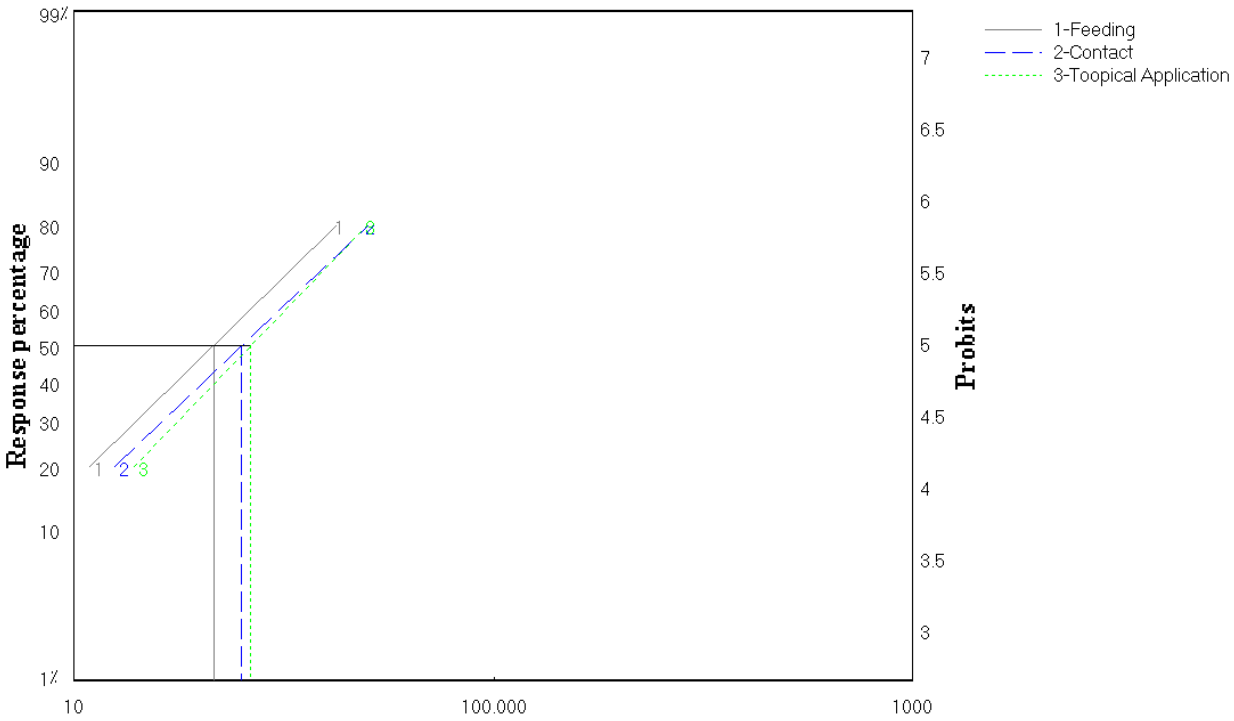


Fig. 1: Concentrations of mortality probit lines of Methomyl in case of feeding, topical application and Contact method of methomyl for the 2nd larval instar of *Spodoptera littoralis*.

The results show a difference in the toxicity to *S. littoralis* and the differences in LC_{25}, LC_{50}, LC_{90} and LC_{95} values may be different modes of action of the tested methods.

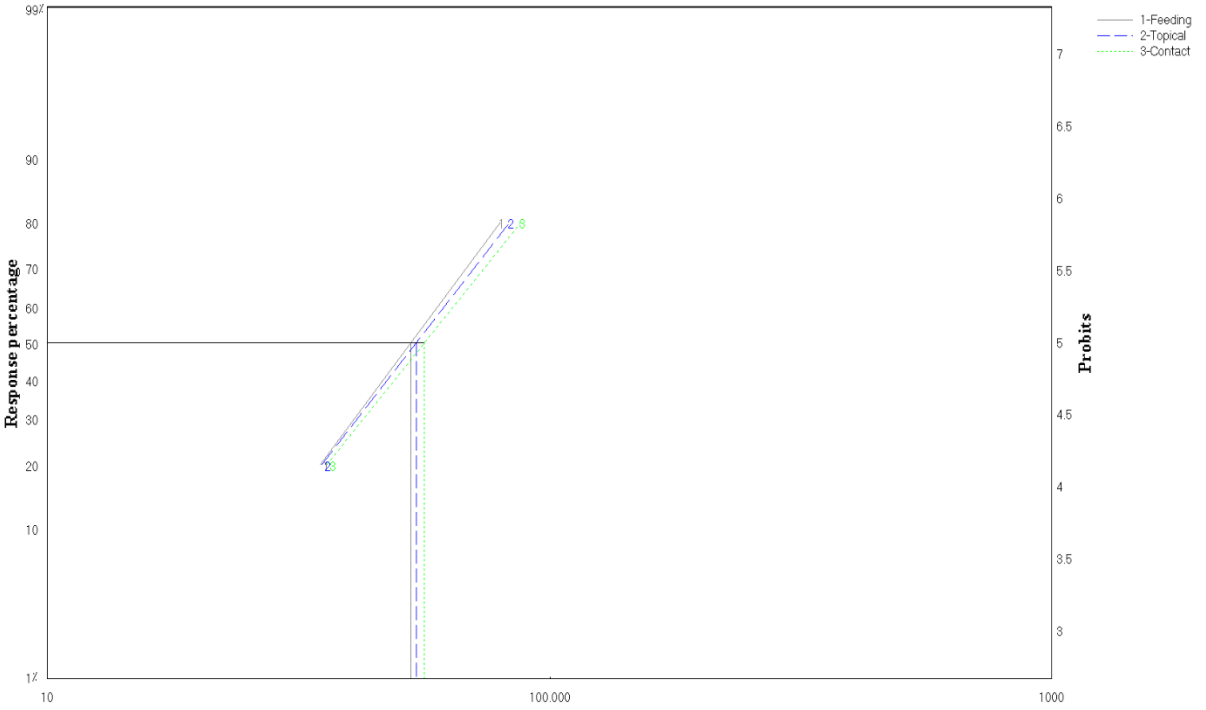
Table (6), and Fig. 2 showed that the LC_{25}, LC_{50}, LC_{90} and LC_{95} values of Methomyl for the 4th larval instar of *S. littoralis* in case of feeding, Contact topical application and method. The LC_{25} values were 38.01, 38.43 and 39.46 ppm for these treatments, respectively. While the LC_{50} values were 52.91, 54.28 and 56.38 ppm, respectively. In addition the LC_{90} values were 99.23, 104.62 and 111.07 ppm, respectively and the LC_{95} values were 118.59, 126.01 and 134.61 ppm, respectively.

From these data it can noticed that the highest effect of the tested methods was feeding method followed by topical application method and Contact method.

Table 6: Lethal concentrations of methomyl against 4th instar larvae of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Method	Day	LC_{25} (Low-Up.)	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Toxicity index	Slope ±SE
Feeding	**2**	**38.01 (34.68-40.79)**	**52.91 (50.12-55.84)**	**99.23 (89.41-114.45)**	**118.59 (104.35-141.62)**	**100**	4.6934 ±0.4151
Topical application	**3**	**38.43 (34.96-41.32)**	**54.28 (51.33-57.44)**	**104.62 (93.36-122.57)**	**126.01 (109.58-153.38)**	**97.48**	4.4974 ±0.4119
Contact	3	39.46 (35.88-42.43)	56.38 (53.25-59.86)	111.07 (98.09-132.39)	134.61 (115.63-167.23)	93.86	4.3519 ±0.4118

Fig. 2: Concentrations of `mortality probit lines of Methomyl in case of feeding, topical application and Contact



method of Methomyl for the 4th larval instar of *Spodoptera littoralis*.

The results show a difference in the toxicity to *S. littoralis* and the differences in LC_{25}, LC_{50}, LC_{90} and LC_{95} values may be different modes of action of the tested methods.

Table (7), and Fig. 3 showed that the LC_{25}, LC_{50}, LC_{90} and LC_{95} values of Spinetoram for the 2nd larval instar of *S. littoralis* in case of feeding, topical application and Contact method. The LC_{25} values were 8.12, 11.23 and 12.97 ppm for these treatments, respectively. Whereas the LC_{50} values were 15.94, 21.76 and 29.40 ppm, respectively. In addition, the LC_{90} values were 57.55, 76.45 and 139.16 ppm, respectively and the LC_{95} values were 82.81, 109.16 and 216.21 ppm, respectively.

From these data it can noticed that the highest effect of the tested methods was feeding method followed by topical application method and Contact method.

Table 7. Lethal concentrations of Spinetoram against 2nd instar larvae of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Method	Day	LC_{25} (Low-Up.)	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Toxicity index	Slope ±SE
Feeding	**3**	**8.12 (5.83-10.14)**	**15.94 (13.37-18.26)**	**57.55 (47.60-75.71)**	**82.81 (64.83-119.23)**	**100**	2.2992 ±0.2527
Topical application	**3**	**11.23 (8.68-13.45)**	**21.76 (19.06-24.43)**	**76.45 (61.61-104.92)**	**109.16 (83.19-163.77)**	**73.29**	2.3483 ±0.2530
Contact	3	12.97 (9.59-15.8)	29.40 (25.57-34.18)	139.16 (97.41-250.72)	216.21 (138.92-451.82)	54.23	1.8983 ±0.2495

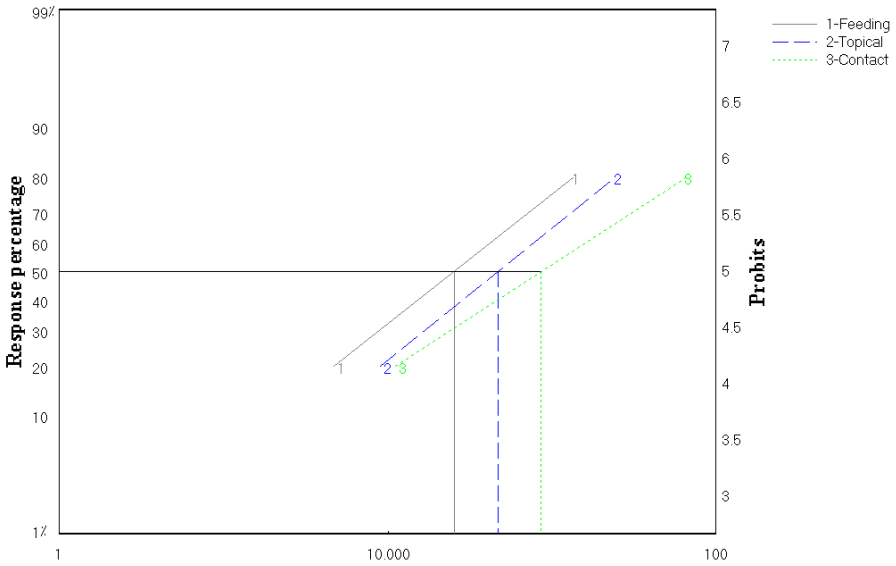


Fig. 3. Concentrations of `mortality probit lines of Spinetoram in case of feeding, topical application and Contact method of Methomyl for the 2nd larval instar of *Spodoptera littoralis*.

The results show a difference in the toxicity to *S.littoralis* and the differences in LC_{25}, LC_{50}, LC_{90} and LC_{95} values may be different modes of action of the tested methods.

Table (8), and Fig. 4 showed that the LC_{25}, LC_{50}, LC_{90} and LC_{95} values of Spinetoram for the 4th larval instar of *S. littoralis* in case of feeding, topical application and Contact method. The LC_{25} values were 11.82, 13.14 and 14.98 ppm for these treatments, respectively. Whereas the LC_{50} values were 25.18, 29.34 and 35.76 ppm, respectively. Whereas the LC_{90} values were 105.93, 135.18 and 186.91 ppm, respectively and the LC_{95} values were 159.19, 208.42 and 298.69 ppm, respectively.

From these data it can noticed that the highest effect of the tested methods was feeding followed by topical application and Contact method.

Table 8. Lethal concentrations of Spinetoram against 4th instar larvae of *Spodoptera littoralis* in case of feeding, topical application and Contact method.

Method	Day	LC_{25} (Low-Up.)	LC_{50} (Low-Up.)	LC_{90} (Low-Up.)	LC_{95} (Low-Up.)	Toxicity index	Slope ±SE
Feeding	**3**	**11.82**	**25.18**	**105.93**	**159.19**	**100**	2.0539 ±0.2491
Topical application	**3**	**13.14 (9.79-15.93)**	**29.34 (25.58-34.02)**	**135.18 (95.57-238.44)**	**208.42 (135.60-424.03)**	**85.79**	1.9321 ±0.2503
Contact	3	14.98 (11.17-18.10)	35.76 (30.76-43.37)	186.91 (120.98-397.29)	298.69 (175.29-757.45)	70.41	1.7844 ±0.2530

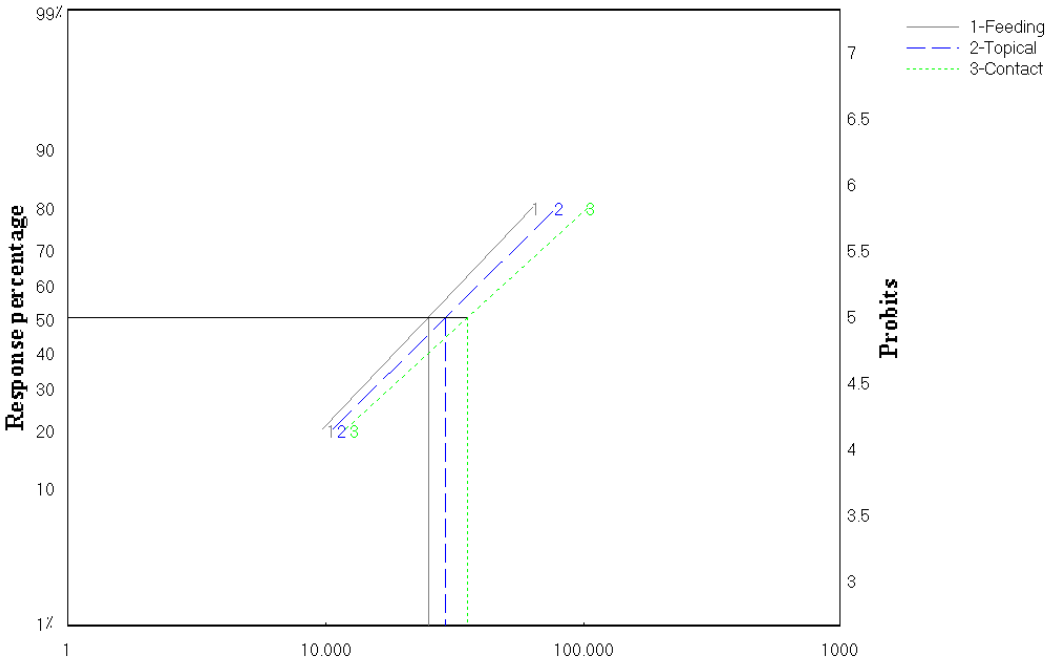


Fig. 4. Concentrations of mortality probit lines of Spinetoram in case of feeding, topical application and Contact method of Methomyl for the 4th larval instar of *Spodoptera littoralis*.

The results show a difference in the toxicity to *Spodoptera littoralis* and the differences in LC_{25}, LC_{50}, LC_{90} and LC_{95} values may be different modes of action of the tested methods.

3.2. Biochemical studies on the laboratory strains:

The LC_{50} values of Methomyl and Spinetoram were tabulated in **Table (9),** and demonstrated Methomyl and Spinetoram on the activities of AChE, GST, alpha esterases and alkaline phosphatase in the total homogenate of 4th instar *Spodoptera littoralis* larvae after the treated larvae.

Results from **Table (9),** indicated that the Methomyl and Spinetoram decreased the stimulation of AChE activity significantly compared with the control, the values of AChE were 101 and 114 ug AChBr/min/g.b.wt, respectively, compared with the 183 in the control. While, the GST enzyme levels increased significantly for Methomyl and Spinetoram, the values of GST were 50.7 and 43.2 mmol sub. Conj./min/g.b.wt, respectively, compared with 21.8 in the control. Also, alpha esterase levels showed slightly increased by 58.2 in case of Methomyl while, Spinetoram showed a no significant increase by 48.2 ug α-naphthol/min/g.b.w compared with 54.5in the control. The Alkaline phosphatase levels were increased significantly to 6199 U /g.b.wt when treated by Methomyl while, Spinetoram showed a no significant increase by 5841 U /g.b.wt compared with 526 U /g.b.wt in the control.

Table 9. Changes of acetylcholinesterase (AchE), glutathione s-transferase (GST), α-esterases(α-EST)activities and Alkaline phosphatase in the body homogenate of tested insects treated with some insecticides at LC_{50} after 24 h of treatment.

Chemical Materials	Resistance Enzymatic activity (Mean ± SD)			
	AChE (ug AChBr/min/g.b.wt)	**GST (mmol sub. Conj./min/g.b.wt)**	**Alpha esterases (ug α-naphthol/min/g.b.wt)**	**Alkaline phosphatase (U /g.b.wt)**
Methomyl	*101±3.8c*	*50.7±3.2a*	*58.2±0.9b*	*6199±92a*
Spinetoram	*114±5.1c*	*43.2±1.8b*	*48.2±1.1c*	*5841±36b*
Control	*183±6.1a*	*21.8±1.8c*	*45.5±3.5c*	*5926±37.9b*

These results agreement with **Moustafa *et al.*, 2003** reported that, the treatment with Methomyl against cotton leaf worm *Spodoptera littoralis* 4th instar larvae reduction in the activities of AChE

enzyme **(Abdel-Aal and A. E. 2012)**. Indicated that the acetylcholinesterase activity decreased significantly for Methomyl treatment. **(Salgado *et al.*, 1998)**. Methomyl act as carbamate insecticide through excitation of the insect nervous system, which in turn cause alteration in the function of nicotinic and GABA gated ion channels which leads to involuntary muscle contractions and tremors. According to such an activity, it was expected that such insecticide may produce cytotoxic action either in neurons or non-target cells.

The cytotoxic action in neurons may alter the neurotransmitter mechanisms through interfering processes of methomyl with the production of acetylcholine in the synaptic region which affect in turn the activity of the acetycholinesterase to be in form of false inhibition. **(Assar *et al.*, 2003)** was reported Acelylcolinesterase (AchE) activity significantly decreased with spinetoram.

The effect of treatment with Methomyl and Spinetoram led to increasing in the activity of several enzymes such as esterases which protect insect from insecticide poisoning as a part of defense mechanism, or added stress on enzyme expression system to synthesize new and higher amount of detoxification enzymes where could be the possible reasons for the arrested growth and mortality (Wheeler and Isman 2000). Also, the increasing activity of esterase enzymes post treatment and decreasing with high dose that may be due to the decrease of body weight defense against insecticide stress (Muthusamy et al., 2011).

Based on the LC_{50} values of Methomyl and Spinetoram, the data in **Table (10),** demonstrated Methomyl and Spinetoram on the activities of Total proteins, Total carbohydrates, GOT and GPT in the total homogenate of 4th instar *S. littoralis* larvae after the treated larvae were exposed for 24 hours. The findings showed that Methomyl and Spinetoram decreased the stimulation of Total proteins and GOT enzaym activity significantly about the control, whereas Total carbohydrates did not significantly differ from the control For Methomyl and Spinetoram. However, The GPT did not significantly differ from the control For Methomyl while Spinetoram decreased the The GPT levels about the control, the values of Total proteins were 31.7 and 28.6 mg /g.b.wt, respectively. While the values of Total carbohydrates were 24.8 and 23.7 mg /g.b.wt, respectively .while the values of GOT were 59.2 and 52.2 U /g.b.wt. While the values of GPT 7.6 and 5.9 U /g.b.wt for Methomyl and Spinetoram respectively.

The treatment of *Spodoptera littoralis* larvae with Methomyl and caused variable reduction in GOT with control **(Abdel Hafez *et al.*, 1993)**, in addition, reduction in protein levels in the 4th instar larvae of *Spodoptera littoralis* treated with LC50 of Spinetoram for 2 days. The reduction in protein content could be linked to the formation of mechanical lipoproteins used for repairing damaged cells, tissues, and organs **(Saravana Bhavan & Geraldine, 2001; Ribeiro *et al.*, 2001; Mosleh *et al.*, 2003),** or it could be due to the mobilization of amino acids to meet energy demands during insecticide-induced stress. Furthermore, the decrease in protein levels might be a result of the destructive impact on certain cerebral neurosecretory cells in the brain responsible for protein secretion in the treated larvae of *S. littoralis* **(Hamouda and Dahi, 2008),** who demonstrated that Spinetoram has a neurotoxic effect, causing distinct histopathological changes in the nerve and neurosecretory cells of *S. littoralis*. Additionally, the treated larvae may produce hormones that utilize protein**. (Hassan *et al.*, 2014)** Our results induced highly significant inhibition in carbohydrate levels with Spinetoram **Also, (El-Sheikh, 2012**) state that the total carbohydrates content in 6th larval instar treated with spinosad significantly decreased.

Table 10. Changes of Total proteins, Total carbohydrates, GOT and GPT in the body homogenate of tested insects treated with some insecticides at LC_{50} after 24 h. of treatments.

Chemical Materials	Metabolites Enzymatic activity (Mean ± SD)			
	Total proteins (mg /g.b.wt)	**Total carbohydrates (mg /g.b.wt)**	**GOT (U /g.b.wt)**	**GPT (U /g.b.wt)**
Methomyl	*31.7±1.5b*	*24.8±0.7a*	*59.2±2.5a*	*7.6±0.5a*
Spinetoram	*28.6±0.32b*	*23.7±1.7a*	*52.2±2.8b*	*5.9±0.7b*
Control	*36.2±1.8a*	*24. ±1.4a*	*61±2a*	*7.8±0.5a*

Based on the LC_{50} values of Methomyl and Spinetoram, the data in **Table (11),** demonstrated Methomyl and Spinetoram on the activities of Proteases, Amylase and Lipase in the total homogenate of 4th instar *S. littoralis* larvae after the treated larvae were exposed for 24 hours.

The findings showed that Methomyl and Spinetoram decreased the stimulation of Proteases

and Lipase enzyme activity significantly about the control, whereas Amylase enzyme did not significantly differ from the control For Methomyl. While Spinetoram decreased Amylase enzyme levels about the control.

The values of Proteases were 43 and 32.2 ug alanine/min /g.b.wt, respectively. While the values of Amylase were 10 and 7 ug glucose/min /g.b.wt, respectively. While the values of Lipase 182 and 198.7 mU /g.b.wt for Methomyl and Spinetoram respectively. **(El Saidy, 1990)**, methomyl inhibited amylase activity indirectly**. (Assar *et al.*, 2003)** was reported a significant decrease in the activity of amylase activity was induced by the spinetoram.

Table 11. Changes of Proteases, Amylase and Lipase in the body homogenate of tested insects treated with some insecticides at LC_{50} after 24 h. of treatments.

Chemical Materials	Digestive Enzymatic activity (Mean ± SD)		
	Proteases (ug alanine/min /g.b.wt)	**Amylase (ug glucose/min /g.b.wt)**	**Lipase (mU /g.b.wt)**
Methomyl	43±1.8b	10±0.15a	182±7b
Spinetoram	32.2±2.6c	7±0.4b	198.7±7.1b
Control	58.3±3a	10.2±0.75a	270±11a

Methomyl and Spinetoram had inhibition effect on Total proteins, Total carbohydrates, GOT, GPT Proteases , Amylase and Lipase in the total homogenate of 4th instar *S. littoralis* larvae after the treated larvae were exposed for 24 hours.

The death of insect results from combination factors such as mechanical damage which resulting from tissue invasion, depletion of nutrient resources, enzymatic disturbance and toxicities.

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دراسة مقارنة على اليرقات في الطور الثاني والرابع من دودة ورق القطن، المعاملة بالميثوميل والسبينيتورام في المختبر

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قسم وقاية النبات – كلية الزراعة جامعة بنها

تاثير مبيدين حشريين، وهما ميثوميل وسبينيتورام، على اليرقات في الطورين الثاني والرابع من دودة تم LC_{50}عئد تركيزات ( ) :Lepidoptera (Boisd.). Noctuidaeورق القطن تحت ظروف المختبر لتقييم نسبة الوفيات والتأثيرات البيوكيميائية. أظهرت النتائج أن معدل وفيات اليرقات كان مرتبطًا بشكل إيجابي مع زيادة تركيزات المبيدات الحشرية المختبرة، وطرق الاختبار، والوقت بعد التعرض. معدل الوفيات في ميثوميل كان أعلى من سبينيتورام. تسببت المركبان اللذان تم اختباره في انخفاض كبير في تحفيز إنزيم الأستيل كولينستراز (AChE) والبروتينات الكلية، والكربوهيدرات الكلية، وإنزيمات GOT وGPT، والبروتيازات، والأميليز، والليباز، كما تسببت في زيادة كبيرة في نشاط إنزيمGST ، والأستراز ألفا، والفوسفاتاز القلوي.