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TOXICOLOGICAL AND BIOLOGICAL EFFECTS OF SOME INSECTICIDES ON THE COTTON LEAFWORM, *Spodoptra littorallis* (Boisd.)

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ABSTRACT: Toxicity of five insecticides belonging to different groups of chemicals, namely: chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar to cotton leafworm were evaluated under the field and laboratory conditions. The effect of the tested chemicals on some biological aspects of 2nd and 4th instar larvae of the cotton leafworm, *Spodoptra littoralis* (Boisd.) were investigated. The lethal concentrate (LC_{50} and LC_{90}) values were calculated after 24, 48, and 72 hr., post-treatment. The obtained results showed that there was a wide range of toxicity of the tested pesticides; however the insecticide bestban showed to be the highest toxic compound effect against 2nd and 4th instar larvae, with LC_{50} and LC_{90} values after 72 hr., post-treatment recording 0.05, 0.22 mg/l and 0.15, 1.10 mg/l for 2nd and 4th instar larvae, respectively. On the other hand, the biopesticide biovar exhibited the lowest toxicity to the tested larvae at both levels of toxicity (LC_{50}; LC_{90} values after 72 hr., recording 414; 856 mg/l and 1100; 1740 mg/l) for 2nd and 4th instar larvae, respectively. The other tested insecticides occupied an intermediate position between the highest and the lowest toxic pesticides. Regarding the biological effects, results showed that, the tested insecticides induced a clear reduction in mean larval weight and mean pupal weight compared with the control. The tested insecticides affected the larval duration, pupal duration, pupation, adult emergence and adult longevity.

Key words: Cotton leafworm, toxicity, biological aspects.

INTRODUCTION

The cotton leafworm, *S. littoralis* (Boisd.) is considered a major polyphagous pest, widely distributed throughout Africa, Mediterranean Europe, and several parts of Asia **(Azab *et al*., 2001)**. Larvae of this pest can feed on ~90 economically important plant species belonging to 40 families **(Brown and Dewhurst, 1975)**. In Egypt, *S. littoralis* is one of the most destructive pests of cotton which is considered the most valuable crop in the country. Over the past 25 years, the intensive use of broad-spectrum insecticides against *S. littoralis* has led to the development of resistance to many used pesticides **(Rizk *et al*., 1990; Aydin and Gurkan, 2006)**.

Larvae feed not only on cotton leaves, but also attack other economically important crops such as cucumber, potato, okra and eggplants. Several control measures have been directed to combat this insect. The most widely used method of control is the chemical insecticides, together with other methods which sometimes failed to achieve a complete protection of the cotton crop against this pest.

The intensive use of the chemical compounds has resulted many problems such as pest population outbreaks and chemical resistance, endangering human health and overall the environment . Therefore, the authorities decided to reduce chemicals use and tried to introduce another alternative control methods such as biocontrol agents and others through integrated pest management programs. The present study was carried out to evaluate acute and sublethal effects of chlorpyrifos, methomyl, emamectin benzoate, protecto, biovar against the cotton leafworm, *S. littoralis*.

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MATERIALS AND METHODS

Tested Insecticides

Commercial formulation of the tested insecticides were used, these insecticides were as follows:

Chlorpyrifos (Bestban 48% EC). Methomyle (Agrannet 90% SP). Emamectin benzoate (Emamectin 1.9% EC). *Bacillus thuringiensis kurstaki* (Protecto 9.4% WP). *Beauveria bassiana* (Biovar*).*

Chemical abstracts name *(B.bassiana)*

Test insect

A laboratory strain of the cotton leafworm, *S. littoralis* was reared in the laboratory on castor bean leaves under laboratory conditions of 26 ± 2°C and 65 ± 5% RH **(El-Defrawi *et al*., 1964)**. The culture of the cotton leafworm was initiated from freshly collected egg masses supplied from the Division of Cotton Leafworm, Plant Protection Research Institute, Dokki, Egypt.

Bioassay Tests

Under laboratory conditions

The efficiency of the tested insecticides; chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar were assessed against the 2nd and 4th instar larvae of *S. littoralis*. Serial concentrations of each tested insecticide were prepared using distilled water. Disks (9 cm. diameter) of castor bean leaves were dipped in the tested concentrations for 10 seconds then left to dry and offered to larvae, which starved for 4-6 hours before treatment **(Merdan, 1968).** Larvae and disks were placed into glass jars (5 pounds). Each treatment was replicated 3 times (10 larvae per). Control disks were dipped in distilled water only. The larvae were allowed to feed on treated disks for replicate 24 hr., or 48 hr., then transferred to fresh untreated ones. Mortality percentages were recorded after 72 hr., for all insecticides except chlorpyrifos after 24 hr. The corrected mortality of larvae was calculated using Abbott's formula **(Abbott, 1925)**. The LC_{50}, LC_{90} and slope values of the tested compounds were calculated using Finney's equation **(Finney, 1971)**, through software computer program.

Toxicity Index and Relative Potency were calculated according to Sun equations **(Sun, 1950)** as following:

Toxicity index = LC_{50} or LC_{90} of the most efficient compound ÷ LC_{50} or LC_{90} of the other compound × 100

Relative potency = LC_{50} or LC_{90} of the other compound ÷ LC_{50} or LC_{90} of the most efficient compound

Under filed conditions

The purpose of this experiment was to evaluate the initial and residual effects of the tested insecticides (chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar) at the recommended concentrations applied using a knapsack sprayer against the 2nd and 4th instar larvae of the cotton leafworm, *S. littoralis*. Samples of castor bean leaves were picked up at random for each treatment directly after spraying (zero time) after 1, 3, 5, 7, 11 and 14 days post treatment. The collected samples were sealed in paper bags and transferred to the laboratory **(Aly, 1999)**. Ten 2nd and 4th larvae were placed in each glass jar of 500 ml capacity and allowed to feed on the field collected treated leaves for 2 days. The alive larvae were transferred to other clean jars containing untreated fresh castor bean leaves for 3 days. Three replicates were used for each treatment. This experiment was conducted at laboratory conditions of 26°C (as an average) and 65±5% RH. Cumulative mortalities were calculated at the end of experiment and corrected according to Abbott's Formula **(Abbot, 1925)**. The mortalities at zero time were considered as initial kill, while the mean of the cumulative mortalities of the remaining tested periods were considered as residual effect.

Biological studies

Newly moulted 2nd and 4th larval instars were segregated from the stock colony in clean glass Petri dishes and starved for 24 hr., **(Nasr, 1999).** Five concentrations for each pesticide were used.The concentrations were prepared by dissolving the tested pesticide in distilled water to get the appropriate concentration as a stock solution and the other concentrations were prepared by dilution. Pieces of castor bean leaves were treated by the leaf- dipping technique in the different concentrations of the

tested compound and left to dry under the air of laboratory for 1 hr., and then introduced to larvae for feeding. Thirty of starved larvae, distributed in three replicates (10 larvae/ replicate) were used for each concentration and allowed to feed for 24 hr., on treated castor bean leaves before introducing fresh leaves. The same technique described above was used in control group except that larvae were allowed to feed on castor bean leaves that dipped only in distilled water. Daily inspections were carried out to supply replicates with fresh leaves until pupation. Number of individuals that succeeded to develop were recorded, mean larval and pupal weight, larval mortality (%), larval duration, pupation (%), pupal duration and pupal malformation were recorded.

Equations used for estimating different bio-metric records of *S. littoralis*

Pupation (%) = Total number of pupae ÷ Total number of treated larvae × 100

Pupae deformation (%) = Number of abnormal pupae ÷ Number of (normal + abnormal) pupae × 100

Statistical Analysis

Data were statistically analyzed according to **CoStat® Statistical Software (2005)**. In addition, the correlation and regression analyses were done by using Cosatat. The significance of various treatments was evaluated by Duncan's multiple range test ($p < 0.05$) **(Snedecor and Cochran, 1980)**.

RESULTS AND DISCUSSION

The results presented in Table 1 show the toxicity of five insecticides (chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar) against the 2nd larval instar of the cotton leafworm, *S. littoralis* at different exposure times. This experiment was conducted at laboratory conditions of 26°C and 65 ± 5% RH.

Among the tested insecticides, chlorpyrifos was the most effective compound followed by methomyl while biovar was the least effective one after 24 hr., 48 hr., and 72 hr., of exposure.

The results indicated that there was a negative relationship between the time post treatment and LC_{50} and LC_{90} values of all the tested insecticides.

The LC_{50} values were 0.18, 0.49, 35.11, 500 and 900 mg/l for chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar, respectively after 24 hr., of exposure. Increasing the period of exposure from 48 to 72 hr. decreased the LC_{50} values to reach 0.1, 0.22, 21.17, 380 and 654 and 0. 05, 0.1, 16.86, 300 and 414 mg/l after 48 and 72 hr., for chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar, respectively.

On the other hand, the corresponding LC_{90} for the tested insecticides after the three periods of exposure (24, 48 and 72 hr.) were 1, 1, 90, 1100 and 1500 ; 0.89, 0.9, 88.55, 860 and 1030 and 0.22, 0.58, 69.31, 920 and 856 mg/l for chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar, respectively.

Results in Table 2 show that LC_{50} values of all tested insecticides were 0.8, 1.40, 95.41, 1400 and 1800 mg/l for chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar, respectively after 24 hr., of exposure. As the period of exposure increased from 48 to 72 hr., the LC_{50} values were decreased to reach 0.3, 0.88, 44.6, 1180 and 1400 mg/l at 48 hr., and 0.15, 0.4, 28.75, 900 and 1100 mg/l at 72 hr., for chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar, respectively.

Furthermore, the LC_{90} for the tested insecticides after the different periods of exposure (24, 48 and 72 hr.) were 1.99, 3.5, 257. 46, 2600 and 3050; 1.5, 1.6, 108.33, 1 830 and 2200 as well as 1.10, 0.98, 89.77, 1205 and 1740 mg/l for chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar, respectively.

Generally, the 2nd larval instar was found to be more sensitive to the tested compound than 4th instar. The low values of toxicity lines slope indicate a degree of heterogeneity for the susceptibility of the treated larvae to the tested compounds. The obtained results are in agreement with those obtained by **Badr (2000), Culter *et al*. (2005) and Han *et al*. (2006)**.

The low susceptibility of 4th larval instar comparing with 2nd instar may be due to the changes in anatomy, physiology and size through which the compounds passes as well as to difference in liability of toxicant penetration **(Busvine, 1971)**.

Table 1. Acute toxicity of some insecticides against the 2nd larval instar of *Spodoptera littoralis* (Boisd.) at different periods of exposure under laboratory conditions of 26°C (as an average) and 65 ± 5% RH

Insecticide	Exposure period (hr.)	LC_{50} mg/l	LC_{90} mg/l	Slope value	Toxicity index	Relative potency
Chlorpyrifos	24	0.18	1	0.701	100	1
	48	0.1	0.89	0.7351	100	1
	72	0.05	0.22	0.725	100	1
Methomyl	24	0.49	1	1.055	19.15	2.7
	48	0.22	0.9	1.048	45.45	2.2
	72	0.1	0.58	1.147	50	2
Emamectin benzoate	24	35.11	90	1.76	0.51	195
	48	21.17	88.55	2.06	0.47	211
	72	16.86	69.31	2.087	0.30	337
Protecto	24	500	1100	0.86	0.036	2777
	48	380	860	1.06	0.026	3800
	72	300	920	1.08	0.025	6000
Biovar	24	900	1500	0.86	0.02	5000
	48	654	1030	1.06	0.015	6540
	72	414	856	1.08	0.012	8280

Table 2. Acute toxicity of some tested insecticides against 4th instar larvae of *Spodoptera littoralis* (Boisd.) at different periods of exposure under laboratory conditions of 26°C (as an average) and 65± 5% RH

Insecticide	Exposure period (hr.)	LC_{50} mg/l	LC_{90} mg/l	Slope value	Toxicity index	Relative potency
Chlorpyrifos	24	0.8	1.99	0.81	100	1
	48	0.3	1.5	0.63	100	1
	72	0.15	1.10	0.82	100	1
Methomyl	24	1.40	3.5	1.5	57	1.75
	48	0.88	1.6	1.7	34.1	2.9
	72	0.4	0.98	1.2	37.5	2.7
Emamectin benzawat	24	95.41	257.46	0.96	0.84	119
	48	44.6	108.33	0.86	0.67	148
	72	28.75	89.77	0. 87	0.32	191
Protecto	24	1400	2600	1.86	0.06	1750
	48	1180	1830	1.56	0.025	3933
	72	900	1205	1.38	0.017	6000
Biovar	24	1800	3050	0.96	0.04	2250
	48	1400	2200	1.16	0.02	4666
	72	1100	1740	1.28	0.014	7333

Mortality of 2nd and 4th larval instar of *S. littoralis* after feeding for 2 days on insecticidal treated eggplant leaves are shown in Table 3.

Among the insecticides used, chlorpyrifos was the most effective compound followed by methomyle while biovar was the least toxic one as indicated in the periods post treatment.

The mortality percentages of 2nd instar larvae were 100, 95, 73, 62, 52 and 42, 90, 75, 69, 51, 42 and 33; 70, 60, 48, 34, 24 and 20; 8, 18, 16, 30, 52 and 36 and 10, 18 , 62 , 68, 38 and 18 after 1, 3, 5, 7, 11 and 14 days for chlorpyrifos, methomyl, emamectin benzoate, protecto and biovar, respectively, while the residual effect averages were 64.8, 54, 51.2, 32 and 42.8%, for the same toxicants, respectively.

In general, mortality percentage of 4th larval instar were less than that of the 2nd larval instar (Table 3) for all tested compounds after all tested periods post treatment with the same order of toxicity.

The Efficiency of the tested insecticides against *S. littoralis* after feeding the 2nd and 4th larval instar on the treated eggplant leaves are presented in Table 4.

As mentioned before in season 2016, chlorpyrifos was the most effective compound followed by methomyl while biovar was the least effective one after the recorded exposure periods post treatment (Table 4).

The mortality percentages of 2nd larval instar were more than that recorded for 4th larval instar in both tested seasons (Tables 3 and 4).

On the basis of LC_{50} and LC_{90}, the present results indicated that, all the tested insecticides have larvicidal activities against both 2nd and 4th instar larvae with the exception of dipel 2x that caused low toxic effect up to 5 days. Chlorpyrifos have the highest larvicidal and the most toxic insecticide tested against the 2nd and 4th larval instar. The same result was obtained by **Abd El-Latief (2001)** when tested various insecticides against eggs and larvae of *S. littoralis* who mentioned that, dipel 2x and thuringiensin had slight or low insecticidal activities until 5 days against the 2nd and 4th larval instar of *S. littoralis.*

Some Biological Aspects of *Spodoptera littoralis* as Influenced by Insecticidal Treatment

Results in Table 5 show that all the tested insecticides caused a significant decrease in larval and pupal duration, larval and pupal weight, pupation, adult emergence and adult longevity as well as an increase of malformed pupae. Also, these effects were more pronounced for bestban, agrannet, emamectin than all tested pesticides.

The larval duration was 7.35, 8.0, 8.85, 10.55 and 12.5 days for bestban, agrannet, emamectin benzawat, protecto and biovar, respectively comparing with the control (12 days), while pupal duration was 5.8, 5.2 , 5.8, 7.5 and 8.34 days for the same previous insecticides, respectively in compared with the control (8.0 days).

The percentage of malformed pupae ranged from 10 to 66% compared with 4% in the control. On the other hand, the tested insecticides induced a significant suppression in pupation and adult emergence. Also, there was a significant difference between the effect of the tested insecticides on pupation (%).

However, the pupation varied from 12 to 75% as compared to 95% for the control one. The adult emergence percentages ranged from 42 to 88% as compared to 92% for the control. Decrease in adult emergence could be due to the fact that the toxic substances block the maturation of imaginal discs which are primordial for many adult integument structure in endopetrygote insect **(Schneidermann, 1972)**.

In general, it was observed that bestban was the most effective in all the mentioned measured parameters, which, may be attributed to effect on converting ingested and digested food (**Senthil and Kalaivanu, 2005).** Pupal mortalities in this study were obvious and recorded after treatment of both 2nd and 4th larval instars that used, there were close-dependent effect on pupation and pupal mortalities, these results are in harmony with those obtained by **Biddinger *et al*. (1998), Butter *et al*. (2003) and Shalokhe *et al*. (2008)**. These results are in agreement with the results obtained by **Zidan *et al.* (1996)** who found that *B. thuringiensis* had prolonged larval duration and adult longevity.

Table 3. Mortality percentage of 2nd and 4th cotton leafworm larval instar exposed in laboratory to insecticidal treated cotton leaves after different intervals of spraying under field conditions (during 2016 growing season)

Pesticide	Mortality (%) of 2nd larval instar post treatment (in days)								Mortality (%) of 4th larval instar post treatment (in days)							
	1 day (initial effect)	3	5	7	11	14	Mean of residual effect	General mean	1 day (initial effect)	3	5	7	11	14	Mean of residual effect	General mean
Chlorpyrifos	100	95	73	62	52	42	64.8	71	82	78	60	55	40	33	53.2	57.5
Methomyl	90	75	69	51	42	33	54	61.5	75	70	58	42	34	30	46.8	52.5
Emamectin benzoate	70	60	48	34	24	20	51.2	45	60	80	82	42	24	18	49.2	39
Protecto	8	18	16	30	52	36	32	20	15	30	42	56	32	22	36.4	18.5
Biovar	10	18	62	68	38	18	42.8	14	10	22	60	52	18	15	33.4	12.5
Control	0	0	0	0	0	0	-	-	0	0	0	0	0	0	-	-
LSD	2.12	0.88	1.31	1.69	1.1	2.01	-	-	0.64	0.74	0.81	0.79	1.37	0.74	-	-

Table 4. Mortality percentage of 2nd and 4th cotton leafworm larval instar exposed in laboratory to insecticidal treated cotton leaves after different intervals of spraying under field condition (during 2017 growing season)

Pesticide	Mortality (%) of 2nd larval instar post treatment (in day)								Mortality (%) of 4th larval instar post treatment (in day)							
	1 day (initial effect)	3	5	7	11	14	Mean of residual effect	General mean	1 day (initial effect)	3	5	7	11	14	Mean of residual effect	General mean
Chlorpyrifos	100	85	65	51	44	22	53.4	76.7	85	77.5	75	50	42	28	54.5	69.75
Methomyl	100	78	58	46	46	18	49.2	74.6	81	72	70	44	30	33	49.8	65.4
Emamectin benzoate	85	72	44	31	26	22	39	62	70	55	48	33	26	21	36.6	53.3
Protecto	10	40	34	50	70	42	47.2	28.6	8	25	48	66	71	41	50.2	29.1
Biovar	9	22	42	56	69	61	50	29.5	6	22	42	56	62	38	44	25
Control	0	0	0	0	0	0	-	-	0	0	0	0	0	0	-	-
LSD	0.92	0.74	0.94	0.81	0.79	0.92	-	-	0.64	0.88	0.79	0.92	1.05	0.81	-	-

Table 5. Changes in some biological aspects of the cotton leafworm, *Spedoptora littoralis* as influenced by insecticidal treatment

Insecticide	Mean larval weight (g)	Larval duration (day)	Mean pupal weight (g)	Pupal duration (day)	Pupation (%)	Malformed pupae (%)	Adult emergence (%)	Adult longevity (day)
Chlorpyrifos	0.33	7.35	0.22	5.8	12	45	42	3
Methomyl	0.38	8	0.2	5.2	15	66	46	2
Emamectin benzoate	0.44	8.85	0.26	5.8	22	45	50	3.5
Protecto	0.64	10.55	0.36	7.5	62	8.5	86	6
Biovar	0.72	12.5	0.39	8.34	75	10	88	6
Control	0.67	12	0.38	8.0	95	4	92	7.5
LSD	0.074	0.72	0.058	0.66	0.92	0.54	0.86	0.79

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التأثيرات التوكسيكولوجية والبيولوجية لبعض المبيـــدات الحشـــرية على دودة ورق القطـــن

نورهان زيدان عواد - على احمد على أيوب – احمد السيد السبكى – محمد عبد العال هنداوى

قسم وقاية النبات – كلية الزراعة – جامعة الزقازيق – مصر

تم اختبار خمسة من المبيدات الحشرية التى تنتمى إلى مجاميع مختلفة وهى كلوربيرفوس، ميثومايل، ايمامكتين بنزوات، بيوفار وبرتكتو على دودة ورق القطن تحت ظروف الحقل والمعمل، كما تم دراسة تأثير المبيدات المختبرة على بعض النواحى البيولوجية لدودة ورق القطن، تم حساب قيمة التركيز القاتل لـ ٥٠% و٩٠% للمبيدات المختبرة معمليا بعد ٢٤، ٤٨ و٧٢ ساعة من المعاملة، أظهرت النتائج أن هناك مدى واسع من السمية للمبيدات المختبرة على دودة ورق القطن وكان المبيد الحشرى كلوربيرفوس أكثر المبيدات المختبرة سمية على العمر اليرقى الثانى والرابع لدودة ورق القطن، كانت قيم التركيز القاتل ل٥٠ و ٩٠% للمبيدات المختبرة (كلوربيرفوس ، بيوفار) بعد ٧٢ ساعة من المعاملة هى ٠.٥ ، ٠.٢٢ و ٠.١٥، ١.١ مجم/لتر للعمر اليرقى الثانى والرابع على التوالى، على الجانب الاخر كان المبيد الحيوى بيوفار اقل المبيدات المختبرة سمية على اليرقات المختبرة بعد٧٢ ساعة مسجلا قيم موت ل٥٠ و ٩٠% ٤١٤، ٨٥٦ مجم/لتر للعمر اليرقى الثانى، ١١٠٠ و١٧٤٠ مجم/لتر للعمر اليرقى الرابع على التوالى، احتلت باقى المبيدات المختبرة مراكز وسطية بين المبيدين الأعلى والأقل فى السمية، فى الدراسات البيولوجية اظهرت النتائج ان المبيدات المختبرة أحدثت خفض فى وزن اليرقات ووزن العذارى مقارنة بالكنترول، كما اثرت المبيدات المختبرة على مدة الطور اليرقى وطور العذارى ونسب التعذر وخروج الحشرات الكاملة ومدة بقائها.

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