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EFFICIENCY OF SOME INSECTICIDES SEQUENCE ON COTTON BOLLWORMS AND HISTOPATHOLOGICAL EFFECTS OF SOME BIOCIDES ON PINK BOLLWORM LARVAE

SALAMA, M. ABD EL-MOHSEN[1], M. A. ABD EL-BAKI[1], JEHAN B.A. EL-NAGGAR[2] and E.Y. EL-NAGAR[2]

1. *Pesticides Dept., Fac. Agric., Kafrelsheikh Univ.*
2. *Plant Prot. Res. Inst., ARC, Egypt.*

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Abstract

The experiments were carried out during the cotton seasons of 2008 and 2009 in Sakha Agricultural Research Station Farm, Kafr El-Sheikh Governorate to evaluate the efficiency of certain insecticidal sequences against pink and spiny bollworms larvae. The aim of the insecticidal sequences is to get the most effective control one against the bollworms. The data revealed that the infestation of cotton fields with both bollworms in both successive seasons started with few number of 1st and 2nd instar larvae on late July, but increased gradually till the end of each season. In general all treatments had slight and moderate reduction in bollworms where the reduction rates ranged between 34.18 and 52.43%. The sequences of biocides (Spinosad, Azadirachtin and *Bacillus thuringiensis*) was efficient in reducing larval population when sprayed three times alone or alternative with other three sprays at 15 days intervals. Spinosad in three successive sprays was the best sequences in controlling bollworms induced 48.66% average reduction, using the conventional insecticides with biocides, IGRs and antimolting compounds caused good average reduction on larval population. While the sequence of methomyl followed by oxymatrine + prosuler and lambdacyhalothrin induced the least reduction which being 38.02% reduction.With respect to the histopathological effects of the tested biocides, midgut histological sections were carried out on the 2nd and 4th instar larvae of Pink bollworm (PBW) with field concentration of the three tested biocides. Spinosad was the most effective compounds in causing aberrations in the midgut layers. Also, the pathological effect of the tested biocides was more serious on the 2nd instar larvae than the 4th one. Accordingly, spinosad, IGRs, antimolting compounds and biocides could be included with conventional insecticides to get the highest reduction in bollworms population.

INTRODUCTION

Cotton bollworms, the pink bollworm (PBW), *Pectinophora gossypiella* (Saunders) and the spiny bollworm (SPW), *Earias insulana* (Boisd.) are considered the most serious pests attacking cotton plants during flowering as well as fruiting stages and cause great losses of cotton yield and quality. Menally and Mullins (1996) indicated that the loss amount caused by *P. gossypiella* to cotton arises to one million kentar annually in Egypt. As a result of continued and unregulated application of insecticides, insects began to develop high levels of resistance to insecticides. In response to this problem effort to control or suppress bollworm damage to growing cotton frequently involve using insecticides of different groups in rotation program which may be useful to delay the resistance problem. Khidr *et. al*. (1996), El-Sorady *et. al.* (1998), and Abd El-Mageed *et. al*. (2007) indicated that application of insecticides in sequential use induced higher reduction in larval number as compared with the lower reduction resulting from several applications with the same insecticide alone. Use of the microbial insecticides, derived from *Bacillus thuringiensis* are non-toxic to human (Corlett, 1961), plants, animals, fishes and for parasitoids and predators (Burges and Hussey, 1971), natural products of plant origin is a new trend that preserve the environment from contamination with harmful toxicant.

Azadirachtin exhibits extremely low acute mammalian toxicity but it is very effective as control agent for many insect groups (Champagne *et al*., 1989). Also, biotic compound played an important role in pest control, among these compounds spinosad, it is a soil dwelling bacterium called *Saccharopolyspora spinosa*. Spinosad posses less rich than most insecticides to mammals, birds, fish and beneficial insects. It was used for control of Lepidoptera insect (Temarak, 2007, Ghure *et. al*., 2008 and Gosalwad *et. al*., 2009).

The present work is an attempt to evaluate the effectiveness of sequence insecticides mixtures with insect growth regulators IGRs and some biocide against cotton bollworms, (pink and spiny bollworms) infesting cotton green bolls in the cotton fields. Also, to study the histopathological effects of some biocides against either 2nd and 4th instar larvae of PBW, *P. gossypiella*.

MATERIALS AND METHODS

1. Insecticides

All tested insecticides used in this study were in formulated forms:

a. Organophosphates

a.1. Chlorpyrifos (Dursban 48% EC). It was supplied by Dow Agro Sciences, applied at rate of 1 L/fed.

a.2. Profenofos (Curacron 72% EC). It was supplied by syngenta, applied at rate 750 mL/fed.

b. Synthetic pyrethroids

b.1. Deltamethrin (Decis 2.5% EC) it was supplied by Bayer crop Science, applied at rate 750 ml/fed.

b.2. **Lambda-cyhalothrin** (Karate 2-5% EC). It was supplied by Samtrade Co., at rate 750 ml/fed.

c. **Carbamate**

c.1. **Methomyl** (Lannate 90 WP). It was supplied by Bayer Crop Science, applied at rate 300 gm/fed.

d. **Oxadizine**

d.1. **Indoxacarb:** (Steward 15% EC). It was supplied by Du Pont Agriculture, applied at rate 26.25 ml/100 L.

e. **Insect growth regulators (IGRs):**

e.1. **Chlorfluazuron** (Atabron 5% EC). It was supplied by Ishinara Sangyo, applied at rate 400 ml/fed.

e.2. **Hexaflumuron** (Consult 10% EC). It was supplied by Dow Agro Science, Applied at rate 200 ml/fed.

e.3. **Oxymatrine** 0.4% + prosuler 0.2% (Kingo 0.6% S.L). It was supplied by King Bo, China, applied at rate 200 ml/fed.

f. **Biocides**

f.1. ***Bacillus thuringiensis*** (Agerin 6.4% WP). It was supplied by Plant Genetic Engineering Research Institute, applied at rate 500 gm/fed.

f.2. **Spinosad** (Tracer 24% SC). It was supplied by Dow Agro Sciences, applied at rate 50 ml/fed.

f.3. **Azadirachtin** (Neem-Azal 0.15% EC). It was supplied by cyclo-forune Grtis Tagros, applied at rate 600 ml/fed.

2. Design of field experiments

The effect of different insecticides in 19 rotations against the pink bollworm and spiny bollworm (SBW), *E. insulana* (Boisd.) infesting cotton plants was studied to select the best sequence. The experiment was carried out at Sakha Agricultural Research Station, Kafr El-Sheikh Governorate during 2008 and 2009 growing cotton seasons. The cultivated cotton variety was Giza 86. The area was arranged in a complete randomized block design with four replicates for each treatment. Each replicate was one kirate (1/24 feddan). Tested compounds were sprayed using a knapsack sprayer (CP3) at rate of 200 Liter/feddan. All treatments were done when the percent of bollworms infestation exceeded 3%. Table (1) summarized the different sequential treatments and dates of spraying. The interval between any two successive applications was two weeks.

Table 1. Represent different sequential treatments during 2008 and 2009 cotton season.

No.	Sequence of treatment		
	1st spray **10/8/2008** **29/7/2009**	**2nd spray** **24/8.2008** **11/8/2009**	**3rd spray** **7/9/2008** **25/8/2009**
1	King Bo. (Oxymatrine+ prosaler)	Lambdacyhalothrin	Methomyl
2	Indoxacarb	Chlorpyrifos	Deltamethrin
3	Chlorfluazuron	Methomyl	Deltamethrin
4	Hexaflumuron	Deltamethrin	Profenofos
5	Chlorpyrifos	Deltamethrin	King Bo. (Oxymatrine+ prosaler)
6	Methomyl	King Bo. (Oxymatrine+ prosaler)	Lambda cyhalothrin
7	Profenfos	Lambda cyhalothrin	Methomyl
8	Spinosad	Spinosad	Spinosad
9	*B. thuringiensis* (Agerin)	*B. thuringiensis* (Agerin)	*B. thuringiensis* (Agerin)
10	Azadirachtin (Neem)	Azadirachtin (Neem)	Azadirachtin (Neem)
11	Spinosad	*B. thuringiensis* (Agerin)	Azadirachtin (Neem)
12	*B. thuringiensis* (Agerin)	Spinosad	Azadirachtin (Neem)
13	Azadirachtin (Neem)	*B. thuringiensis* (Agerin)	Spinosad
14	Spinosad	Lambda cyhalothrin	Methomyl
15	Indoxacarb	Spinosad	Lambdacyhalothrin
16	Chlorfluazuron	*B. thuringiensis* (Agerin)	Chlorpyrifos
17	Hexaflumuron	Azadirachtin (Neem)	Deltamethrin
18	*B. thuringiensis* (Agerin)	Deltamethrin	King Bo. (Oxymatrine+ prosaler)
19	Azadirachtin (Neem)	King bo	Lambdacyhalothrin
20	Untreated		

2.a. Determination of bollworms infestation

For assessing the infestation by the pink and spiny bollworms, 100 green bolls were collected weekly at random from both diagonals of the inner square area for each plot (25 bolls for each replicate) according to the method of Shaaban and Radwan (1974).

Laboratory experiment

a. Rearing of pink bollworm

Newly hatched larvae of *P. gossypiella* were obtained from the Bollworms Research Department, Plant Protection Research Institute, Dokki, Giza, Egypt and reared several generations on modified artificial diet at 27 $\pm$ 1^{o}C and 75 $\pm$ 5% R.H. as described by Abd El-Hafez *et. al.* (1982).

b. Histopathological studies

Detection of chronic effects of the tested biocides against either 2nd or 4th instar larvae of pink bollworm treated at newly hatched larvae was as follows:

The newly hatched larvae of PBW were fed on diets containing field recommended concentrations of the tested biocides. These rates were 0.25 mg/L, 3.0 ml/L, 3 g/L for spinosad, Azadirachtin and Agrin, respectively, then held at 27 $\pm$ 1^{o}C and 75 $\pm$ 5 R.H. for two days after treatment. The survivors 2nd and 4th larvae were transferred to glass vials (2 x 7 cm) containing untreated diet, the survived 2nd and 4th instar larvae (7 and 14 days old) were removed and fixed in formalin 10% and processed to paraffin sections according to the method of Lee (1950). The same procedure was adopted on untreated larvae as comparison. All histopathological study was done in the Dept. of Zoology, Faculty of Science, Cairo University.

Statistical analysis

The reduction percentages of bollworms infestation were calculated according to the equation of Henderson and Tilton (1955). All data were subjected to one-way analysis of variance (ANOVA) followed by Duncan multiple range test (Duncan, 1955) to determine the significant differences among treatments mean values at 0.05 probability .

RESULTS AND DISCUSSION

The effectiveness of the insecticidal sequence for the reduction in the green boll infestation caused by pink and spiny bollworms were summarized and discussed as follows:

Data presented in Table (2) showed that the numbers of bollworms larvae per 25 green bolls during 2008 cotton season. It is clear that the infestation with both pink and spiny bollworms started with few numbers of 1st and 2nd instar larvae from late July and slightly increased gradually till the end of season. Also, all insecticides treatments decreased significantly the population of both pests compared to the untreated check as the mean number of bollworms. Larvae/25 bolls in treated plots ranged between 1.42 and 4.08 larvae, whereas it reached to 10.29 larvae in untreated plots.

Comparing between the efficiency of the tested insecticidal sequences against both bollworms, the data presented in Table (3) revealed that spraying of Hexafluzuron followed by methomyl and profenofos (sequence 4) was the most effective insecticidal treatment induced 50.05% reduction in larval population, while the sequence number (16) included chlorfluzuron, B. thuringiensis and chlorpyrifos was the least effective sequence with percent reduction of 34.18% comparing between the different insecticidal sequences against bollworms infestation, Based on general mean reduction in bollworms infestation the potency of these sequences could be arranged descendingly as the following sequence number: 4 (50.05%, 13 (46.99%), 8 (44.88%), 17 (44.72%), 11 (43.78%), 1 (43.49%), S7 (43.22%), 10 (43.10%), 12 (41.06%), 3 (40.27%), 18 (40.07%), 5 (39.67%), 9 (38.92%), 6 (37.38%), 15 (37.12%), 19 (37.12%), 2 (37.04%), 14 (36.09%) and 16 (34.18%) reduction in larval population. With regard to 2009 cotton season, data presented in Table (4) showed similar trend of results as in 2008 season, but the effect of tested insecticidal sequences against both bollworms were relatively more than in the first season, the mean number of bollworms larvae per 25 boll in treated plots ranged between 1.79 and 3.71 larvae, while it reached to 10.25 larvae in the untreated plots. Data presented in Table (5) indicated that repetitive spraying of spinosad (biocide) in the three spraying (sequence 8) was the most efficient insecticidal treatment induced 52.43% reduction in larval population compound with other sequences, on contrary the sequence number (6) was the least effective sequence with percent reduction of 38.65%. Based an general mean reduction in boll worms infestation the potency of these sequences could be arranged, discerningly as the following sequence number: 8 (52.43%), 11 (47.76%), 18 (46.83%), 16 (46.16%), 17 (45.83%), 12 (45.82%), 7 (43.94), 1 (43.82%), 13 (43.78%), 9 (43.58%), 14 (43.17%), 2 (42.77%), 19 (42.74%), 15 (42.63%), 10 (41.99%), 3 (40.76%), 5 (39.86%), 4 (39.09%) and 6 (38.65%) reduction in larval population (Table 5).

Table 2. Effect of different sequence of insecticides on the number of bollworms larvae/25 cotton green bolls during 2008 cotton season

No. of seq.	Sequences			After 1st spray				After 2nd spray				After 3rd spray				General mean
				Before	Week after			Before	Week after			Before	Week after			
	1st	2nd	3rd	spray	1 w	2w	Mean	spray	1 w	2w	Mean	spray	1 w	2w	Mean	
1	Oxymatrine + Prosuler	Lambdacyhalothrin	Methomyl	1.50	2.00	2.75	1.19 bcd	2.75	3.50	3.25	3.37 de	3.25	2.25	3.00	2.63 efg	2.79 b
2	Indoxacarb	Chlorpyrifos	Deltamethrin	1.25	1.75	2.25	2.00 b-e	2.25	2.75	3.75	3.25 def	3.75	3.50	3.50	3.26 def	2.92 b
3	Chlorfluazuron	Methomyl	Deltamethrin	1.50	2.50	2.50	2.50 bc	2.50	2.75	3.00	2.87 e-h	3.00	2.50	2.75	2.63 efg	2.66 b
4	Hexaflumuron	Deltamethrin	Profenofos	1.50	1.50	2.25	1.87 cde	2.25	1.25	3.00	2.13 hi	3.00	2.50	2.75	2.63 efg	2.21 b
5	Chlorpyrifos	Deltamethrin	Oxymatrine + Prosuler	1.00	1.50	1.75	1.63 def	1.75	2.50	2.75	2.63 e-h	2.75	2.25	2.50	2.37 fgh	2.25 b
6	Methomyl	Oxymatrine + Prosuler	Lambdacyhalothrin	1.00	1.50	2.0	1.75 c-f	2.00	1.75	3.00	2.37 f-i	3.00	3.50	3.50	3.50 def	2.54 b
7	Profenofos	Lambdacyhalothrin	Methomyl	1.00	1.50	1.75	1.63 def	1.75	1.50	2.50	2.0 hi	2.50	2.00	2.50	2.25 gh	1.95 b
8	Spinosad	Spinosad	Spinosad	1.25	0.75	1.25	1.00 f	1.25	1.50	1.50	1.5 i	1.50	1.50	1.75	1.63 h	1.38 b
9	*B. thuringiensis*	*B. thuringiensis*	*B. thuringiensis*	1.50	2.50	3.00	2.75 b	3.00	3.50	4.00	3.75 cd	4.00	3.75	3.25	3.5 cde	3.33 b
10	Azadirachtin	Azadirachtin	Azadirachtin	1.25	1.25	2.75	2.00 b-e	2.75	4.00	5.00	4.5 bc	5.00	3.00	3.50	3.25 def	3.25 b
11	Spinosad	*B. thuringiensis*	Azadirachtin	1.00	1.00	2.00	1.50 ef	2.00	1.25	2.75	2.00 hi	2.75	2.50	3.25	2.87 d-g	2.13 b
12	*B. thuringiensis*	Spinosad	Azadirachtin	1.25	1.50	2.50	2.00 b-e	2.50	2.50	2.75	2.62 e-h	2.75	2.50	3.25	2.87 d-g	2.50 b
13	Azadirachtin	*B. thuringiensis*	Spinosad	1.50	2.00	3.50	2.75 b	3.50	3.50	5.25	4.37 bc	5.25	2.75	3.25	3.00 d-g	3.38 b
14	Spinosad	Lambdacyhalothrin	Methomyl	1.00	1.50	1.75	1.60 def	1.75	1.75	2.75	2.25 ghi	2.75	2.75	3.25	3.00 d-g	2.29 b
15	Indoxacarb	Spinosad	Lambdacyhalothrin	1.25	1.50	2.00	1.75 c-f	2.00	3.00	3.25	3.12 d-g	3.25	2.25	3.75	3.50 def	2.63 b
16	Chlorfluazuron	*B. thuringiensis*	Chlorpyrifos	1.50	2.50	3.00	2.75 b	3.00	4.50	5.00	4.75 b	5.00	4.75	3.50	4.13 c	3.88 b
17	Hexaflumuron	Azadirachtin	Deltamethrin	1.50	1.00	3.00	2.00 b-e	3.00	3.50	3.25	3.37 de	3.25	3.00	3.00	3.00 d-g	2.79 b
18	*B. thuringiensis*	Deltamethrin	Oxymatrine + Prosuler	1.25	1.25	3.00	2.12 b-e	3.00	3.50	4.50	3.25 bcd	4.50	4.00	3.50	3.75 cd	3.29 b
19	Azadirachtin	Oxymatrine + Prosuler	Lambdacyhalothrin	1.50	1.00	3.50	2.50 b-e	3.50	5.00	5.00	4.25 b	5.00	5.50	4.50	5.00 b	4.08 b
20	Control			1.50	5.00	5.00	5.00 a	5.00	8.25	11.50	6.62 a	11.50	14.50	17.50	16.00 a	10.29 a

Mean followed by the same letter are not significantly different at 0.05 level of probability according to DMRT

Table 3. Reduction percentages in bollworms applied with different insecticidal sequences during cotton season of 2008

No. of seq.	No. of sequence			1st spray			2nd spray			3rd spray			General mean of reduction
	1st	2nd	3rd	% red after			% red after			% red after			
				1 w	2 w	Mean	1 w	2 w	Mean	1 w	2 w	Mean	
1	Oxymatrine + Prosuler	Lambdacyhalothrin	Methomyl	60.00	45.00	52.50 bc	22.78	48.62	35.7 a-d	45.08	39.35	42.23 b	43.49 g
2	Indoxacarb	Chlorpyrifos	Deltamethrin	57.92	46.00	51.96 bc	25.92	27.55	26.73 cde	25.98	38.84	32.21 bcd	37.04 bc
3	Chlorfluazuron	Methomyl	Deltamethrin	50.00	50.00	50.00 bc	33.35	47.83	40.59 abc	20.68	39.75	30.21 bcd	40.27 e
4	Hexaflumuron	Deltamethrin	Profenofos	70.00	55.00	62.50 ab	59.59	42.04	50.81 a	33.91	39.76	36.84 bc	50.05 i
5	Chlorpyrifos	Deltamethrin	Oxymatrine + Prosuler	55.00	62.50	58.75 bc	30.99	31.69	31.34 b-e	35.11	40.27	37.69 b	39.67 de
6	Methomyl	Oxymatrine + Prosuler	Lambdacyhalothrin	55.00	57.14	56.07 bc	46.97	34.78	40.87 abc	7.47	23.33	15.40 e	37.38 bcd
7	Profenofos	Lambdacyhalothrin	Methomyl	55.00	47.50	51.25 bc	48.24	31.69	39.97 a-d	27.91	34.29	31.10 bcd	43.22 fg
8	Spinosad	Spinosad	Spinosad	80.00	70.00	75.00 a	27.25	47.84	37.37 a-d	20.87	23.33	22.10 de	44.88 gh
9	*B. thuringiensis*	*B. thuringiensis*	*B. thuringiensis*	50.00	40.00	45.00 c	29.29	42.02	35.65 a-d	25.63	46.60	36.12 bc	38.92 cde
10	Azadirachtin	Azadirachtin	Azadirachtin	70.00	34.00	52.00 bc	11.85	36.77	24.31 de	52.00	54.00	53.00 a	43.10 fg
11	Spinosad	*B. thuringiensis*	Azadirachtin	70.00	40.00	55.00 bc	62.12	40.21	51.16 a	27.90	22.33	25.12 cde	43.78 g
12	*B. thuringiensis*	Spinosad	Azadirachtin	64.00	40.00	52.00 bc	39.39	52.77	46.08 ab	27.91	22.33	21.12 cde	41.06 ef
13	Azadirachtin	*B. thuringiensis*	Spinosad	60.00	30.00	45.00 c	39.39	34.78	37.08 a-d	58.45	59.32	58.93 a	46.99 hi
14	Spinosad	Lambdacyhalothrin	Methomyl	55.00	47.50	51.00 bc	39.39	31.69	35.54 a-d	20.68	22.33	21.50 de	36.09 ab
15	Indoxacarb	Spinosad	Lambdacyhalothrin	64.00	52.00	58.00 bc	9.10	29.34	19.22 e	45.08	23.22	34.15 bc	37.12 bc
16	Chlorfluazuron	*B. thuringiensis*	Chlorpyrifos	50.00	40.00	45.00 c	9.10	27.53	18.331 e	24.64	54.00	39.32 b	34.18 a
17	Hexaflumuron	Azadirachtin	Deltamethrin	80.00	40.00	60.00 bc	29.29	52.90	41.90 abc	26.80	39.35	33.07 bcd	44.72 gh
18	*B. thuringiensis*	Deltamethrin	Oxymatrine + Prosuler	70.00	28.00	49.00 bc	29.29	34.78	32.03 b-e	29.50	48.89	39.19 b	40.07 e
19	Azadirachtin	Oxymatrine + Prosuler	Lambdacyhalothrin	80.00	30.00	55.00 bc	13.43	37.89	25.66 cde	20.68	40.85	30.76 bcd	37.12 bc

Mean followed by the same letter are not significantly different at 0.05 level of probability according to DMRT

Table 4. Effect of different sequences of insecticides on the number of bollworms larvae/25 cotton green bolls during 2009 cotton season.

No. of seq.	Sequences			After 1st spray				After 2nd spray				After 3rd spray				General mean
				Before spray	Week after			Before spray	Week after			Before spray	Week after			
	1st	2nd	3rd		1 w	2w	Mean		1 w	2w	Mean		1 w	2w	Mean	
1	Oxymatrine + Prosuler	Lambdacyhalothrin	Methomyl	1.00	1.50	1.50	1.50 cde	1.50	2.00	3.50	2.75 f-i	3.50	2.50	2.25	2.37 fg	2.21 b
2	Indoxacarb	Chlorpyrifos	Deltamethrin	1.00	1.50	2.00	1.75 cd	2.00	3.00	3.00	3.00 e-h	3.00	2.25	2.75	2.50 fg	2.42 b
3	Chlorfluazuron	Methomyl	Deltamethrin	1.25	1.50	3.50	2.50 b	3.50	4.00	4.50	4.25 bc	4.50	4.25	4.75	4.50 b	3.79 b
4	Hexaflumuron	Deltamethrin	Profenofos	1.00	1.25	1.50	1.37 cde	1.50	2.00	3.00	2.50 g-j	3.00	2.75	3.50	3.12 c-g	2.33 b
5	Chlorpyrifos	Deltamethrin	Oxymatrine + Prosuler	1.00	1.25	2.00	1.63 cde	2.00	2.50	3.50	3.00 e-h	3.50	2.75	3.50	3.12 c-g	2.58 b
6	Methomyl	Oxymatrine + Prosuler	Lambdacyhalothrin	1.00	1.50	1.75	1.63 cde	1.75	2.50	3.50	3.00 e-h	3.50	2.50	3.00	2.75 d-g	2.46 b
7	Profenofos	Lambdacyhalothrin	Methomyl	1.00	1.25	1.75	1.50 cde	1.75	1.75	2.25	1.75 ij	2.25	2.25	2.75	2.50 fg	2.00 b
8	Spinosad	Spinosad	Spinosad	1.00	0.75	1.25	1.00 e	1.25	1.25	2.50	1.87 j	2.50	2.00	3.00	2.50 fg	1.79 b
9	*B. thuringiensis*	*B. thuringiensis*	*B. thuringiensis*	1.25	1.50	2.25	1.87 bc	2.25	2.50	4.50	3.50 c-j	4.50	3.00	5.25	4.13 bc	3.17 b
10	Azadirachtin	Azadirachtin	Azadirachtin	1.00	0.75	1.75	1.25 cde	1.75	2.00	3.00	2.50 g-j	3.00	3.25	3.50	3.37 c-g	2.38 b
11	Spinosad	*B. thuringiensis*	Azadirachtin	0.75	0.75	1.50	1.13 de	1.50	2.00	2.75	3.37 hij	2.75	2.00	2.50	2.25 g	1.92 b
12	*B. thuringiensis*	Spinosad	Azadirachtin	1.25	0.75	2.25	1.50 cde	2.25	2.50	3.00	2.75 f-i	3.00	2.75	4.00	3.37 c-g	2.51 b
13	Azadirachtin	*B. thuringiensis*	Spinosad	1.00	0.75	2.50	1.63 cde	2.50	3.25	4.50	3.87 bcd	4.50	2.75	4.75	3.75 bcd	3.08 b
14	Spinosad	Lambdacyhalothrin	Methomyl	1.25	1.50	2.00	1.75 cd	2.00	3.00	3.75	3.37 def	3.75	2.25	3.00	2.63 efg	2.58 b
15	Indoxacarb	Spinosad	Lambdacyhalothrin	1.50	1.50	3.50	2.50 b	3.50	3.75	5.00	4.37 b	5.00	4.00	4.00	4.00 bc	3.63 b
16	Chlorfluazuron	*B. thuringiensis*	Chlorpyrifos	1.25	1.50	1.50	1.50 cde	1.50	2.00	3.50	2.75 f-i	3.50	2.75	2.50	2.63 efg	2.29 b
17	Hexaflumuron	Azadirachtin	Deltamethrin	1.25	1.00	2.50	1.75 cd	2.50	2.50	4.00	3.25 d-g	4.00	2.75	4.50	3.63 b-e	2.88 b
18	*B. thuringiensis*	Deltamethrin	Oxymatrine + Prosuler	1.25	1.25	2.00	1.63 cde	2.00	3.00	4.50	3.75 b-e	4.50	4.00	5.00	4.54 b	3.29 b
19	Azadirachtin	Oxymatrine + Prosuler	Lambdacyhalothrin	1.25	1.50	3.00	2.25 cde	3.00	4.00	4.00	4.00 b-e	4.00	2.75	4.50	3.63 b-e	3.29 b
20	Cont.			1.00	3.25	4.50	3.87 a	4.50	8.50	11.75	10.00 a	11.75	14.50	19.00	16.75 a	10.25a

Table 5. Reduction percentages in bollworms applied with different insecticidal sequences during cotton season of 2009

No. of seq.	No. of sequence			1st spray			2nd spray			3rd spray			General mean of reduction
	1st	2nd	3rd	% red after			% red after			% red after			
				1 w	2 w	Mean	1 w	2 w	Mean	1 w	2 w	Mean	
1	Oxymatrine + Prosuler	Lambdacyhalothrin	Methomyl	53.84	66.66	60.25 b-e	29.41	10.66	20.04 e	42.12	60.24	51.18 a	43.82 e
2	Indoxacarb	Chlorpyrifos	Deltamethrin	53.84	55.55	54.69 c-f	20.00	44.68	32.34 cd	39.22	43.32	41.27 abc	42.77 de
3	Chlorfluazuron	Methomyl	Deltamethrin	63.10	37.50	50.30 ef	39.49	50.76	44.75 ab	18.96	34.72	26.84 def	40.76 bc
4	Hexaflumuron	Deltamethrin	Profenofos	61.53	66.66	64.09 a-d	29.40	23.38	26.39 cde	25.72	27.85	26.78 def	39.09 a
5	Chlorpyrifos	Deltamethrin	Oxymatrine + Prosuler	55.00	47.00	51.00 def	30.99	31.69	31.34 cde	36.33	38.16	37.25 bcd	39.86 ab
6	Methomyl	Oxymatrine + Prosuler	Lambdacyhalothrin	55.00	40.00	47.50 f	24.40	23.39	23.89 e	42.12	47.00	44.56 ab	38.65 a
7	Profenofos	Lambdacyhalothrin	Methomyl	61.38	61.11	61.24 a-e	47.05	50.72	48.88 a	18.06	24.42	21.24 f	43.94 e
8	Spinosad	Spinosad	Spinosad	76.92	72.22	74.57 a	47.05	57.45	52.29 a	35.17	25.79	30.48 c-f	52.43 h
9	*B. thuringiensis*	*B. thuringiensis*	*B. thuringiensis*	63.10	60.00	61.55 a-e	41.19	23.41	32.30 cd	45.98	27.85	36.92 bcd	43.58 de
10	Azadirachtin	Azadirachtin	Azadirachtin	76.92	61.11	69.02 ab	39.52	34.33	36.93 bc	12.21	27.85	20.03 f	41.99 cd
11	Spinosad	*B. thuringiensis*	Azadirachtin	76.92	66.66	71.79 ab	29.41	29.78	29.59 cde	41.07	43.78	42.42 abc	47.76 g
12	*B. thuringiensis*	Spinosad	Azadirachtin	81.52	60.00	70.76 ab	41.19	48.94	45.06 ab	25.72	17.53	21.62 ef	45.82 f
13	Azadirachtin	*B. thuringiensis*	Spinosad	76.92	44.44	60.68 a-e	25.88	30.22	28.05 cde	50.48	34.72	42.60 abc	43.78 e
14	Spinosad	Lambdacyhalothrin	Methomyl	63.10	64.48	63.79 a-d	47.05	23.42	35.23 bcd	35.17	25.79	30.48 c-f	43.17 de
15	Indoxacarb	Spinosad	Lambdacyhalothrin	69.23	48.15	58.69 b-f	41.19	23.41	32.30 cd	45.97	27.85	36.91 bcd	42.63 de
16	Chlorfluazuron	*B. thuringiensis*	Chlorpyrifos	63.00	73.35	68.17 abc	39.52	34.34	36.93 bc	28.62	38.15	33.38 b-e	46.16 f
17	Hexaflumuron	Azadirachtin	Deltamethrin	75.38	55.60	65.49 abc	29.41	29.78	29.59 cde	41.07	43.77	42.42 abc	45.83 f
18	*B. thuringiensis*	Deltamethrin	Oxymatrine + Prosuler	69.23	64.48	66.85 abc	41.19	48.94	4506 ab	39.60	17.54	28.57 def	46.83 fg
19	Azadirachtin	Oxymatrine + Prosuler	Lambdacyhalothrin	63.10	46.71	54.00 c-f	31.18	30.22	30.70 cde	50.48	34.73	42.61 abc	42.74 de

Means followed by the same letter are not significantly different at 5% level according to DMRT test.

The average of percent reduction of bollworms during 2008 and 2009 cotton seasons are presented in Table (6). It is clear that, the sequence number (8) repetitive spraying of spinosad in three successive sprays had the highest reduction in the number of larvae in bolls recording 48.66% reduction. While the sequence number (6) include methomyl followed by oxymatrine + prosuler and lambdacyhalothrin induced the lowest reduction recording (38.02% reduction), the potency of these sequences could be arranged, descendingly as follows sequence number: 8> 11> 13> 17> 4> 1> 7> 18> 12> 10> 9> 3> 16> 19> 2> 15> 5> 14> 6.

Generally, repetitive spraying of bioinsecticides in three successive sprays induced variable effectiveness against bollworms larvae, the sequence 8 (spinosad repetitive three spraying induced 48.66% reduction, sequence (*B. thuringiensis*) repetitive three sprays induced 41.25% reduction and sequence 10 (Azadirachtin) repetitive three sprays caused 42.55% reduction. The sequence 11 use bioinsecides, spinosad, *B. thuringiensis* and Azadirachtin, seq.12 use *B. thuringiensis*, spinosad and azadirachtin and S13 use Azadirachtin, *B. thuringiensis* and spinosad induced good effect in control bollworms causing 45.85%, 43.40% and 45.39% reduction, respectively. Using the conventional insecticides in sequence with antimolting compounds (chlorfluazuron oxymatrine + prosuler and Hexaflumuron) slightly caused high reduction in larval population which being 40.25, 43.66 and 44.57% reduction, respectively, sequences number seq.1, seq.4 and seq.5 (Table 6). Similar observation was noticed that conventional insecticides in sequence with biocides and IGRs caused good reduction in bollworms larvae induced 39.72, 39.88, 40.19, 45.28, 43.64 and 39.94% reduction, respectively. Sequence number 14, 15, 16, 17, 18 and 19 (Table 6).

From the practical point of view, it is advisable to avoid using repetitive spraying of the same compound 3 times to minimize the selection pressure and the rate of developing resistance strain to such compound. Thus, for selecting the proper compound to be used in sequences, one should be aware of such compound have different mode of action. They belong to different chemical groups have no positive cross resistance between each other.

Table 6. Average reduction percentages of bollworms during cotton seasons of 2008 and 2009

No. of seq.	No. of sequence			1st spray			2nd spray			3rd spray			General mean of reduction
	1st	2nd	3rd	% red after			% red after			% red after			
				1 w	2 w	Mean	1 w	2 w	Mean	1 w	2 w	Mean	
1	Oxymatrine + Prosuler	Lambdacyhalothrin	Methomyl	59.42	55.83	57.63 bcd	26.10	29.64	27.87 b	43.60	49.80	46.70 ab	43.66 de
2	Indoxacarb	Chlorpyrifos	Deltamethrin	56.88	50.75	53.81 bcd	22.96	36.12	29.54 b	32.60	41.08	36.84 b-e	39.91 b
3	Chlorfluazuron	Methomyl	Deltamethrin	56.55	43.75	50.15 d	36.42	49.32	42.87 b	19.82	37.24	28.53 cde	40.52 b
4	Hexaflumuron	Deltamethrin	Profenofos	65.77	60.83	63.30 b	44.50	32.71	38.61 b	29.82	33.81	31.81 cde	44.57 efg
5	Chlorpyrifos	Deltamethrin	Oxymatrine + Prosuler	55.00	54.75	54.87 bcd	22.21	31.69	26.95 b	35.72	39.22	37.47 bcd	39.77 b
6	Methomyl	Oxymatrine + Prosuler	Lambdacyhalothrin	55.00	48.57	51.78 cd	35.69	28.88	26.84 b	24.80	35.17	29.98 cde	38.02 a
7	Profenofos	Lambdacyhalothrin	Methomyl	58.19	54.31	56.25 bcd	47.56	44.31	45.94 a	27.78	29.36	28.57 cde	43.58 def
8	Spinosad	Spinosad	Spinosad	78.46	71.11	74.78 a	37.15	52.65	44.90 a	28.02	24.56	26.29 cde	48.66 h
9	*B. thuringiensis*	*B. thuringiensis*	*B. thuringiensis*	56.55	50.00	53.27 bcd	35.24	32.72	33.98 b	35.81	37.23	36.52 b-e	41.25 bc
10	Azadirachtin	Azadirachtin	Azadirachtin	73.46	47.56	60.51 bcd	25.69	35.55	30.62 b	32.11	40.93	36.52 b-e	42.55 cd
11	Spinosad	*B. thuringiensis*	Azadirachtin	73.46	53.00	63.33 bc	45.77	35.00	40.38 b	34.49	33.06	33.77 b-e	45.85 g
12	*B. thuringiensis*	Spinosad	Azadirachtin	72.26	50.00	61.13 bcd	40.25	50.89	45.57 b	26.82	19.93	23.37 e	43.40 de
13	Azadirachtin	*B. thuringiensis*	Spinosad	68.46	37.22	52.84 bcd	32.61	32.50	32.53 b	54.47	47.02	50.75 a	45.39 fg
14	Spinosad	Lambdacyhalothrin	Methomyl	59.05	55.99	57.52 bcd	43.22	27.56	35.39 b	27.93	24.06	25.99 cde	39.72 b
15	Indoxacarb	Spinosad	Lambdacyhalothrin	66.62	50.08	58.35 bcd	25.15	26.38	25.76 b	45.51	25.54	35.53 b-e	39.88 b
16	Chlorfluazuron	*B. thuringiensis*	Chlorpyrifos	56.50	56.68	56.59 bcd	24.31	30.94	27.63 b	26.63	46.08	36.35 b-e	40.19 b
17	Hexaflumuron	Azadirachtin	Deltamethrin	77.69	47.80	62.59 bc	29.35	41.34	35.34 b	33.94	41.56	37.75 bc	45.28 fg
18	*B. thuringiensis*	Deltamethrin	Oxymatrine + Prosuler	69.62	46.25	57.94 bcd	35.24	41.86	38.55 b	34.55	33.20	33.87 b-e	43.46 de
19	Azadirachtin	Oxymatrine + Prosuler	Lambdacyhalothrin	71.55	38.36	54.95 bcd	22.31	34.06	28.18 b	35.58	37.75	36.66 b-e	39.94 b

Means followed by the same letter are not significantly different at 5% level according to DMRT test.

These results agreed with the previous finding of Johnson et el. (2000), Haidar *et. al.* (2002), Omar *et. al.* (2005), Ghure *et. al.* (2008) and Gosalwad *et. al.* (2009) showed that the newer insecticides molecules i.e. (indoxacarb, spinosad, Emamectin benzoate, lambdacyhalothrin, polytrin c and endosulfan) significantly reduced bollworms infestation in cotton. However, Emamectin benzoate was the most effective followed by spinosad.

Abd El-Mageed *et. al.* (2007) reported that the sequence of betacyfluthrin, malathion and spinosad and the sequence of lufenuron, malathion and spinosad induced the highest reduction (81.04 and 86.08%) in infestation of pink bollworm larvae, respectively. Abdel-Rahman *et. al.* (2005) reported that the Azadirachtin Azal formulations applying at higher dose T/S (20 and 25 ppm) were the most effective against P. gossypiella also, Sarode *et. al.* (2000) and Gupta (2001) who found that Azadirachtin products and formulation of B. thuringiensis were effective against the bollworms and can be used as alternative to chemical insecticides. In addition, Mirmoayedi *et. al.* (2010) showed that three bioinsecticides, B. thuringiensis, Azadirachtin and spinosad were effective against spiny bollworm. Also, spinosad gave most effective followed by Azadirachtin and B. thuringiensis. Fadare and Amusa (2003) reported that application of microbial (biotrop, diple and thuricide) followed by chemical insecticides were better to control bollworms than other combinations evaluated. El-Metwally *et. al.* (2003) showed that synthetic pyrethroids (Fenpropathrin, esfenvalerate and lambdacyhalothrin) gave the highest reduction of bollworms infestation followed by IGRs, flufenoxuron, hexaflumzuron mixed with O.P chlorpyrifos, while IGRs alone gave least reduction.

On the other hand, our results disagree with that obtained by Jeyakumar and Gupta (2002) who reported Azadirachtin and Bacillus thuringiensis treatments alone was not effective against bollworms. But, treatments by neem + B. thurienginsis alternatively with synthetic pesticides were more effective in reducing bollworm infestation.

Histopathological effects of some biocides on pink bollworm larvae (PBW)

The histopathological structure of midgut in larvae of lepidoptera is well documented (Chapman, 1988). Figures (1 and 2) exhibit the various organs and tissues of fourth abdomen segment of the untreated 2nd and 4th instar larvae of PBW.

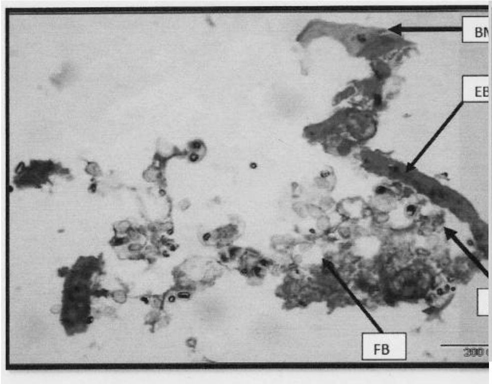


Fig. 1. Tissue and cells organs of the midgut of the 2nd instar larvae on control (untreated)

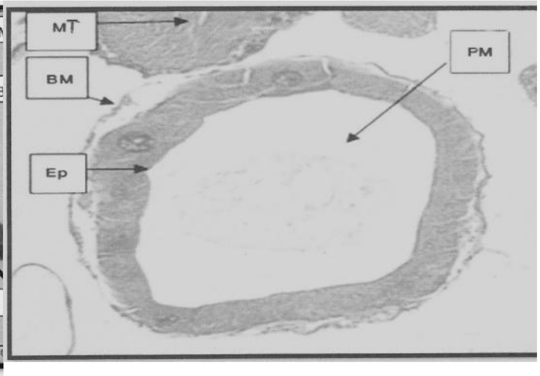


Fig. 2. Tissues and cells organs of the midgut of the 4th instar larvae on control (untreated)

1. The histopathological effects of B. thuringiensis

Figures (3 and 4) show the effect of B. thuringiensis on 2nd and 4th instar larvae of PBW. These effects are complete destruction of midgut vigorous degeneration of fat bodies (IFB and OFB), sometimes degeneration of some epidermal cells and midgut in the 2nd and 4th instar larval compared with untreated larvae.

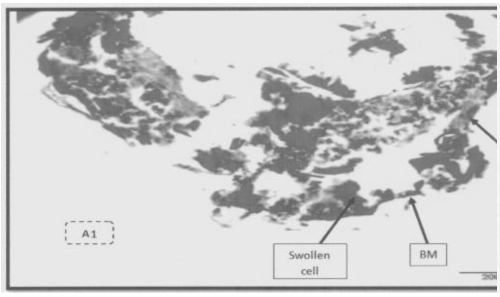


Fig. 3. The histopathological effects of B. thuringiensis (1644×102 IU/L) on the tissues and cells organs of the midgut of the 2nd instar larvae

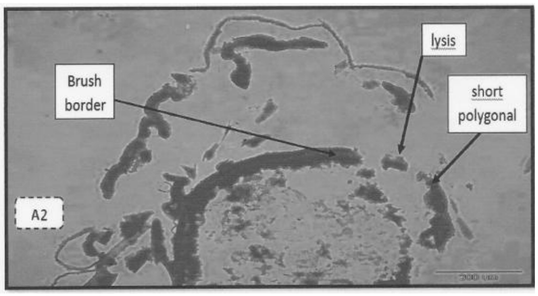
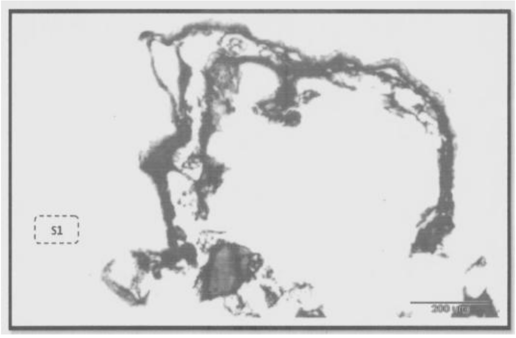


Fig. (4): The histopathological effects of B. thuringiensis (1644×102 IU/L) on the tissues and cells organs of the midgut of the 4th instar larvae

2. Histopathological effects of spinosad

Spinosad caused adverse effects on 2nd and 4th instar larvae of PBW. Fig. (5) Shows these changes included degeneration, vacuolation and shrinkage of the epithelial cells. The lysis of the anterior mid-gut progressed through swollen clear cells vacuoles, nuclear degenerated Fig. (6) Show the serious lesion alterations included analyze and destroyed of epithelial cells and dissolve of nuclei of epithelial cells. Changes were observed in the anterior and posterior region of the midgut, included separation of the epithelial cells from the basement membrane with damage of peritrophic membrane.



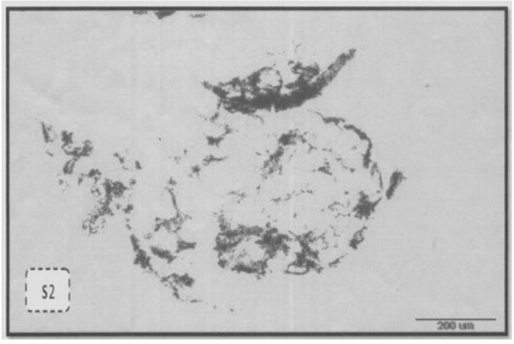


Fig. 5. The histopathological effects of Spinosad (60 ppm) on the tissues and cells organs of the midgut of the 2nd instar

Fig. 6. The histopathological effects of Spinosad (60 ppm) on the tissues and cells organs of the midgut of 4th instar

3. Histopathological effects of Azadirachtin

Figure (7, 8) shows the effect of Azadirachtin on 2nd and 4th instar larvae of the PBW. These effects are, figure (7) show the lysis of the anterior midgut progressed through swollen clear cells, vacuoles, nuclear degeneration, disruption of the junction of complexes and nuclear lysis, degeneration of the microvilli acceleration of the lysis of clear cells was perceptible at the level of their bruch border, basal membranes, nucleus and cytoplasmic organells, partial lysis of midgut began through local detachment among small groups of dark cells bearing a dilated basal membrane.

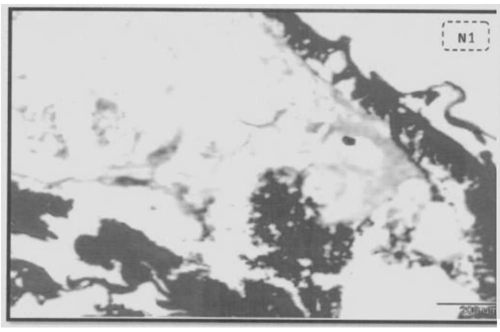


Fig. 7. The histopathological effects of Azadirachtin (4.5 ppm) on the tissues and cells organs of the midgut (2nd instar)

Fig. 8. The histopathological effects of Azadirachtin (4.5 ppm) on the tissues and cells organs of the midgut (4th instar)

Figure (8) show the serious damage of the epithelial columnar cells, some cells appear slightly hypertrophied with a perceptible beginning of vacuolization at apical level cells of midgut show hardly any morphological deviation from normal ones. Generally, the histopathological effects of tested biocides were more serious on

2nd instar larvae than 4th one Also, this previous observation was more evident when larvae were treated with field concentration of spinosad figures (5, 6).

The current results are in good harmony with the previous finding of Zidan *et. al.* (1998) who found that (MPVII) caused morphological changes to the midgut, epithelial cells layer (EP) of treated larvae of P. gossypiella and Earias insulana, Rashmi and Singh (2004) studied the histological effect of BTK on the midgut of fourth instar larvae of H. armigera. They found that there was a hypertrophied columnar cell in the epithelium, PM pushing into sloughing cells. Hussein *et. al.* (2002) studied the histological and histochemical changes of vertimec and neemazal-natural product on P. gossypiella and E. insulana in laboratory conditions. They found that the two compounds caused destruction for the midgut epithelial cells. The histochemical studies revealed that both compounds affected on the polysaccharides in midgut epithelium. Also, Abdel Rahman *et. al.* (2005), Omar *et. al.* (2006) and Abd El-Halim *et. al.* (2008) who found that B. thuringiensis var kurstaki, Azadirachtin formulation and spinosad had histopathological effects on midgut of P. gossypiella and E. insulana. Abd El-Hafez *et. al.* (2009) found that the LC50 of B. thuriengiensis (Bt), Ecotech, Pro (BtK+Bta) and Bioclean (Beauveria basiana) had histopathological effects on midgut of PBW. Abd El-Wahed *et. al.* (2011) studied the histological effects of LC50 (Protecto, profect and Viruset) on midgut of 6th instar larvae of S. littoralis treated as 4th instar larvae and found that, Profect was the most effective product in causing aberrations in the midgut layers, followed by Protecto and viruset.

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فعالية تتابع بعض المبيدات على مكافحة ديدان اللوز فى القطن والتأثيرات الهستوباثولوجية لبعض المبيدات الحيوية على دودة اللوز القرنفلية

محمد عبد المحسن سلامة[1] ، محمد عبد السلام عبد الباقى[1] ، جيهان بدوى أحمد النجار[2] ، عصام يوسف عزب النجار[2]

١. *كلية الزراعة ــ جامعة كفرالشيخ*
٢. *معهد بحوث وقاية النباتات ــ مركز البحوث الزراعية ــ الجيزة*

أجريت هذه التجارب فى موسم 2008 ، 2009م فى مزرعة محطة البحوث الزراعية بسخا ــ كفرالشيخ لتقييم بعض تتابعات المبيدات على تعداد يرقات دوده اللوز القرنفلية والشوكية وكذلك النسبة المئوية للإصابة فى اللوز الأخضر وذلك بهدف الحصول على أحسن تتابع فى مكافحة ديدان اللوز ولقد أوضحت النتائج أن الإصابة بديدان اللوز فى كل من الموسمين تبدأ فى أواخر شهر يوليو تم تزداد تدريجيا تصل لأقصاها فى نهاية الموسم.عامه اوضحت النتائج انه كان تأثير التتابعات تأثيراً بين منخفض الى متوسط على ديدان اللوز. تتابع المبيدات الحيوية (الاسبينوساد ــ ازدراختين ــ باسيلس ثيرونجينسس) كانت أكثر تأثيرا فى خفض تعداد يرقات ديدان اللوز عندما رشت ثلاث مرات متتالية منفردة أو بالتبادل مع بعضها كل 15 يوم. وجد أن رش مبيد الاسبينوساد ثلاث مرات متتالية أعطى أعلى متوسط لنسبة خفض فى التعداد 48.66% واستخدام المبيدات التقليدية فى تتابع مع المبيدات الحيوية ، منظمات النمو ومانعات الانسلاخ أعطت نسب جيدة فى خفض تعداد اليرقات. بينما أعطى التتابع بمبيد الميثوميل ثم أوكسى ماترين + بروسلر ثم لامبادا سيهالوثرين أقل متوسط نسبة خفض فى التعداد سجل 38.02%.

بالنسبة للتأثيرات الهستوباثولوجية لبعض المبيدات الحيوية بالتركيز الحقلى على العمر اليرقى الثانى والرابع لدودة اللوز القرنفلية. أوضحت النتائج بالفحص الميكروسكوبى لقطاعات الحلقة البطنية الرابعه وجود تغيرات غير طبيعية فى طبقات المعى المتوسط وأن مركب الاسبينوساد كان أكثر المركبات تأثيرا وكذلك التأثيرات الباثولوجية للمبيدات الحيوية المختبرة كانت أكثر خطورة على العمر اليرقى الثانى منها عن العمر اليرقى الرابع. وفقا لذلك فإن مبيد الاسبينوساد وبعض المبيدات الحيوية الأخرى يمكن أن تستخدم مع المبيدات الحشرية التقليدية ومنظمات النمو ومانعات الانسلاخ للحصول على أعلى معدل لنسبة الخفض فى تعداد اليرقات