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Susceptibility of field and laboratory strains of pink and spiny cotton bollworms larvae to different insecticides

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ABSTRACT

The susceptibility of field and laboratory strains of pink (*Pectinophora gossypiella)* and spiny (*Earias insulana*) cotton bollworms larvae to three different insecticides was studied. The significant differences between insecticidal-treatments and control are due to the high mortality of treated larvae before entering the bolls. Based on the LC_{50} values, it is quite clear that cypermethrin was the most toxic insecticide against both strains, followed by profenofos and thiodicarb in a descending order. The LC_{50} values in case of the laboratory strain are: 17, 90 and 300 ppm for cypermethrin, profenofos and thiodicarb, respectively. Based on the toxicity index values, it is apparent that cypermethrin is the most potent compound against the 2nd larval instar of both strains followed by profenofos and thiodicarb with toxicity index values of 100, 18.9 and 5.7, respectively (in case of lab strain) and 100, 16.8 and 6.9, respectively (in case of field strain). Based on the tolerance levels, the data showed that no single case of tolerance was observed since the calculated tolerance values are: 1.53, 1.72 and 1.25 for cypermethrin, profenofos and thiodicarb, respectively. The data also showed that no change in the reactions of the lab and field strains to the three tested insecticides occurred as their degree of homogeneity (D.H.) values are equal to 0.96. 0.95 and 1.03 (which is almost equal to 1.0). Cypermethrin was the most toxic insecticide against both laboratory and field strains followed by profenofos and thiodicarb in a descending order. The data revealed that cypermethrin is the most toxic compound against both second and fourth instars larvae followed by profenofos and thiodicarb. Based on the initial mortality cypermethrin is more toxic against both bollworms followed by profenofos while thiodicarb is the least toxic compound in this respect. Based on the residual percent mortality, the data confirmed the previous trend of result. In other words, cypermethrin has relatively long residual action followed by profenofos and thiodicarb. In term of figures, in case of pink bollworm, their percentages mortality is, 66.77, 63.72 and 33.39, respectively. The corresponding values, in case of spiny bollworm, are: 57.00, 55.54 and 34.88, respectively. Based on the residual toxicity, one could figure out that, apart of thiodicarb, the spiny bollworm showed little tolerant towards both profenofos and cypermethrin in comparison with the pink bollworm.

Keywords: Cotton bollworms, Susceptibility, *Pectinophora gossypiella, Earias insulana*, Insecticides, Field strain, Laboratory strain

INTRODUCTION

Production of cotton is ancient dating back thousands of years (Gulati and Turner, 1928). In Egypt, cotton is a very important crop that cultivated mainly for fibers in industry and seeds for oil which is of great value (Kamal, 1951). In 2001 cotton-season, the total cultivated area reached about 750,000 feddans produced about 6.5 million kentar fibers, 4.5 million kentar for exportation (Cotton Research Institute, Ministry of Agriculture).

In Egypt, many insects and mites are reported to attack cotton crop, although very few are so consistently serious as to be considered key pests. The crop is subjected to attack from the time seeds are planted until harvest about 7 to 10 months later. All the plant parts may be attacked, but the most serious pests attack primarily the fruiting portions; squares, blooms and bolls, reducing both quantity and quality of the harvested lint and seeds. Newsom and Brazzel (1968) reported that more than 80% of the losses attributable to cotton pests were caused by species that attack the fruits. In Egypt, during the late cotton-season, cotton plants suffer from the infestation with pink bollworm, *Pectinophora gossypiella* (Saunders) and the spiny bollworm, *Earias insulana* (Boisd.). Both bollworms are of the most serious insects that constitute a major part of the pest complex on cotton in Egypt. Metwally *et al.* (1980) indicated that the loss caused by *P. gossypiella* to cotton arises to one million kentar annually.

Although new insecticides give excellent control to resistant strains of insects, no one can accurately predict how long resistant insect populations will take to develop. The problem of tolerance and resistance of cotton bollworms to insecticides had attracted the attention of many investigators all over the world (Haynes et al., 1986; Hirano et al., 1993 and Payne et al., 1999). However, in Egypt, very little work had been done on the resistance or tolerance of these pests to insecticides. In this sense, it is very important to carry out some experiments to evaluate the status of tolerance of the two cotton bollworms to three currently used insecticides in Kafr El-Sheikh

Governorate namely profenofos, thiodicarb and cypermethrin before being used in the suggested IPM programme.

MATERIALS AND METHODS

Tested organisms:

Pink bollworm, *Pectinohora gossypiella* (Saunders): A susceptible strain of *P. gossypiella* (Saunders) was obtained from the Plant Protection Research Institute, Agricultural Research Center, Dokki, Egypt. The field strain of the pink bollworm was originally collected from infested bolls in cotton plantation in the farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate.

Insect rearing: The full grown larvae obtained from infested cotton bolls at the end of the cotton-season, were held at 26 $\pm$ 1°C and 80 $\pm$ 5% Relative Humidity (R.H) until pupation. The pupae were sexed daily and kept under the previous conditions. Ten pairs of newly emerged moths were transferred into 20 x 15 cm glass jars which served as feeding and oviposition chambers. The jars contained 10% sucrose solution provided through cotton swabs hung from muslin cloth. The sugar solution was renewed every 48 hours for moths feeding. The jars were covered with muslin secured with rubber bands and their bottoms were covered with screening mesh for stimulating oviposition. Eggs were deposited through the screening mesh on a piece of paper placed in an open Petri-dish that served as oviposition site. The jars were maintained at a temperature of 26 $\pm$ 1°C and 80 $\pm$ 5 R.H., and were examined daily for collecting eggs. Paper and muslin containing eggs were kept in 12 x 3.5 cm glass vials and covered with pieces of cotton wool until hatching.

Newly hatched larvae were fed individually in glass vials (2 x 7.5 cm) filled to one-third with kidney beans artificial diet (Abd El-Hafez *et al.*, 1982) covered with absorbent cotton and held under the same conditions as mentioned above. The larvae continued feeding on the artificial diet for about 20 days, and then usually pupated on its top or between the diet and the vial-wall. The vials were examined daily for transferring the pupae individually to clean vials to be incubated until moth emergence.

Spiny bollworm, *Earias insulana* (Boisd.): A field strain of spiny bollworm was originally collected from infested bolls in cotton plantations in the farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate.

Insect rearing: The rearing of spiny bollworm was carried out in the laboratory at Sakha Research Station, Kafr El-Sheikh Governorate under constant temperature (30 ± 1°C) and relative humidity (70 ± 5%) on natural diet of okra (*Hibiscus osculentus*) pods. Infested cotton bolls were dissected and the full grown larvae of *E. insulana* were placed in wooden bedding cages supplied daily with fresh okra pods for larval feeding. The pre-pupal stage was transferred to clean disinfected glass jars contained fine clean sand in their bottoms for pupation. The pupae were collected daily and sexed and kept in glass jars (20 x 15 cm) covered with muslin and secured with rubber band and kept under the previous conditions until moths emergence. Ten pairs of newly emerged moths were transferred into 20 x 15 cm glass jars supplied with okra pods for oviposition. The jars also contained 10% sucrose solution provided through cotton swabs hung from muslin cloth. The sugar solution was renewed every 48 hours for moths feeding. The jars were covered with muslin secured with rubber bands. The deposited eggs were collected daily and kept in glass jars under the pre-set conditions. The newly hatched larvae were supplied with fresh okra pods for feeding.

Insecticides used: All tested insecticides used in this study were in formulated forms, supplied from Kafr El-Zayat Pesticides and Chemical Co., Egypt. However, the chemical group, type of formulation and their recommended dosages per feddan are presented in Table (1).

Table (1): Tested insecticides, their type of formulation and their recommended doses/feddan.

Tested insecticides	Chemical group	Type of formulation	Dosage a.i/fed.
Profenofos	Organophosphate	E.C. 72%	375 ml.
Thiodicarb	Oxime carbamate	Fl. 37.5%	500 gm.
Cypermethrin	Synthetic pyrethroid	E.C. 20%	75 ml.

Susceptibility of bollworms to tested insecticides: The residual film method was used to determine the LC_{50} values of different insecticides. One milliliter of acetone solution of the toxicant under test was uniformly distributed on the surface of 9 cm diameter Petri-dish. After complete dryness, five full-grown larvae (4^{th} instar) and 2^{nd} instar larvae of both bollworms were transferred and left to dose themselves by crowling on the deposited film. The Petri-dish was covered and mortality counts were recorded 24 hours after exposure. Six replicates of 5 larvae each were used for each concentration. Seven concentrations per insecticide were used to compute its LC-P line. Dosage mortality lines were plotted and the LC_{50} and slope values were calculated according to Litchfield and Wilcoxon method (1949).

The larvae required for the tests were collected from laboratory strain (Plant Protection Institute) and infested bolls were obtained from cotton plants without any insecticidal treatments. The insecticides were tested according to Guirguis and Watson (1981).

RESULTS AND DISCUSSION

Susceptibility of 2nd instar larvae of field and laboratory strains of pink bollworm to the three different insecticides: The susceptibility of 2nd instar larvae of the field and laboratory strains of pink bollworm to profenofos, thiodicarb and cypermethrin was studied under laboratory conditions and the data are reported in Table 2. Based on LC_{50} values, it is quite clear that cypermethrin was the most toxic insecticide against both strains, followed by profenofos and thiodicarb in a descending order (Table 2). The LC_{50} values in case of the laboratory strain are 17, 90 and 300 ppm for cypermethrin, profenofos and thiodicarb, respectively. The same trend of toxicity was observed in the case of evaluating these compounds against 2nd instar larvae of field strain.

For more precise evaluation of acute toxicity, a toxicity index was calculated for each compound by giving the most toxic insecticide in a test a grade of 100. Then the other two compounds were graded relative to this by comparing their LC_{50} values as described by Sun (1950). Based on the toxicity index values (Table 2) it is quite clear that Cypermethrin is the most potent compound against the 2nd instar of both strains followed by profenofos and thiodicarb with toxicity index values of 100, 18.89 and 5.67, respectively (in case of laboratory strain) and 100, 16.77 and 6.93, respectively (in case of field strain).

Based on the tolerance levels, the data presented in Table (2) showed that no single case of tolerance was also observed since the calculated tolerance values are: 1.53, 1.72 and 1.25 for cypermethrin, profenofos and thiodicarb, respectively. With respect to the values of the degree of homogeneity (D.H), the obtained data showed that no change in the reactions of the lab and field strains to the three tested insecticides occurred as their D.H. values are equal to 0.96, 0.95, and 1.03 (which are almost equal to 1.0) (Table 2). Moreover, there is no significant difference between the LC_{50} of laboratory-strain and that of field strain for any tested compound since both values are being within the same confidence limits (Table 2).

Table (2): LC_{50} values, tolerance level, degree of heterogeneity and toxicity index of 2nd instar of pink bollworm to tested insecticides.

Insecticide	LC_{50} value (ppm)		Slope value		T.L.	D.H.	T.I	
	Lab strain	Field strain	Lab strain	Field strain			Lab.	Field.
Profenofos	90	155	1.50	1.42	1.72	0.95	18.9	16.8
Thiodicarb	300	375	2.28	2.35	1.25	1.03	5.7	6.9
Cypermethrin	17	26	2.41	2.31	1.53	0.96	100	100

T.L. = Tolerance level. D.H. = Degree of homogeneity T.I. = Toxicity index.

Susceptibility of 4th instar larvae of field and laboratory strains of pink bollworm to three different insecticides: Three insecticides belonging to organophosphate (profenofos), oxime carbamate (thiodicarb) and synthetic pyrethroid (cypermethrin) were assayed for their toxicity to laboratory and field strains of pink bollworm. The data are presented in Table 3. Cypermethrin was the most toxic insecticide against both laboratory and field strains followed by profenofos and thiodicarb in a descending order (Table 3). The LC_{50} values in case of the susceptible strain are: 22.5, 170 and 320 ppm for cypermethrin, profenofos and thiodicarb, respectively. The corresponding values in case of the field strain are: 25, 250 and 430 ppm, respectively.

The tolerance level for each compound was calculated by dividing the LC_{50} value of the field strain of any compound by the LC_{50} value of the laboratory strain of the same compound. The data are presented in Table (3). Based on the tolerance levels, the data revealed that no single case of tolerance was observed as their calculated tolerance values are 1.11, 1.47 and 1.34 for cypermethrin, profenofos and thiodicarb, respectively.

Table (3): LC_{50} values, tolerance level, degree of heterogeneity and toxicity index of 4th instar of pink bollworm to tested insecticides.

Insecticide	LC_{50} value (ppm)		Slope value		T.L.	D.H.	T.I	
	Lab strain	Field strain	Lab strain	Field strain			Lab.	Field.
Profenofos	170	250	1.72	1.73	1.47	1.00	13.2	10.0
Thiodicarb	320	430	1.88	1.94	1.34	1.03	7.0	5.8
Cypermethrin	22.5	25.0	2.68	2.75	1.11	1.03	100	100

T.L. = Tolerance level. D.H. = Degree of homogeneity T.I. = Toxicity index.

The degree of homogeneity (D.H) was also calculated as described by Salama and Hosney (1979) by dividing the slope value of tested strain (field strain) by the slope value of susceptible strain (Lab. strain). If D.H. value is 1.0, it means that there is no change in pink bollworms in their response to the insecticide, the values less than 1.0 indicates that their populations become more heterogeneous, while more than 1.0 shows a tendency towards homogeneity. The data are also presented in Table (3). Reviewing the obtained results it could be concluded that the two strains of pink bollworm showed no change in their reactions to the three tested insecticides, since their D.H. values are almost equal 1.0. However, these results confirmed that no single case of tolerance was observed, and the differences between the LC_{50} values of the susceptible (Lab.) and field strain are insignificant as indicated by the confidence limits. In other words, the LC_{50} for cypermethrin, for instance, in case of the field strain is 25 ppm and the confidence limits for the LC_{50} for the same compound in case of the lab-strain ranged between 19.56- 31.95 (Table 3). However, the insignificant differences between the LC_{50} values of the laboratory and field strain were quite evident in all tested compounds (Table 3).

In general, reviewing our results concerning the susceptibility of the 2nd and 4th instar larvae of both field and laboratory strains of pink bollworm, it is quite fair to conclude the following points:

1. Cypermethrin is the most toxic compound against 2nd and 4th instar larvae while thiodicarb is the lowest toxic compound in this respect.

2. No significant differences were observed between laboratory and field strains either in their reactions towards tested compounds or in their degrees of homogeneity.

The current results agreed fully with the previous finding of Watson et al. (1981) who reported that the synthetic pyrethroids were the most effective insecticides. Moreover, Gupta (1990) found that fenvalerate proved to be highly effective in reducing bollworms. Feshawi *et al.* (1991) concluded that thiodicarb gave lower reduction of the pink bollworm.

Susceptibility of the 2nd and 4th instars larvae of field strain of spiny bollworm to three different insecticides: The susceptibility of 2nd and 4th instars larvae of field strain to profenofos, thiodicarb and cypermethrin was studied under laboratory conditions. The results are recorded in Table (4). The data revealed that cypermethrin is the most toxic compound against both second and fourth instars larvae followed by profenofos and thiodicarb in a descending order. In term of figures, the LC_{50} values in case of the second instar are: 20, 90 and 375 ppm for cypermethrin, profenofos and thiodicarb, respectively. The corresponding values in case of the fourth instar are: 36, 230 and 530 ppm, respectively (Table 4).

Table (4): Toxicity of tested insecticides against 2nd and 4th instar larvae of spiny bollworm (field strain)

Insecticide	LC_{50} value (ppm)		Slope value		T.I	
	Field strain 2nd instar	Field strain 4th instar	Field strain 2nd instar	Field strain 4th instar	Field 2nd instar	Field 4th instar
Profenofos	90	230	1.60	1.80	22.2	15.65
Thiodicarb	375	530	2.35	3.03	5.3	6.79
Cypermethrin	20	36.0	2.00	2.60	100	100

T.I. = Toxicity index.

Based on the toxicity index values, it is quite clear that the toxicity of cypermethrin against the 2nd instar larvae is 4.5 fold more toxic than profenofos and 18.87 folds more toxic than thiodicarb. However, the same trend of results was observed in case of comparing the toxicity of cypermethrin with the other tested compounds against the 4th instar as cypermethrin is 6.39 and 14.73 folds more toxic than profenofos and thiodicarb, respectively.

Table (5): LC_{50}, slope and toxicity index values of 2nd and 4th instar larvae of pink and spiny bollworms

Insecticide	Field strains of cotton bollworms													
	2nd instar							4th instar						
	Pink			Spiny			LC_{50}	Pink			Spiny			LC_{50}
	LC_{50}	Slope	T.I.	LC_{50}	Slope	T.I.	ratio	LC_{50}	Slope	T.I.	LC_{50}	Slope	T.I.	ratio
Profenofos	155	1.4	16.8	90	1.6	22.2	0.58	250	1.7	10	230	1.8	15.7	0.92
Thiodicarb	300	2.4	6.9	375	2.4	5.3	1.25	430	1.9	5.8	530	3.0	6.8	1.23
Cypermethrin	26	2.3	100	20	2.0	100	0.77	25	2.8	100	36	2.6	100	1.44

LC_{50} ratio = LC_{50} spiny / LC_{50} Pink T.I. = Toxicity index.

Comparing the LC_{50} values of the 4th instar larvae of pink and spiny bollworms, it is quite evident that with the exception of profenofos, the 4th instar of spiny was relatively more tolerant to Thiodicarb and cypermethrin with values of 1.23 and 1.44, respectively. On the other hand, the 2nd instar larvae of spiny were relatively more tolerant to profenofos and cypermethrin with values of 0.58 and 0.77, respectively (Table 5). The obtained results are in agreement with those of Watson et al. (1981) who found that the synthetic pyrethroids were the most effective insecticides for controlling bollworms. In addition, Mourad et al. (1991) and Ayad et al. (1993) found that the O.P. insecticide Bolstar followed by the carbamate thiodicarb were less toxic with respect to pyrethroids. Moreover, Khidr et al. (1996) found that all treatments of pyrethroids during the three experimental seasons gave a high degree of control against cotton bollworms.

Evaluation of tested compounds against bollworms under laboratory conditions: To gain more accurate information about the relative effectiveness of the tested compounds particularly biocides, it is preferable to run the bioassay tests under laboratory and field conditions. Therefore, the residual and accumulative toxicities of tested compounds were evaluated under laboratory conditions.

Residual toxicity of tested compounds against both bollworms under laboratory conditions: The residual toxicity of tested insecticides against pink and spiny bollworms was evaluated and the data are presented in Tables (6 and 7), respectively. Based on the percent mortality, the data clearly indicate the following points:

1. Based on the initial mortality, cypermethrin is more toxic against both bollworms followed by profenofos while thiodicarb is the least toxic compound in this respect (Tables 6 and 7).

2. Based on the residual percent mortality, the data presented in Tables (6 and 7) confirmed the previous trend of results. In other words, cypermethrin has relatively long residual action followed by profenofos and Thiodicarb. In term of figures, in case of pink bollworm, their percentages mortality was 66.77, 63.72 and 33.39, respectively. The corresponding values, in case of spiny bollworm, are: 57.00, 55.54 and 34.88, respectively.

3. Based on the residual toxicity, one could figure out that, a part of thiodicarb, the spiny bollworm showed little tolerant towards both profenofos and cypermethrin in comparison with the pink bollworm (Tables 6 and 7).

Table (6): Residual toxicity of the tested insecticides against newly hatched pink bollworm larvae.

Treatment	% mortality at indicated days after spraying									Average of % mortality
	1	2	3	4	5	6	7	8	9	
Profenofos	85	80	80	75	70	63.3	50	40.2	30	63.72
Thiodicarb	68.3	60	50.3	33.3	30	20.3	16.7	13.3	8.3	33.39
Cypermethrin	100	90	79.7	68.3	55	55	52	51.7	49.2	66.77

Table (7): Residual toxicity of the tested insecticides against newly hatched spiny bollworm larvae.

Treatment	% mortality at indicated days after spraying									Average of % mortality
	1	2	3	4	5	6	7	8	9	
Profenofos	85.0	83.3	75.0	6.2	50.0	40.0	37.3	37.3	30.0	55.54
Thiodicarb	63.3	60.0	50.0	48.3	38.3	24.0	20.0	10.0	0.0	34.88
Cypermethrin	90.0	85.0	72.0	61.0	51.7	41.7	38.3	40.0	33.3	57.00

Accumulative toxicity of the tested insecticides against both bollworms under laboratory conditions: To evaluate the accumulative effect of each compound, treated flowers were daily picked from each treatment and offered daily to the same larvae (pink or spiny) for feeding. This was continued for 9 days. The data are presented in Tables (8 and 9) for pink and spiny bollworm, respectively. Reviewing these results, one could conclude the following points:

1. Based on the initial mortality (1 day), profenofos is the most toxic compound followed by cypermethrin while thiodicarb is the least toxic compound in this respect.

2. Based on the residual toxicity values, all tested insecticides considered long acting compounds either against pink or spiny bollworms.

3. The current result confirmed that the three tested insecticides are promising for controlling both bollworms if they are used at the proper time in a protective control programme.

Table (8): Accumulative toxicity of the tested insecticides against newly hatched pink bollworm larvae fed daily on treated flowers.

Treatment	% mortality at indicated days after spraying									Average of % accumulative toxicity
	1	2	3	4	5	6	7	8	9	
Profenofos	75.0	90.0	100	100	100	100	100	100	100	96.11
Thiodicarb	63.75	63.75	87.5	90.0	100	100	100	100	100	89.44
Cypermethrin	73.75	87.5	100	100	100	100	100	100	100	95.69

Table (9): Accumulative toxicity of the tested insecticides against newly hatched spiny bollworm larvae fed daily on treated flowers.

Treatment	% mortality at indicated days after spraying									Average of % accumulative toxicity
	1	2	3	4	5	6	7	8	9	
Profenofos	80.0	80.0	85.0	87.5	88.8	95.0	97.5	100	100	90.42
Thiodicarb	67.5	67.5	76.3	77.5	80.0	83.8	90.0	92.5	100	81.67
Cypermethrin	77.5	78.75	85.0	87.5	90.0	95.0	97.5	100	100	90.13

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الملخص العربى

حساسية السلالة المعملية و الحقلية لدودة اللوز القرنفلية ودودة اللوز الشوكية لمبيدات حشرية مختلفة

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* قسم المبيدات كلية الزراعة جامعة طنطا
**معهد بحوث وقاية النباتات مركز البحوث الزراعية الدقى

تمت دراسة حساسية سلالات معملية وحقلية من دودة اللوز القرنفلية والشوكية لثلاثة مبيدات من مجاميع مختلفة. واوضحت النتائج ان مبيد السيبرمثرين اكثر المبيدات سمية على العمر اليرقى الثانى والرابع لدودة اللوز القرنفليةوالشوكية بينما يلية فى السمية مبيد البروفينفوس ثم مبيد الثيوديوكارب. وبناءاً على قيم معامل السمية اوضحت النتائج ايضا كفاءة مبيد السيبرمثرين على كلا السلالتين المعملية والحقلية لدودة اللوز القرنفلية والشوكية. كما اوضحت النتائج عدم وجود فروق معنوية بين السلالات فى الاستجابة للمبيدات الثلاثة. كما اوضحت النتائج عدم ظهور اى حالة من التحمل سواء فى العمر اليرقى الثانى او الرابع.

وبمقارنة قيم LC_{50} للعمر اليرقى الرابع لكل من ديدان اللوز القرنفلية والشوكية فان النتائج توضح بانه باستثناء مبيد البروفينفوس فان العمر اليرقى الرابع لدودة اللوز الشوكية كان اكثر تحملا لمبيدى الثيوديوكارب و السيبرمثرين وعلى العكس من ذلك لدودة اللوز الشوكية فان العمر اليرقى الثانى يعتبر نسبيا اكثر تحملا لمبيد البروفينفوس و السيبرمثرين. وعلى اساس السمية المتبقية كان مبيد السيبرمثرين له اثر متبقى طويل نسبيا بالمقارنة بالبروفينفوس و الثيوديوكارب. وابدت دودة اللوز الشوكية تحمل اقل لكل من البروفينفوس و السيبرمثرين بالمقارنة بدودة اللوز القرنفلية على اساس الاثر الباقى. واثبتت النتائج ان المبيدات الثلاثة لها تاثيرات قوية ومشجعة لاستخدامها فى مكافحة دودة اللوز القرنفلية والشوكية.