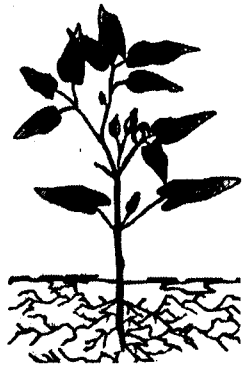

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LETHAL EFFECTS OF SPINOSAD AND AZADIRACHTIN ON THE COTTON LEAFWORM, *SPODOPTERA LITTORALIS* (BOISD.) (LEPIDOPTERA: NOCTUIDAE)

[22]

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Keywords: *Spodoptera littoralis*, Spinosad, Azadirachtin, Fecundity, Egg viability, Longevity

ABSTRACT

Laboratory experiments have been conducted to determine the lethal effects of Spinosad and Azadirachtin on the cotton leafworm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae by a diet incorporation bioassay. Newly molted third instar larvae were exposed through ingestion to different concentrations. Larval mortality of *S. littoralis* increased with the concentration resulting a LC_{50} values of 1.38 µg/ml and 0.14 µg/ml for Spinosad and Azadirachtin respectively, the mortality times decreased with the concentration increase. Sublethal effects were studied by treating of third instar larvae with a concentration equivalent to the respective LC_{50}. Both insecticides significantly increased larval development period from treatment until pupation, but the effect of Azadirachtin was greater than Spinosad. The pupation period was significantly prolonged in male pupae when the larvae were treated with Spinosad, whereas with Azadirachtin this effect happened in both sexes. Egg viability was reduced with Azadirachtin, and the oviposition period was affected by Spinosad. No significant differences were found in pre-oviposition periods, spermatophore number, fecundity and adult longevity.

INTRODUCTION

The Egyptian cotton leaf worm, *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) is a key pest of cotton and other many crops in the Mediterranean area and Middle Eastern countries (**Campion *et al* 1977; Nasr *et al* 1984; Ahmad 1988; Dominguez 1993**). The insect infests more than 112 host plants belonging to 44 families (**Moussa *et al* 1960**) makes it a model of serious polyphagous pests. The control of this pest is focused to the searching of new insecticides with biological and ecological qualities.

Spinosad (Dow Agrosciences LLC) is a mixture of spinosyns A and D produced during fermentation of the soil actinomycete *Saccharopolyspora spinosa* Mertz & Yao (**Dutton *et al* 2003**). It is a neurotoxin with a novel mode of action involving the nicotinic acetylcholine receptor and probably GABA receptor as well (**Salgado 1998**). This insecticide is highly active by ingestion and causes cessation of feeding followed later by tremors, paralysis and death of susceptible insects. Spinosad-based products have been registered in more than 30 countries for control of pests belonging to Lepidoptera, Diptera, some Coleoptera, ants and thrips (**Thompson *et al* 2000**). Spinosad has very low mammalian toxicity (**Breslin *et al* 2000**), and is classified by the United States Environmental Protection Agency as an environmentally and toxicologically reducing risk material (**Thompson *et al* 2000**). As a biorational pesticide, Spinosad now represents an important option for pest control in a growing number of crops produced under systems of integrated pest management (IPM). The adoption of Spinosad-based products by IPM practitioners is due to its effectiveness as an insecticide combined with its relatively low toxicity to natural enemies (**Miles & Dutton 2003**).

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There are little information on lethal and sublethal effects of Spinosad on *S. littoralis*. Some authors has reported toxicity on *S. littoralis* larvae (**Sannino 2001; Sannino & Piro 2003; El-Awa 2003 and Lechuga *et al* 2004**). **El-Awa (2003)** found that Spinosad reduces the fecundity of *S. littoralis* females came from treated larvae.

On the other hand, insecticides derived from the Neem tree seed extracts which originated from *Azadirachta indica* A. Juss. (Meliaceae), have been shown to cause several effects on a number of insect species (**Mordue & Blackwell 1993**). Some studies have shown that Azadirachtin increase the larval development period in noctuid larvae, such as *S. littoralis* (**Martínez & Van Emden 2001**), *S. mauritia* (**Jagannadh & Nair 1992**), and *H. armigera* (**MaDeling *et al* 2000**). Moreover, the fecundity and egg viability were reduced when *S. littoralis* (**Gelbic & Némec 2001; El-Awa 2003**) and *S. exempta* (**Tanzubil & McCaffery 1990**) larvae were treated with Azadirachtin.

Sublethal effects may be as important as lethal effects in crop protection programs as a result of feeding suppression, delaying development, and reducing reproductive potential of survivors. Thus, sublethal effects of Spinosad and Azadirachtin may have a great importance in regulating population of a target species, particulary in polivoltine species.

The objectives of this study were to determine: (1) lethal effects of Spinosad and Azadirachtin on *S. littoralis* larvae; and (2) sublethal effects of both insecticides on the insect development and reproduction.

MATERIALS AND METHODS

1. Insects

A laboratory colony of *S. littoralis* have been collected from Clover crop in Menofiya Governorate (Egypt). Insects were raised for ten generations in the laboratory before being bioassayed to avoid genetic drift, selection, and inbreeding (**Boller & Chanmers 1977**). Larvae were reared on an artificial diet containing alfalfa powder (**Vargas-Osuna 1985**) and maintained at 25±2 °C, 65±5 % RH and a photoperiod of 16:8 h (L:D).

2. Insecticides

Spinosad: Active material is (spinosyn A & D). Trade name SPINOSAD480 that contains 480 g/l of active ingredient and is formulated like concentrated suspension.

Azadirachtin: ALIGN that contains 3.2% of active ingredient derived from seed kernels of the Neem tree, *Azadirachta indica*.

3. Lethal Effects

Third instar *S. littoralis* larvae were treated by diet incorporation method. Newly molted 3rd-instar larvae were placed in individual plastic cups and fed on diet disks (9 mm in diameter), which were treated with different Spinosad and Azadirachtin concentrations.

4. Sublethal Effects

Newly molted 3rd-instar larvae were treated with concentrations 1.38 µg/ml and 0.14 µg/ml for Spinosad and Azadirachtin, respectively, during 24-48 hrs. Control larvae were fed on untreated disks of diet. Males from the night after emergence were paired with 1 or 2-day-old virgin females in a filter paper cylinder (12 cm in diameter and 24 cm high), one pair per cylinder, and fed on a 15% honey solution and maintained in the containers until they died. The four possible crosses between treated and untreated female and male were performed. Egg production was recorded daily and eggs were allowed to hatch. When females died, they were dissected to determine the presence of spermatophores in the bursa copulatrix.

All bioassays were conducted at 25±2°C. Mortality was recorded every twenty four hours.

5. Statistical Analysis

Median lethal concentrations (LC_{50}s) were determined by linear regression analysis and a test was made for parallelism according to the relative potency estimation method (**Finney, 1971**), using the microcomputer program POLO-PC (**Russell *et al* 1977**). The Median lethal times (LT_{50}s) were calculated from truncated data on the proportion of the test population that died by treatment (**Biever & Hostetter 1971**).

The larval and pupal development, preoviposition period, oviposition period, total fecundity, egg viability and adult longevity data were analysed by ANOVA and comparison of means by the least significant difference test (LSD).

RESULTS

A- Lethal Effects

The mortality increased with the concentration of Spinosad and Azadirachtin (**Table 1**). Most of the *S. littoralis* larvae treated with Spinosad died in the same instar of treatment, but the mortality of treated ones with Azadirachtin was in the last instars.

Analysis by probit regression line revealed the following equations and LC_{50}s (with 95% confidence limits): $y = 1.48 x + 4.79$, $\chi^2 = 7.52$ (3 df) and 1.38 µg/ml (0.82 - 2.81) for Spinosad (**Fig. 1**); $y = 1.23x+6.05$, $\chi^2 = 9.30$ (3 df) and 0.14 µg/ml (0.06 -0.32) for Azadirachtin (**Fig. 2**). The adjustment was acceptable using the χ^2 test. No differences were found between regression line slopes. On the bases of the relative potency, Azadirachtin was ten times more toxic than Spinosad (**Table 2**).

Median lethal times were significantly affected by the concentration either Spinosad ($p=0.0366$; LT_{50}= 4.1days at 1 µg/ml and 1.1 days at 4 µg/ml), or Azadirachtin ($p=0.039$; LT_{50}= 9.7 days at 0.32 µg/ml and 8 days at 1.6 µg/ml) (**Table 3**).

B- Sublethal Effects

Development period of treated larvae were prolonged significantly with Spinosad (20.54 days, $p<0.0001$) and Azadirachtin (26.40 days, $p=0.0024$) (**Table 4**). The pupation periods were also longer than control, while male pupae tended to develop slower than females (**Table 5**); Spinosad treatment caused significant differences only in male pupae ($p=0.0030$), but Azadirachtin affected either males ($p<0.0001$) or females ($p=0.0208$).

Most of females mated and laid viable eggs (**Table 6**). Mean number of eggs per female were increased in matings where one or both adults came from larvae treated with Spinosad, but the differences were not significant. However, with Azadirachtin the highest fecundity was only found in combinations of treated female and male (1747.5 eggs). The maximum value of egg viability ocurred in combinations of female treated with Spinosad and untreated male (95.55%). With Azadirachtin, the combinations of treated female with untreated male reduced significantly ($p=0.0280$) the egg viability. The mean number of spermatophore per female was not affected by the treatments (**Table 7**).

Mean of pre-oviposition period and number of viable eggs were not significantly different among mating combinations in either insecticides. Mean of oviposition period weresignificantly ($p=0.0007$) longer only in mated combinations of female and male treated with Spinosad (**Table 8**). The mean of males and females longevity were not affected by the insecticide treatments (**Table 9**).

DISCUSSION

Spinosad

The median lethal concentration (LC_{50}) of Spinosad is in agreement with **Lechuga *et al* (2004)** using the same bioassay method, and most of larvae died in the same instar in which they were treated. Similar results were found by **Yee & Toscano (1998)**, who reported that first, third and fifth instar larvae of *S. exigua* died after 24hrs of exposure to high doses of Spinosad, and reduced the leaf consumption.

The treatment of *S. littoralis* larvae not only exerted its lethal action, but also caused an extended effect on larval development and pupation period. When sublethal concentration of Spinosad was applied to newly molted third instar *S. littoralis* larvae, the larval development time was prolonged and the pupation period was reduced. The effect on larval development could be related to the reduction of cosumption reported in *S. exigua* larvae by **Yee & Toscano (1998)**.

In our bioassay conditions, the female fecundity and egg vibility were not affected, although the oviposition period was reduced in all mating combinations. Reduction of fecundity has been reported by **El-Awa (2003)** when fourth instar larvae were treated with a concentration equivalent to the LC_{25}.

Azadirachtin

Azadirachtin have higher activity than Spinosad for third-instar *S. littoralis* larvae, but the mortality period after treatment was prolonged until 20 days. The results are in agreement with **Martínez &Van Emden (2001)**. This long time of mortality of *S. littoralis* larvae is related to the complex mode of action, mainly to its antifeedant activity (**Mordue, 2004**).

Sublethal concentration of Azadirachtin caused a prolonged larval development period, similar results are obtained by **Jagannadh & Nair (1992)**

Table 1. Mortality of *S. littoralis* larvae treated in third instar with Spinosad and Azadirachtin

Insecticide	Concentration (µg/ml)	N	Mortality		
			n	Time(days)	%
Spinosad	0	90	0	0	0
	0.25	90	14	1-7	15.6
	0.5	90	18	1-7	20.0
	1	90	45	1-7	50.0
	2	90	46	1-7	51.1
	4	90	71	1-7	78.9
Azadirachtin	0	90	0	0	0
	0.0128	90	10	2 - 21	11.1
	0.064	90	39	2 - 19	43.3
	0.32	90	60	2 -18	66.7
	1.60	90	72	2 -18	80.0
	8.00	90	89	2 - 14	98.9

N = Treated larvae number. n = Died larvae number

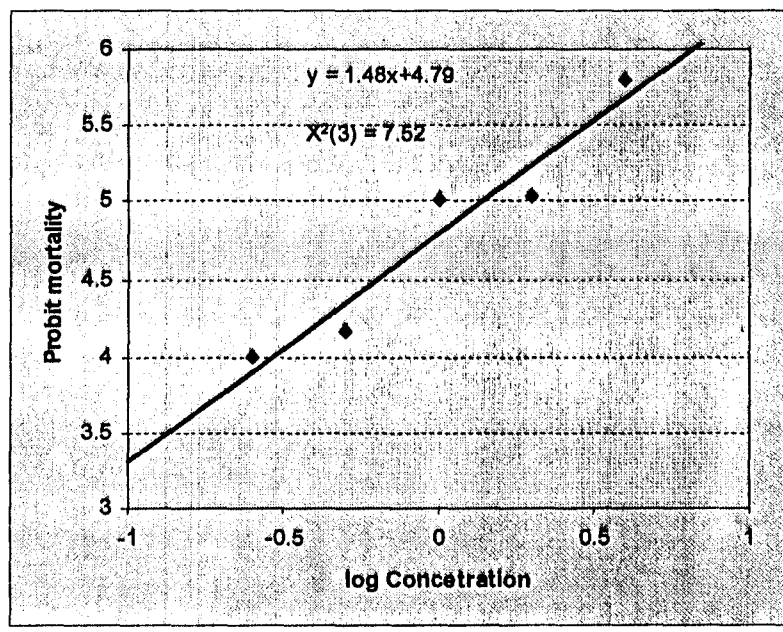

Fig. 1. Probit regression line for third instar larvae of *S. littoralis* treated with Spinosad

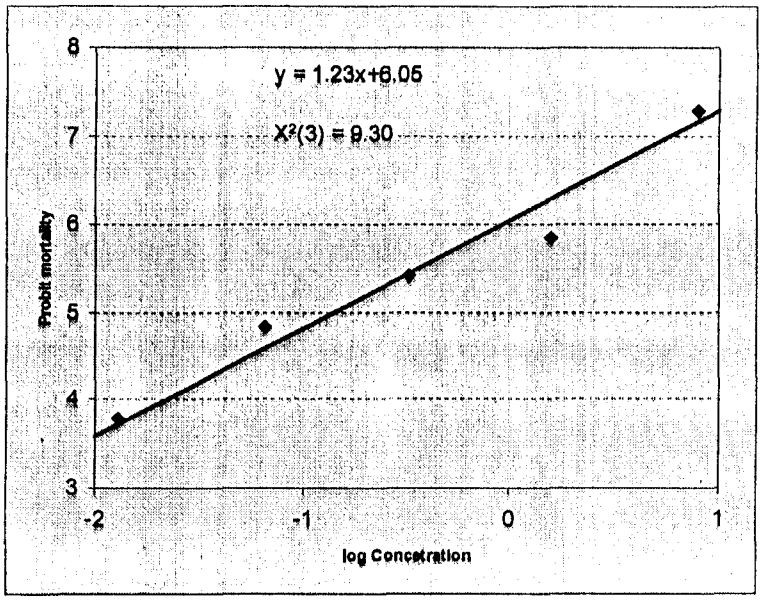

Fig. 2. Probit regression line for third instar larvae of *S. littoralis* treated with Azadirachtin

Table 2. Regression lines subjected to parallelism and LC_{50} values on third instar of *S. littoralis* larave treated with Azadirachtin and Spinosad

Treatment	χ^2	df	Regression line equation	LC_{50} (µg/ml)	Confidence Limits 95%	
					Lower	Upper
Azadirachtin	18.99	7	y = 1.30x +6.10	0.14	0.08	0.24
Spinosad			y = 1.30x+ 4.80	1.43	0.92	2.29

Table 3. Median lethal time of *S. littoralis* larvae treated in third instar with Spinosad and Azadirachtin

Insecticide	Concentration (µg/ml)	Replication	LT50	Mean (days)
Spinosad	1	1	3.1	4.1 a
		2	5.1	
	2	1	2.1	2.6 ab
		2	3.1	
	4	1	1.2	1.1 b
		2	1.1	
		3	1.0	
Azadirachtin	0.32	1	9.0	9.7 a
		2	9.0	
		3	10.0	
	1.60	1	8.5	8.0 b
		2	8.0	
		3	7.5	

Means followed by the same letter are not significantly different (LSD, p= 0.05)

Table 4. Effect of Spinosad and Azadirachtin on larval development of *S. littoralis*

Insecticide	Concentration (µg/ml)	N	Time (days)		
			Mean	Interval	± s.e.
Spinosad	Control	111	19.98 a	12-16	0.11
	1.38	70	20.54 b	12-15	0.14
Azadirachtin	Control	59	20.25 a	18-26	0.26
	0.14	55	26.40 b	23-31	0.27

Means followed by the same letter are not significantly different (LSD, p = 0.05)
s.e.. = standard error.

Table 5. Effects of Spinosad and Azadirachtin on pupal development of *S. littoralis*

Insecticide	Sex	Concentration (µg/ml)	N	Time (days)	
				Mean	± s.e.
Spinosad	♀	Control	48	13.04 a	0.09
		1.38	30	12.90 a	0.12
	♂	Control	47	14.96 a	0.09
		1.38	36	14.50 b	0.11
Azadirachtin	♀	Control	25	17.76 a	0.21
		0.14	23	16.09 b	0.22
	♂	Control	23	19.57 a	0.20
		0.14	21	17.52 b	0.21

Means followed by the same letter are not significantly different (LSD, P = 0.05)

Table 6. Response of *S. littoralis* females came from larvae treated with Spinosad and Azadirachtin according to different mating combinations

Combination	N	Numbers of females		
		Mated	Eggs laid	Eggs viable laid
♀ x ♂	18	17	17	16
♀ x $♂_S$	12	12	12	12
$♀_S$ x ♂	10	10	10	10
$♀_S$ x $♂_S$	13	13	13	13
♀ x ♂	12	12	12	12
♀ x $♂_A$	10	10	10	8
$♀_A$ x ♂	9	9	9	8
$♀_A$ x $♂_A$	8	8	8	7

N = Mated female number. S = Spinosad treatment. A = Azadirachtin treatment

Table 7. Reproductive potential of *S. littoralis* larvae treated with Spinosad and Azadirachtin according to different mating combinations

Combination	N	Mean No of Eggs / femal	Mean % Viability	No Sperms /♀
♀ x ♂	17	1876.5 a (200-3550)	88.78 a (75-100)	1.2 a
♀ x ♂$_s$	12	(1945.4 a) (1200-2475)	91.98 a (76.5-98.4)	1.1 a
♀$_s$ x ♂	10	2371.5 a (1600-2900)	95.55 a (88.6-100)	1.1 a
♀ $_s$ x ♂$_s$	13	2274.6 a (1275-3850)	86.95 a (68.2-96.5)	1.2 a
♀ x ♂	12	1552.9 a (650-2270)	75.23 a (41.9-90.3)	1.3 a
♀ x ♂$_A$	10	1516.2 a (225-2712)	54.53 ab (0-100)	1.2 a
♀$_A$ x ♂	,9	.1272.2 a (275-2475)	36.81 b (0-79.6)	1.1 a
♀ $_A$ x ♂$_A$	8	1747.5 a (915-3200)	62.03 ab (30-96.1)	1.1 a

Means followed by the same letter are not significantly different (LSD, p = 0.05).
N = Mated female number. S = Spinosad treatment. A = Azadirachtin treatment.

Table 8. Oviposition periods of *S. littoralis* females came from larvae treated with Spinosad and Azadirachtin according to different mating combinations

Combination	N_1	Pre-oviposition period (days)	Oviposition period (days)	N_2	No Egg viablity (days)
		Mean	Mean		Mean
♀ x ♂	17	1.18 a (0-2)	3.35 a (2-6)	16	3.44 a (2-6)
♀ x ♂$_s$	12	0.67 a (0-2)	4.42 b (3-6)	12	4.42 a (3-6)
♀$_s$ x ♂	10	0.20 a (0-1)	4.70 b (4 -7)	10	4.70 a (4 -7)
♀ $_s$ x ♂$_s$	13	0.85 a (0-1)	4.69 b (4 -5)	13	4.46 a (3-5)
♀ x ♂	12	0.25 a (0-1)	4.17 a (2-5)	12	4.17 a (2-5)
♀ x ♂$_A$	10	0.60 a (0-2)	3.90 a (1-8)	8	3.70 a (3-8)
♀$_A$ x ♂	9	0.78 a (0-4)	4.22 a (2-6)	8	2.78 a (1-5)
♀ $_A$ x ♂$_A$	8	0.88 a (0-4)	4.25 a (2-6)	7	4.13 a (2-6)

Means followed by the same letter are not significantly different (LSD, p = 0.05).
N1 = Female number. N2 = Fertil female number.
S = Spinosad treatment. A = Azadirachtin treatment.

Table 9. Adult longevity of *S. littoralis* males and females developed from larvae treated with Spinosad and Azadirachtin according to different mating combinations

Combination	N	Males Mean	Females Mean
♀ x ♂	17	10.00 a (5-13)	9.47 a (6-13)
♀ x $♂_S$	12	10.75 a (7-16)	10.17 a (8-14)
$♀_S$ x ♂	10	10.60 a (9-13)	8.80 a (7-10)
$♀_S$ x $♂_S$	13	11.15 a (9-15)	10.15 a (9-12)
♀ x ♂	12	9.00 a (7-11)	8.08 a (6-11)
♀ x $♂_A$	10	9.40 a (8-14)	8.30 a (6-14)
$♀_A$ x ♂	9	8.78 a (7-13)	8.78 a (5-11)
$♀_A$ x $♂_A$	8	9.38 a (6-12)	9.00 a (6-11)

Means followed by the same letter are not significantly different (LSD, p = 0.05).
S = Spinosad treatment. A = Azadirachtin treatment.

on *S. mauritia*, **MaDeling *et al* (2000)** on *Helicoverpa armigera*, and **Kumar *et al* (1997)** on *S. litura*. The pupation period was reduced in both sexes, as has been reported in fifth instar *S. litura* larvae treated with exudate from reddish terminal leaves **(Kumar *et al* 1997)**.

In the present work, the fecundity was not affected by Azadirachtin treatments. El-Awa (2003) reported that a commercial insecticide (Achook) based on Azadirachtin reduced the fecundity of *S. littoralis* when fourth instar larvae were treated with the estimated LC_{25} value. Extracts of Neem seed caused a significant reduction of fecundity and other reproductive parameters on *S. littoralis* **(El-Meniawi *et al* 1999)** and *S. litura* **(Kaur *et al* 2001)**.

The egg viability was also reduced, as it was reported previously in *S. littoralis* larvae treated either with Azadirachtin **(Gelbic & Nemec, 2001; El-Awa, 2003)** or with extracts of Neem seeds **(Dimetry *et al* 1998; El-Meniawi *et al* 1999)**. However, our results show that both sexes were affected by treatment with Azadirachtin.

Adult longevity of *S. littoralis* treated with Azadirachtin was not affected. Nevertheless, reduction of longevity has been reported in *S. litura* when larvae were treated with an exudate from reddish terminal leaves **(Kumar *et al* 1997)**.

The sublethal effects of Azadirachtin in *S. littoralis* are probably related to significant reductions or delays in ecdysteriod titres of the haemolymph due to a blockage of release of prothoracicotropic hormone from the brain-corpus cardiacum complex **(Mordue & Blackwell 1993)**.

The obtained results show the usefullness of the use of these insecticides in IPM programs for the control of *S. littoralis*. Spinosad have a faster action than Azadirachtin, whereas the last one have a long-term effects mainly on the egg-viability of adults came from treated larvae.

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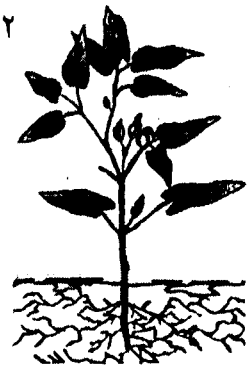

حوليات العلوم الزراعية
جامعة عين شمس ، القاهرة
مجلد(٥٣)، عدد (١)، ٢٧٣-٢٨٢، ٢٠٠٨

تأثير الجرعه المميتة لكلآ من سبينوساد و أزاديراكتين على دودة ورق القطن

[٢٢]

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أجريت تجارب معملية لدراسة تأثير الجرعة المميتة لنوعين من المبيدات هما سبينوساد (مبيد حيوى) وأزاديراكتين (مبيد طبيعى) على يرقات دودة ورق القطن . تم تغذية العمر اليرقى الثالث لهذه الافة على غذاء يحتوى على تركيزات مختلفة لهذين المبيدين ووجد من الدراسة أن الجرعة النصفية المميتة (الجرعة التى تسبب الموت لـ ٥٠% من اليرقات) كانت ١,٣٨ ميكروجرام / ملليمتر لمبيد سبينوساد من البيئة الغذائية ، ٠,١٤ ميكروجرام/ ملليمترم لمبيد أزاديراكتين من البيئة الغذائية . كما وجد أيضا أن فترات الموت قلت بزيادة التركيز المستخدم . وجد من الدراسة أيضا أن بمعاملة يرقات العمر الثالث بتركيز يساوى الجرعة النصفية المميتة قد أدى الى زيادة فترة الطور اليرقى الى طور العذراء وكان تأثير أزاديراكتين أكبر من سبينوساد كما وجد أيضا أن فترة طور العذراء كانت أطول (فى حالة العذراء الذكور) عند استخدام سبينوساد وكانت أطول فى كلا الجنسين عند استخدام أزاديراكتين . ووجد أيضا أن حيوية البيض قد قلت عند المعاملة بالازاديراكتين ، كما قلت فترة وضع البيض عند المعاملة بسبينوساد.

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