

**INSECTICIDAL EFFICACY OF THE BIOTIC COMPOUND,
SPINOSAD, AGAINST THE SECOND AND FOURTH INSTAR
LARVAE OF THE BLACK CUTWORM, *AGROTIS IPSILON*
(HÜFNAGEL) 0:LEPIDOPTERA: NOCTUIDAE**

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Abstract

The toxic effect of Spinosad against the second and fourth instar larvae of *Agrotis ipsilon* was evaluated in laboratory tests. The 2nd and 4th instar larvae were fed for 48h on castor leaves *Ricinus communis* were dipped for 5 seconds in eight concentrations of the tested compound ranging from 1.95 to 250 ppm. Spinosad exhibited higher toxicity against the second instar larvae than the 4th instar ones. With LC50 values of 7.8 and 30 ppm, respectively. The biological activities of the treated larvae were obviously affected specially with the second instar treatment of *A. ipsilon*. Therefore, the larval and pupal durations were prolonged, the pupation, and adult emergence percentages were depressed, the pupal weight was decreased and hence, pupal and adult malformations percentage were produced. Also, the fecundity and fertility were inhibited and adult longevity was shortened. The sex ratios of males and females of the emerged adult were more affected in respect to control.

INTRODUCTION

The black cutworm, *Agrotis ipsilon* is a serious pest of several important vegetable and field crops in Egypt, such as cotton, soybean, corn, potatoes and tomatos. Control of this pest, usually depends exclusively on conventional insecticides. In the developing countries, pesticides have played an extremely important role to maintain the high agricultural productivity. But the massive application of pesticides has created serious problems such as the build-up of pest resistance, the upsetting of natural balance, acute and chronic hazards to man and animals. The use of natural products of plant origin is a new trend that preserve the environment from contamination with harmful toxicants. The most familiar one of these chemicals in the recent years is azadirachtin that isolated from the fruits of *Melia azedarach* and seeds of *Azadirachta indica* (Meliaceae). Azadirachtin exhibits extremely low acute mammalian toxicity yet it is very effective as control agent for many insect groups (Champagne *et al.* 1989). Besides azadirachtin, other plant-derived extracts and

phytochemicals have been shown to possess insecticidal activity and some morphogenetic effects against Lepidoptera insects especially *A. ipsilon* (Abdel-Rahim,2002 and Abo- El- Ghar *et al.*,1994). Also, biotic compounds played an important role in pests control. Among these compounds , Spinosad , gets its name from the microbe that produces it, a soil-dwelling bacterium called *Saccharopolyspora spinosa*. spinosad possess less risk than most insecticides to mammals, birds, fish, and beneficial insects .It was used for control of Lepidoptera insects (Gomaa,2005 and Temarak,2007). It is already approved for use on more than 100 crops, including apples, almonds ,citrus ,eggplants ,tomatoes ,and cotton.

The aim of the present study is to evaluate the insecticidal properties of a biotic compound , Spinosad, against the second and the fourth instar larvae of *A. ipsilon*.

MATERIALS AND METHODS

1-Insect rearing

Rearing of *A. ipsilon* in the laboratory was followed according to Abdel-Salam(1980).The eggs were maintained in small jars(8x16cm) covered with muslin and provided with a tissue paper in the bottom to absorb humidity and fresh castor leaves, *Ricinus communis* were used ,as food for hatched larvae. The newly hatched larvae were carefully transferred by a fine-hair brush on the fresh castor leaves and a new tissue paper. The jars were daily examined and cleaned, and the food and tissue papers were renewed until the larvae develop to 3rd instars then the tissue paper was put over a saw dust layer in the bottom to absorb humidity for the young larvae. At the 6th larval instars, the number of larvae must be reduced to about twenty larvae per jar to avoid larval cannibalism and incubated at 25°C ,and 70-75% R.H. until the pupation .The newly formed pupae were maintained inside another clean jars over tissue paper until the moths emergence. The newly emerged moths were mated within a large glass jar (11x16 cm) provided with a cotton piece soaked in 10% sugar solution as a feeding source and muslins as oviposition substrate for the moths.The second and fourth instar larvae were used for the bioassay test.

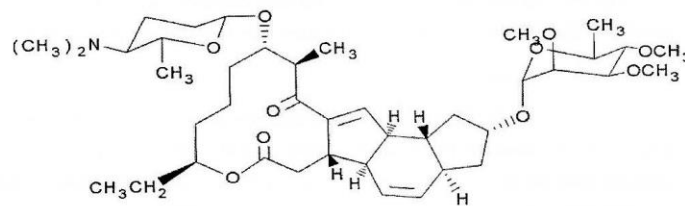
2-Insecticide used

Trade name: The insecticide was introduced by Dow Agro Sciences for control lepidopterous pests in cotton under the trade name Tracer (Thompson *et al.*, 1997)

Chemical name : The name spinosad is derived from combining the characters from Spinosyns A and D

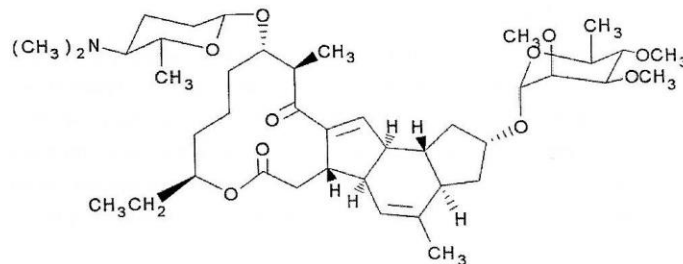
Empirical formulae: Spinosyn A: C₄₁H₆₅N₁₀O₁₀

: Spinosyn D: C₄₂H₆₇N₁₀O₁₀

Structural formulae:

spinosyn A

Molecular weight , Spinosyn A: 731.98
, Spinosyn D: 745.99



spinosyn D

3- Test procedures

Series of different concentrations the tested of the compound 1.95, 3.9 ,7.8 , 15.6 , 31.3 ,62.5 ,125 and 250 ppm were used. The eight concentrations were prepared on the active ingredient basis (p.p.m.) by diluting the compound in the water as a solvent . The leaves of castor dipped for 5 seconds in each concentration ,then left to dry in air current for about 1hr. Also, castor leaves were dipped in only distilled water used as control. About forty larvae in two replicates of each second and fourth instar larvae of each concentration of the tested compound and of control were used .After 48h.,the treated leaves was replaced by another untreated ones and the larvae fed on it until the pupation .The jars were examined daily to determine the larval mortality .Also ,the observed malformations were recorded and photographed.

4-Statistical analysis

The total percent of the larval mortality after 48h were recorded and corrected according to the check by using Abbott formula (Abbott ,1925).The data were then analyzed using the probit analysis (Finney, 1971) and LC-P lines:(toxicity lines) , and the LC₅₀ values were estimated for tested compound. The different biological effects such larval and pupal duration, pupation and adults emergence percentage ,pupal weight as well as adult fecundity, fertility ,longevity ,sex ratio were studied at the LC₅₀ values. The data of the biology statically calculated through SPSS 10 for windows computer program to determined the F-value, P-value and L.S.D(least significant difference at 0.05 or 0.01 freedom degrees)

RESULTS AND DISCUSSION

1-Toxic effect

Data presented in Table (1) demonstrated that the mortality percentages for 2nd and 4th instar larvae of *A. ipsilon* treated with the Spinosad were 100 ,100,90,70,60,50,30,15 and 100,90,70,50,30,10,5%,respectively at the tested concentrations. Spinosad was more effective against the second instar larvae of , where the LC₅₀ value recorded was 7.8 ppm ,and was less effective on the fourth instar , where the LC₅₀ value was 30 ppm (as showed in Table.1and Fig.1) .

Table1. Insecticidal activity of Spinosad against the second and the fourth instar larvae of *Agrotis ipsilon*.

Treatment	2 nd instar					4 th instar					
	Con. ppm	Mortality %	LC ₅₀ Value ppm	Slope function	95%confidence limit		Mortality %	LC ₅₀ value	Slope function	95%confidence limit	
					Upper	lower				Upper	Lower
Spinosad	250	100	7.8	4.2	18.7	3.3	100	30	3.03	66	14
	125	100					90				
	62.5	90					70				
	31.3	70					50				
	15.6	60					30				
	7.8	50					10				
	3.9	30					5				
	1.95	15					0				
	Control	All tested larvae were pupated									

The obtained results were similar to those obtained by Tamarak(2007) who showed that a radiant 12 SC (new generation) of Spinosad was 7 times stronger than Spintor 24 SC(old generation) to control of egg masses of *Spodoptera littoralis* in laboratory tests based on the LC₅₀ values. Also, he found that the radiant 12 SC was 5 times stronger (it was active at 5.76 ppm) than the Spintor 24 SC(it was active at 28.8) in the field. Also, Tamarak (2003) proved that the field strain of the cotton leaf worm *S. littoralis* (known to be tolerant or resistant to most of the conventional insecticides) was to be more susceptible to Spinosad (Spintor,24 SC) than the laboratory strain (known as susceptible to conventional insecticides). Also, Abdel – Rahman *et al.* (2002) demonstrated that the Neem Azal formulations applying at higher doses T/S(20 and 25 ppm) were the most effective against the Pink bollworm , *Pectinophora gossypiella*. Also, Dhawan *et al.* (1998) showed that all the 3 formulations of *B. thuringiensis* subsp. kurstak (Dipel, Bioasp and Biolep), neem, *Azadirachta indica* (Nimbecidine, Neemgold and Neemazal) and the insect growth regulators (lufenuron, flufenoxuron and RH-2485 a diacylhydrazine) were effective against *H. armigera*. at 1.50 kg/ liter as quinalphos insecticide against the young larvae. Salama and Ahmed(1997) recorded a very high insecticidal activity of methanolic extract of *Melia azedarach* at high concentrations against *Spodoptera littoralis* larvae. Percentage of mortality was 100% at 50 p.p.m. and 16% at 10 p.p.m. David *et al.*(1996)reported that the two formulations of Spinosad ,NAF-85 and

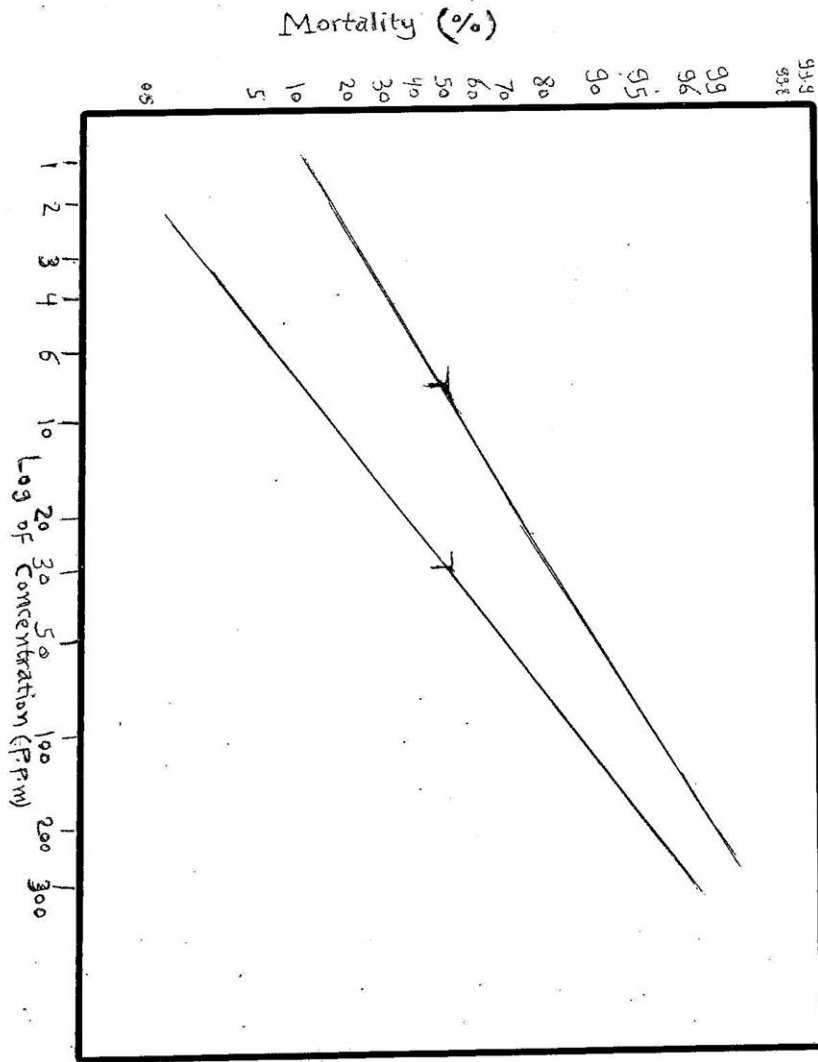


Fig. 1. Lc-pline of 2nd and 4th instar larvae of *Agrotis ipsilon* treated with spinosad.

NAF-127 were effective for control of black cutworm, *Agrotis ipsilon*, and Sod webworms, *Agrostis palustris*, the NAF-85 was active at 15ppm, while NAF-127 was active at 8 ppm. Also Abo-El-Ghar *et al.*(1994) indicated a reduction in the larval survival of 4th instar of *A. ipsilon* at concentrations of 0.01, 0.025 and 0.01, 0.05% of the ethanolic extracts of *Melia azedarach* and *Vinca rosea*, respectively.

Table.2. Latent effect of Spinosad at its LC₅₀ values against the 2nd instar larvae of *A. ipsilon*.

Compound	Larval duration (days) ± SD	% Pupation ± SD		Pupal duration (days) ± SD	Pupal weight (mg) ± S.D	% Moth emergence ± SD	
		Normal	Malfo.			Normal	Malfo.
Spinosad	34 ± 0**	50±10**	14±7.1*	13.5±3.1*	227±72.3**	58.8±21.3**	26±4.0*
Check	16 ±1.7	100±0.0	0.0	7.8± 2	439+ 35.2	100±0.0	0.0± 0
F value	99.040	75.000	4.069	5.115	15.951	9.455	6.497
P value	0.000	0.000	0.055	0.033	0.004	0.014	0.032
L.SDat0.05	3.3	16.3	10.8	4.1	98	24.3	17.9
0.01	5.0	24.7	15.6	5.9	148	36.7	27.1

** = Highly Significant (p<0.01)

S.D.=Standard deviation

L.S.D.= Least significant difference

* Significant (p<0.05)

Malfo.= Malformation%

Table 3. Latent effect of Spinosad at its LC₅₀ values against 4th instar of *A. ipsilon*.

Compound	Larval duration (days) ± SD	% Pupation ± SD		Pupal duration (days) ± SD	Pupal weight (mg) ± S.E	% Moth emergence ± SD	
		Normal	Malfo.			Normal	Malfo.
Spinosad	21.0 ± 1**	52 ± 6**	9±11**	12±2**	424±21**	65± 10**	14±5**
Check	15.0 ± 1.2	100 ± 0.0	0.0±0	7.0+ 2	492 ±16.3	100±0	0.0± 0
F value	36.867	54.280	17.667	6.470	12.682	30.145	28.806
P value	0.000	0.000	0.001	0.018	0.034	0.0000	0.0000
L.SDat0.05	3.1	13.0	3.1	3.5	65	11	6
0.01	4.5	18.7	4.7	5.2	119	15	8.6

** = Highly Significant (p<0.01)

S.D.=Standard deviation

Malfo.= Malformation%

L.S.D.= Least significant difference

2. Latent effect

2.1. Larval and pupal periods

Data in Tables (2 & 3) showed that the larval periods of the second and fourth instars of *Agrotis ipsilon* larvae treated with the LC₅₀ values of Spinosad were significantly (p<0.01) increased. The effect was more pronounced with the treated second larvae instar where the larval period was elongated to 34 day as compared to 16 days for the control, whereas the averaged larval periods for the 4th instar larvae Spinosad- treated and check treatments were 21 and 15 days, respectively.

Also, Table (2 & 3) indicated that the larval treatment of the second and fourth instar of *A. ipsilon* with Spinosad highly significant (p<0.01) increased the pupal periods. The pupal period averaged 13.5 and 12 days for pupae treated as 2nd and 4th larvae instar, respectively with Spinosad, as compared to 7.8 and 7.0 days. for pupae were produced from untreated second and fourth instar larvae, respectively.

These results are similar to those obtained by Abdel -Rahim(2002) who recorded an increase in the larval and pupal periods of *A. ipsilon* as result of the larval treatment with *Ambrosia maritima* extract by a contact method. Abdel - Rahman *et al.* (2002) reported that larval mortality, viable pupation of pink bollworm were

adversely affected by neemazal formulations (T, F and T/S) in dose and time dependent manners of the three tested Nemazal formulations. T/S was the most effective one . The larvae treated with the higher doses of Nemazal T/S (20 and 25 ppm) failed to molt to pupal stage and were termed as permanent larvae. Ivan and Jesus (2000) demonstrated that cotton treated with Spinosad in Texas had fewer damaging bollworm and budworm larvae than plots treated with the other pesticides ,and they suggested that Spinosad prevented small larvae from becoming larger and more damaging .Also, Jagannadh and Nair (1992)recorded a prolongation in the larval period of 5th and 6th instars *Spodoptera mauritia* that injected with azadirachtin at doses 0.5,1 and 2mg .

2.2. Pupation and Pupal weight

Tables(2& 3) demonstrated that the treatment of the 2nd and 4th instar larvae of *A. ipsilon* with Spinosad at its LC₅₀ value significantly ($p < 0.01$) reduced the pupation percentages which were 50 and 52% for pupae treated as 2nd and 4th larvae instar , respectively ,as compared to 100% of the check.

Likewise , the larval treatment of 2nd and 4th instar with Spinosad at the LC₅₀ resulted in highly significant ($p < 0.01$) reduction in the pupal weight of the resulting pupae(Table2 & 3).The treated second larvae instar was the most suppressive on the pupal weight . It averaged 227 mg as compared to 439mg pupal weight of the check .Whereas , the fourth larvae instar treatment had the least effect on the pupal weight ,it averaged 424mg, as compared to 492mg of control.

These results are agree with those obtained by Abdel- Rahim (2002) who recorded a reduction in both pupation and pupal weight of the resulting pupae of *A. ipsilon* treated as 4th instar larvae with *A .maritima* extract by a contact method . Also, Jagannadh and Nair (1992) reported that an injection of 2mg of azadirachtin within *S .mauritica* larvae prevented the normal pupation in the larvae of all age groups(0,1,2-day)

2.3. Moths emergence

Data in Tables (2 &3)showed that the second and fourth instar larvae of *A .ippsilon* treated with Spinosad at the LC₅₀ values induced highly significant ($p < 0.01$) reduction in the moths emergence rate. The treated second larvae instar had the highest effect on the moths emergence rate, where it averaged 59 %,while it reached to 65% for adults treated as 4th larvae instar with this compound ,as compared to100% adults emergence of the check.

These results were agree with those obtained by Abdel – Rahman *et al.* (2002)who reported that the adult emergence of pink bollworm *P .gossypiella* were

adversely affected by Neemazal formulations (T, F and T/S) in dose and time dependent manners of the three tested Neemazal formulations. The percent of individuals failed to turn to active adults reached its maximum values 73.33, 62.5 and 100% at 100 ppm Neemazal T, 300 ppm Neemazal F and 20 ppm Neemazal T/S, respectively. Also ,Abdel-Rahim(2002) recorded that the larval treatment of *A. ipsilon* with *A. maritima* extract induced the highest reduction in the adult emergence by a contact method .Also ,Abo -El - Ghar *et al.*(1994) demonstrated a decrease in the adult emergence of *A .ipsilon* treated as 4th instar larvae with petroleum ether extracts of *L. cylindrica* ,*A. majus* ,*C. elegans* and *V. rosea* ,as compared to control .

2.4. Morphogenetic effects

Data presented in Tables(2&3)demonstrated that the larval treatment of 2nd and 4th instars of *A .ipsilon* with Spinosad at the LC₅₀ values induced highly significant ($p<0.01$) increase in the pupal malformations. The treated second larvae instar induced the highest percent ,it reached 14 % ,as compared to 0% pupae malformations of the check, whereas, it reached 9% for pupae treated as 4th instar larvae with this compound.

With regarded to the adult malformations(Tables 2 & 3) ,it was found that the larval treatment of 2nd and 4th instars of *A .ipsilon* with Spinosad at the LC₅₀ value caused highly significant ($p<0.01$)increase of adult malformations. The treated second larvae instar had the greatest effect in adult malformations inducing, it reached 26% , as compared to 0% of control while ,it reached 14% emergence for adults treated as 4th larvae instar with this compound.

These results are similar to that obtained by Abdel- Rahim (2002)who indicated that *A .maritima* extract was the most potent extract in inducing noticeable malformations in both pupae and adult stages of *A. ipsilon* that treated as 4th instar with this extract by a contact method. Also, Abo- El - Ghar *et.al.*(1994) obtained the same results on the *S .littoralis* .



fig.1. Malformed prepuae had Larval 's legs and black body leading to death



fig.2 : Abnormal pupae showing body shrinkage.



fig.3 : Malformed pupae failed to cast the larval skin.



fig. 4 : larval -pupal intermediates



fig (5 , 6 and 7) : Malformation of adults appeared as adults had strongly deformed poorly developed wings or twisting wings.



fig 8 : or adults had weakly deformed

fig.(9 and 10) : Normal pupae and adults

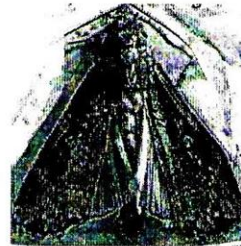


Fig.2. indicated the pupal and adult malformations of *Agrotis ipsilon* treated as second and fourth instar larvae with Spinosad.

Malformations *A. ipsilon* pupae resulting from the larval treatment of 2nd and 4th instars in the present work mostly appeared malformed prepupa had larval ' s legs and blackening of the body leading to death (fig.1)or abnormal pupae showing body shrinkage (fig.2)or malformed pupae failed to cast the larval skin (fig.3) or larval pupal intermediates (fig.4). The malformations of adults ,however appeared often as it had strongly deformed poorly developed or twisting wings (fig.5, 6 and7) or had weakly deformed wings(fig.8),as compared to normal pupae and adults(fig. 9 and10),as showed in Fig.2.

Table. 4. Latent effect of Spinosad at its LC₅₀ values against the 2nd instar larvae of *A. ipsilon* .

Compound	Fecundity	Fertility	Longevity	Adult sex ratio(%)	
	Mean ± S.D eggs/f.	Mean ± S.D. eggs/f.	Mean ± S.D. (days)	Male	Female
Spinosad	37.7 ± 20**	51 ± 6.4**	6 ± 1.2**	45.1	55
Check	1137± 260	1075± 236**	12.3± 1.5	70	30
F value	52.7	56.1	15.633		
P- value	0.0000	0.0000	0.004		
L.S.D at 0.05	301.7	274.5	3.7		
	0.01	457.1	415.9		

** = Highly Significant (p<0.01)

* Significant (p<0.05)

S.D.=Standard deviation

Malfo.= Malformation%

L.S.D.= Least significant difference

Table5. Latent effect of Spinosad at its LC₅₀ values against 4th instar of *A. ipsilon* .

Compound	Fecundity	Fertility	Longevity	Adult sex ratio(%)	
	Mean ±S.D. eggs/f.	Mean ± S.D. eggs/f.	Mean ± S.D. (days)	Male	Female
Spinosad	117 + 92**	60 + 14.1**	7.3+2.4 *	45.5	54.6
Check	1287± 260	1212± 236	11.5 + 2.1	70	30
F value	57.987	70.744	4.006		
P- value	0.0000	0.0000	0.057		
L.S.Dat 0.05	318.2	274.5	3.5		
	0.01	482.1	415.8		

** = Highly Significant (p<0.01)

* Significant (p<0.05)

S.D.=Standard deviation

Malfo.= Malformation%

L.S.D.= Least significant difference

2.5. Adult fecundity and fertility

Treatment of *A. ipsilon* 2nd and 4th instar larvae with Spinosad induced highly significant ($p < 0.01$) reduction in the fecundity of adult females (Tables 4 & 5). The least number of eggs averaged 37.7 eggs / female laid by adults treated as 2nd larvae instar, as compared to 1137 eggs / female laid by untreated adults, whereas the eggs number averaged 117 eggs / female laid by adults treated as 4th larvae instar, as compared to 1287 eggs / female laid by untreated adults.

Likewise, the larval feeding of 2nd and 4th instar larvae of *A. ipsilon* on Spinosad induced highly significant ($p < 0.01$) reduction in the fertility of eggs (Tables 4 & 5)

The least number of viable eggs (51 eggs/ female.) laid by adults treated as 2nd instar larvae with this compound, as compared to 1075 eggs/ female of the check, while the larval treatment of 4th instar larvae inhibited the fertility to average 60 eggs/f female, as compared to 1212 eggs/ female of control.

The effects of Spinosad were comparable to those mentioned by Pineda *et al.* (2004) who reported that both Spinosad and methoxyfenozide reduced in a dose dependent manner the fecundity and fertility of *S. littoralis* adults when treated oral and residually and they concluded that the combination of lethal and sublethal effects of methoxyfenozide and Spinosad might exhibit significant effects on the population dynamics of *S. littoralis*. Also, Abdel-Rahim (2002) demonstrated a significant decrease in the adult fecundity and fertility of *A. ipsilon* treated as 4th instar larvae with both *A. maritima* and *C. fistula* extracts by a contact method. Also, the same results were obtained by Hashem *et al.* (1994) who recorded a reduction in both fecundity and fertility as a result of abnormalities in the ovaries of *S. littoralis* adults fed as 4th instar larvae on artificial diet mixed with 2% of fruit extract of *M. azedarach* for 72h.

2.6. Adult longevity

Tables (4 & 5) indicated that the larval treatment of 2nd and 4th instar larvae of *A. ipsilon* with Spinosad decreased the longevity of the emerged adults. The treatment of second instar larvae highly significantly ($p < 0.01$) reduced the longevity to average 6 days, as compared with 12.3 days adults longevity of the check, while, the larval treatment of 4th instar significantly ($p < 0.05$) decreased the adults longevity to average 7.3 days, as compared to 11.5 days of the check.

These results are in agreement with that obtained by Abdel-Rahim (2002) who demonstrated a significant decrease in the adult longevity of *A. ipsilon* by the larval treatment of 4th instar with *A. maritima* and *T. tipu* extracts by a contact method.

2.7. Adult sex ratio

Data in Table(4&5) showed that the larval treatment of 2nd and 4th instar of *A. ipsilon* with Spinosad at LC₅₀ values shifted the sex ratio ,it decreased the males and increased the females percent, as compared with the check .The effect was similar for adults treated in either 2nd or 4th larvae instar, where it decreased the adult males to 45.1 and 45.5 % for adults treated as 2nd and 4th larvae instar, respectively ,as compared to 70% of adult males of the check. The adult females however, increased to about 55% for the adults treated in both 2nd and 4th larvae instar ,as compared to 30 % of adult females of the check.

These results are similar to those obtained by Abdel- Rahim(2002) who recorded shifting in the sex ratio in favour of females to more than two folds ,as compared to the check of *A. ipsilon* adults treated as 4th instar with *Ambrosia maritime* and *Cassia fistula* extracts .

CONCLUSION

The results of the present work demonstrated that the compound was effective against the survival and biology of *Agrotis ipsilon* , especially it had more toxic effect against the used second instar larvae and also, affect most of the tested biological activities of the insect .Whereas a solution of the compound at LC₅₀ values had the highest effect on the various biological activities of this insect . Thus, the compound may added at concentration,250ppm(lethal concentration of 100% of the two 2nd and 4th instar larvae) around the aerial parts(roots) or sometimes the leaves that may invade by the pest . Therefore, this compound was effective if applied within the cutworm baits or spray as replacement means for the used synthetic insecticides (carbaryl e.g. Sevin or permethrin, chlorpyrifos, or diazinon) for control of the mentioned pest that caused serious effects on the environment.

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تقييم الكفاءة الأبادية للمركب الحيوي (أسبينوساد) ضد يرقات العمر الثاني والرابع للدودة القارضة، اجروتس ايسيلون

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معهد بحوث وقاية النباتات - مركز البحوث الزراعية- الدقى - جيزة

اجريت هذه الدراسات بغرض تقييم النشاط السمي لمركب الأسبينوساد ضد يرقات العمر الثاني والرابع للدودة القارضة. غذيت يرقات العمر الثاني و الرابع لمدة 48 ساعة على ورق خروج تم عمره لمدة 5 ثوانى فى 8 تركيزات للمركب جهزت بناء على نظام التركيز الفعال (p.p.m) بتخفيف المركب فى الماء عند تركيزات تراوحت من 1.95-250 ppm وبناء على قيم التركيز النصفى (LC₅₀) كان المركب لكثير سمية ضد يرقات العمر الثاني حيث بلغت قيمة التركيز النصفى له 7.8ppm. بينما كان المركب أقل تأثيراً على يرقات العمر الرابع حيث بلغت قيمة التركيز النصفى له 30ppm. تأثرت المعايير البيولوجية لليرقات المعاملة اكثر مع معاملة يرقات العمر الثاني وادى ذلك الى طول العمر اليرقى والعزى وانخفاض فى نسب التعذير والأختراق ونقص فى الوزن العزى و انتج نسب من التشوهات العذرية والحشرية وادى الى تثبيط فى معدل وضع البيض ودرجة الخصوبة وقصر فى عمر الحشرة الكاملة . كذلك تأثرت النسب الجنسية للذكور والإناث مقارنة بالكنترول . بينما وجد فى اغلب الأنشطة البيولوجية المختبرة ان معاملة العمر الرابع كانت اقل. تأثيراً.