

THE BIOCHEMICAL EFFECT OF SPINOSAD FOR THE CONTROL OF THE COTTON LEAFWORM *SPODOPTERA LITTORALIS* (BOISD.)

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(Manuscript received April 2004)

Abstract

The present investigation was carried out to evaluate the biochemical compound Spinosad against the cotton leafworm *Spodoptera littoralis* (Boisd.) using of dipping technique. The compound proved its efficacy against the 2nd, 4th and 5th larval instars. The obtained results showed that the 2nd larval instars were the most sensitive to Spinosad at different tested concentrations. The 2ppm concentration could be recommended to control the 2nd and 4th larval instars of *S. littoralis* especially in case of high population densities, while the lower concentration 0.1ppm, and even it could not prevent larval feeding but interrupt development thus prevent the appearance of a second generation. The forth and fifth larval instars were relatively more resistant to high concentrations of Spinosad. The 5th larval instars of *S. littoralis* could not be controlled effectively even with the highest concentration of 8ppm, as it did not prevent larval feeding. Spinosad prevent small larvae from becoming larger and more damaging. The insect dies within several days after ingesting the active ingredient with no recovery. The metamorphosis of the full grown larvae showed failure of pupation, deformed prepupae and pupae. High mortality and malformation of moths occurred. The apparently normal moths were small in size giving few numbers of small egg masses and even those tiny egg masses were sterile. The latent effect of the lowest concentration revealed to no development of the full grown larvae to normal pupae or moths, which means no extra generations of the insect.

INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boisd.) is considered the most serious insect pest of cotton in Egypt. It has developed resistance to many commercial insecticides. Spinosad is the naturally occurring metabolite derived from fermentation of the soil bacterium *Saccharopolyspora spinosa*. Spinosad is a new biochemical insecticide and due to its low toxicity thus showing low impact on the environment, the Environmental Protection Agency (EPA) registered Spinosad as a reduced-risk material to control a variety of insects. The activity spectrum of Spinosad is limited to

Lepidoptera, Thysanoptera and certain Diptera, Coleoptera and Hymenoptera. Targeted Lepidoptera include army worms (*Spodoptera* spp.), cutworms (*Agrostis* spp.), fruitworms (*Heliothis* spp.) and leafrollers (*Tortricidae*). Spinosad has been applied to over 200 different field crops including cotton, especially in situations where the caterpillars were resistant to pyrethroids. Spinosad has provides excellent crop protection with a relatively low toxicity to non-target organisms (EPA, 1997, Dow Agroscience 1997 and Thompson *et al.*, 2000).

The efficacy of Spinosad is at least equivalent to the synthetic pyrethroids, most organophosphate, carbamates and other synthetic chemical insecticides with relatively long residual activity. Spinosad is especially insecticidal to small caterpillars by ingestion and contact, but especially by ingestion. Spinosad has an ovicidal action that could be of great value in pest management and make it an option more unique than most natural pesticides. Spinosad prevent small larvae from becoming larger and more damaging. The insect dies within 1 to 2 days after ingesting the active ingredient with no recovery. Symptoms of exposure are flaccid paralysis, cessation of feeding and mortality, weak tremors and failure to recover. Immobile larvae are typically found immediately adjacent to a minute feeding hole and may take 4-5 days to disappear from leaves (Bret *et al.* 1997, Saunders and Bret 1997, Petersen *et al.* 1997, Cameron and capman 1998 and Tjosvold and Chaney, 2001).

MATERIALS AND METHODS

Spinosad (0.02% E.C.) obtained from Dow AgroSciences Company, was tested against the cotton leafworm *S. littoralis* larvae of the 2nd, 4th and 5th instars on July, 2003 in the laboratory of Economic Entomology, Plant Protec. Res. Inst., Tanta, El-Ghrabia Governorate. Egg masses of the cotton leafworm were obtained from the rearing colonies laboratory, Sakha Agricultural Research Station, Kafer El-Sheikh Governorate. Larvae of *S. littoralis* were fed on Castor leaves, *Ricinus communis* as described by El-Defrawi *et al.*, 1964. The egg masses were maintained on Castor leaves inside glass container until hatching. Larvae were transferred by paintbrush into disinfected glass containers, covered with muslin secured with rubber bands were supplied daily with fresh cleaned Castor leaves. The 2nd instar larvae were treated at concentrations of 8, 4, 2, 0.8, 0.4, 0.3, 0.2, 0.15 and 0.1ppm. The 4th and 5th larval instars were treated at concentrations of 8, 4, 2, 1.5, 1.25, 1, 0.8 and 0.2ppm. The Castor leaves were washed first with clear water and left to air dry then dipped in a

dilution of 500ml containing the different concentrations of Spinosad for three seconds and left to air dry again before feeding to different larval instars. Twenty larvae in three replicates for each tested concentration were put in glass jar (30X25) then covered with muslin and maintained under room temperature - at first they were fed on the treated Castor leaves for two days then fed on fresh untreated leaves. In addition twenty larvae were fed on fresh untreated leaves as control. The different larval instars were monitored daily to calculate the mortality percentages starting from the third day and after feeding with the treated leaves.

As the larvae of each respected concentration reached the prepupal stage, they were collected together and maintained inside appropriate numbers of glass jars until the emergence of moths. The emerged moths were supplied with a piece of cotton wool moistened with 10% sugar solution for feeding and five leaves of Oleander, *Nerium oleander*, on which moths deposited their eggs. The failure in pupation (dead, deformed of prepupae and pupae%), failure in moths emergence (mortality and malformed of moths%), normal prepupae, pupae and apparently normal moths% were recorded. Also, averages numbers of egg masses and hatchability percentages were estimated.

For all experiments, mortality values were corrected according to Abbot's formula (1925), however possible. Duncan's multiple range test, Duncan (1955) at 5% level was used to compare the means of different treatments at the end of the larval stages using MINITAB program.

RESULTS AND DISCUSSION

For the treated 2nd larval instars, data shown in Table 1 revealed that high mortality figures were observed after six days for the first three concentrations (8, 4 and 2ppm). For 0.8ppm concentration, a mortality of 50% was achieved after seven days of treatment and maintained to the end of the larval stage. The other lower concentrations showed varied degrees of mortality at the end of larval period ranged between 21.5 and 8%.

The analysis of variance reveal high significance ($P=0.000$) between 8, 4, 2 and 0.8ppm of Spinosad concentrations and control. While there were no significant difference between 0.4, 0.3 and 0.2 concentrations, although they were significantly different from the control.

The obtained results of the latent effect of Spinosad on the 2nd larval instars are shown in Table 2. No alive prepupae were found for the first three tested concentrations (8, 4 and 2ppm). For 0.8ppm concentration and out of the remaining thirty prepupae 50, 30 and 20% were recorded dead prepupa, dead pupa and deformed ones, respectively. For 0.4ppm concentration 31.9% dead prepupae, 19.1% dead pupae, 17% deformed pupae were recorded, while 32% normal pupae were obtained, which developed totally to malformed moths. Images (1, 2 and 3) show the mortality and deformation of pupae, the incomplete emergence of moths and the mortality of normal moths during mating due to the latent effect of Spinosad treatment, respectively. The apparently normal moths were small in size and laid no egg masses and few moths laid tiny sterile egg masses. For 0.3ppm concentration 12.8% dead prepupae, 12.8% dead pupae, 17% deformed pupae were obtained, and 57.4% normal pupae that developed to 44.4% malformed moths and 55.6% normal moths that failed to put any egg masses. The lower concentrations results showed similar trend without depositing any egg masses.

Table 1. Effect of different concentrations of Spinosad on the 2nd instar larvae of *S. littoralis* in vitro

Concentrations ppm	% of mortalities after.....						Mortality	
	3 days	4 days	5 days	6 days	7 days	12 days	Total no.	C.M*
8	100						20	18.6
4	86.5	91.5	100				20	18.6
2	75	91.5	96.5	100			20	18.6
0.8	18	20	21.5	48	50	50	10	8.8
0.4	10	11.5	15	20	21.5	21.5	4.3	3
0.3	5	11.5	15	18	20	21.5	4.3	3
0.2	0.0	0.0	6.5	16.5	20	21.5	4.3	3
0.15	0.0	0.0	5	10	11.5	11.5	2.3	1
0.1	0.0	0.0	3	8	8	8	1.6	0.3
Control	0.0	0.0	1.5	6.5	6.5	6.5	1.3	0.0

C.M.* = Corrected mortality at the end of the larval stage, according to Abbot.

One-way ANOVA: con.2, con.0.8, con.0.2, con.15, con.0.1, control

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	5	798.019	159.604	929.73	0.000
Error	12	2.060	0.172		
Total	17	800.079			

Table 2. Latent effect of different concentrations of Spinosad on the development of the 2nd instar larvae of *S. littoralis* to pupal and adult stage in vitro

Concentrations ppm	Total no of prepupae.	Pupation %				Moths emergence %		Egg masses	
		Dead prepupa	Dead pupa	Deformed pupa	Normal pupa	Malformed moths	Normal moths	Total no.	Hatchability %
8	0.0								
4	0.0								
2	0.0								
0.8	30	50	30	20	0.0				
0.4	47	31.9	19.1	17	32	100	0.0		
0.3	47	12.8	12.8	17	57.4	44.4	55.6	0.0	
0.2	47	6.4	12.8	12.8	68	18.8	81.2	3	0.0
0.15	53	0.0	9.4	12.8	77.8	14.3	85.7	4	0.0
0.1	55	0.0	5.5	9.1	85.4	6.4	93.6	18	0.0
Control	56	0.0	0.0	0.0	100	3.6	96.4	48	98

The control revealed to 56 prepupae that developed to 3.6% malformed moths and 96.4% normal ones that laid 48 egg masses which hatched afterwards.

Results of Spinosad treatment for the 4th larval instars are shown in Table 3 where all treated larvae were dead after five days for 8ppm and 9 days for 4ppm concentrations. The mortality ranged between 95 and 6.5% for 2, 1.5, 1.25, 1, 0.8 and 0.2ppm concentrations, respectively.

The analysis of variance indicate high significant differences ($P=0.000$) between 8, 4 and 2ppm and other concentrations of Spinosad. Also, significant difference was found between control and 0.2ppm concentration ($P=0.007$). While there were no significant differences between 1.5 and 1.25ppm as well as between 1, 0.8 and 0.2ppm concentrations.

The latent effect of different concentrations of Spinosad on the development of the 4th larval instars of *S. littoralis* to pupal and adult stage are shown in Table 4. No alive prepupae were found on using 8 and 4ppm concentrations. For 2ppm, only three dead prepupae were recorded. Fifty three prepupae were alive in case of 1.5ppm, that categorized as 13.2% dead prepupae, 13.2% dead pupae, 17% deformed pupae, 56.6% normal pupae which developed to 37% malformed moths and 63% normal ones which laid 9 unhatched egg masses. The lower concentrations showed similar trends, without depositing any egg masses. The untreated larvae of

the control treatment developed to 57 prepupae that developed to 1.7% malformed moth and 98.3% normal ones that laid 45 egg masses that hatched afterwards.

Results of the 5th larval instars treatment are shown in Table 5 where lower percentages of mortality were recorded even for the high concentration. The mortality ranged between 71.5 to 10% for the tested concentrations, where there was no mortality for the control.

The analysis of variance indicate significant differences between 0.2ppm concentration and the control ($P=0.026$), while all the higher tested concentrations were highly significant compared to the control. There were no significant differences between 4 and 2ppm as well as between 1.5 and 1.25ppm, 1.25 and 1ppm, 1 and 0.8ppm, 0.8 and 0.2ppm concentrations.

Results of the latent effect on prepupae for the 5th larval instars are shown in Table 6. It shows that 18 prepupae were obtained at concentration of 8ppm, where 11.1% were dead prepupae, 11.1% dead pupae, 44.4% deformed pupae, and 33.3% normal ones which developed totally to malformed moths. For 4ppm, 24 prepupae were obtained which gave 25% deformed pupae and 75% normal pupae that developed to 27.8% malformed moths and 72.2% normal ones, which laid no eggs. Similar trends were found for the other concentrations without hatchability of the laid eggs. The untreated one revealed to 60 prepupae which developed to 98.4% normal moths laid a total of 34 egg masses that hatched afterwards.

Table 3. Effect of different concentrations of Spinosad on the 4th instar larvae of *S. littoralis* in vitro

Concentrations ppm	% of mortalities after...								Mortality	
	3 days	4 days	5 days	6 days	7 days	8 days	9 days	10 days	Total no.	C.M.*
8	8	81.5	100						20	19
4	6.5	76.5	85	85.5	88	95	100		20	19
2	0.0	70	80	85	85.5	93	95	95	19	18
1.5	0.0	6.5	8	10	11.5	11.5	11.5	11.5	2.3	1.3
1.25	0.0	5	5	5	5	10	11.5	11.5	2.3	1.3
1	0.0	5	5	5	5	6.5	6.5	6.5	1.3	0.3
0.8	0.0	0.0	5	5	5	5	6.5	6.5	1.3	0.3
0.2	0.0	0.0	1.5	3	3	5	6.5	6.5	1.3	0.3
Control	0.0	0.0	0.0	1.5	3	3	3	3	1	0.0

C.M.* = Corrected mortality at the end of the larval stage, according to Abbot.

One-way ANOVA: con. 2, con. 1.5, con. 0.2, Control

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	689.220	229.740	900.94	0.000
Error	8	2.040	0.255		
Total	11	691.26			

Table 4. Latent effect of different concentrations of Spinosad on the development of the 4th instar larvae of *S. littoralis* to pupal and adult stage in vitro

Concentrations ppm	Total no. of prepupae	Pupation %				Development of moths %			Eggs masses	
		Dead prepupa	Dead pupa	Deformed pupa	Normal pupa %	*C.M.	Malformed	Normal	Total no.	Hatchability %
8	0.0	0.0	0.0	0.0	0.0					
4	0.0	0.0	0.0	0.0	0.0					
2	3	100	0.0	0.0	0.0					
1.5	53	13.2	13.2	17	56.6	11.1	37	63	9	0.0
1.25	53	3.8	11.3	18.9	66	5.1	14.6	85.4	12	0.0
1	56	0.0	7.1	17.9	75	5.1	12.1	87.9	24	0.0
0.8	56	0.0	3.6	12.5	83.9	1	2.1	97.9	26	0.0
0.2	56	0.0	0.0	1.8	98.2	1	1.8	98.2	32	0.0
Control	57	0.0	0.0	0.0	100	0.0	1.7	98.3	45	100

*C.M. = Corrected mortality of moths, according to Abbot.

Table 5. Effect of different concentration of Spinosad on the development of the 5th instar larvae of *S. littoralis* in vitro

Concentrations ppm	% of mortality after				
	3 days	4 days	5 days	6 days	Total no.
8	6.5	20	71.5	71.5	14.3
4	0.0	10	31.5	60	12
2	0.0	6.5	30	50	10
1.5	0.0	0.0	23	31.5	6.3
1.25	0.0	0.0	23	25	5
1	0.0	0.0	15	21.5	4.3
0.8	0.0	0.0	15	20	4
0.2	0.0	0.0	10	10	2
Control	0.0	0.0	0.0	0.0	0

One-way ANOVA: con. 8, con. 4, con. 2, con. 1.5, con. 1.25, con. 1, con. 0.8, Co

Analysis of Variance

Source	DF	SS	MS	F	P
Factor	7	471.198	67.314	133.63	0.000
Error	16	8.060	0.504		
Total	23	479.258			

Table 6. Effect of different concentration of Spinosad on the development of the 5th instar larvae of *S. littoralis* to pupal and adult stage in vitro

Concentrations ppm	Total no. of prepupae	Pupation %				Moths emergence %		Egg masses	
		Dead prepupae	Dead pupae	Deformed pupae.	Normal pupae	Malformed	Normal	Total no.	Hatchability %
8	18	11.1	11.1	44.4	33.3	100	0.0		
4	24	0.0	0.0	25	75	27.8	72.2	0.0	0.0
2	30	0.0	0.0	20	80	20.8	79.8	3	0.0
1.5	41	0.0	0.0	14.6	85.4	17.1	82.9	6	0.0
1.25	45	0.0	0.0	13.3	86.7	15.4	84.6	8	0.0
1	47	0.0	0.0	12.8	87.2	14.6	85.4	9	0.0
0.8	48	0.0	0.0	12.5	87.5	14.3	85.7	11	0.0
0.2	54	0.0	0.0	11.1	88.9	12.5	87.5	15	0.0
Control	60	0.0	0.0	1.6	98.4	0.0	100	34	100

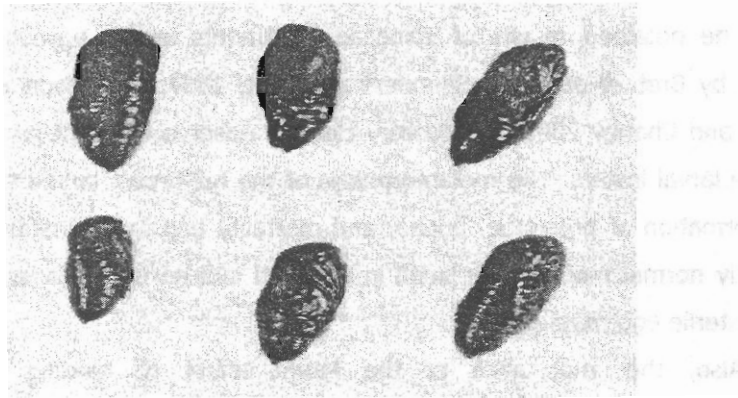


Image 1. Deformed pupae of *S. littoralis* due to the latent effect of Spinosad treatment.

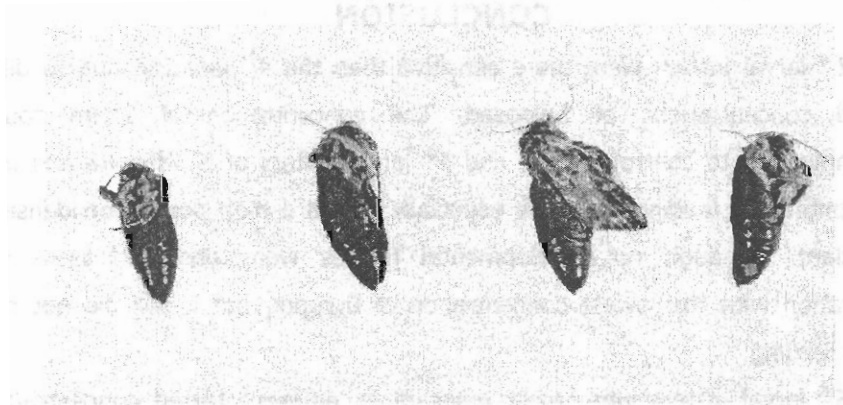


Image 2. Incomplete emergence of *S. littoralis* moths due to the latent effect of Spinosad treatment.

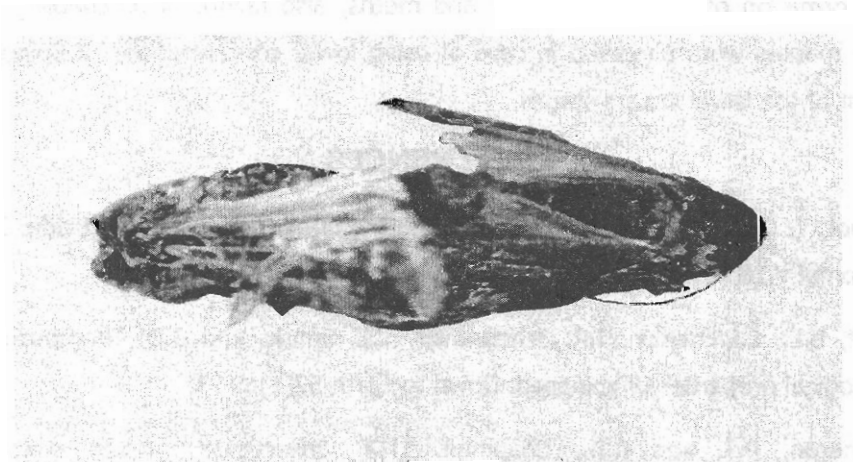


Image 3. Mortality of *S. littoralis* moths during mating due to the latent effect of the latent effect of Spinosad treatment.

The obtained results of spinosad treatments are in agreement with those obtained by Bret *et al.* 1997, Saunders and Bret, 1997, Thompson *et al.* 2000, and Tjosvold and Chaney 2001. It was very clear to observe different larval size even for the same larval instars. The metamorphosis of the full grown larvae showed mortality and deformation of prepupae, pupae, and mortality and malformation of moths. The apparently normal moths were small in size and laid no egg masses and few moths laid tiny sterile egg masses.

Also, the importance of the latent effect of feeding on the lowest concentration revealed that no developmental parameters for the full grown larvae were obtained involving that mean no extra generations are yielded.

CONCLUSION

1. The 2nd larval instars were more sensitive than the 4th larval instars to different tested concentrations of Spinosad. The concentration of 2ppm could be recommended to control the 2nd and 4th larval instars of *S. littoralis* and prevent the continuous feeding of larvae especially where a high population density was abundant. Although no developmental figures were observed to a second generation with the lowest concentration of 0.1ppm, but it still did not prevent larval feeding.
2. The 5th larval instars were most resistant to different tested concentrations of Spinosad and even the high concentration of 8ppm could not control *S. littoralis* effectively.
3. Malformation of prepupae, pupae and moths, also failure of hatchability for the egg masses were observed in case of using lower concentrations of Spinosad for most of the larval instars stages.

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التأثير البيوكيميائي لمركب سبينوساد لمكافحة دودة ورق القطن

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تم تقييم استخدام مركب سبينوساد وهو مركب كيموحيوى معمليا على العمر الثانى والرابع والخامس ليرقات دودة ورق القطن وذلك باستخدام تركيزات مختلفة. ولقد وجد أن العمر الثانى كان أكثر الأعمار حساسية للمركب يليه العمر الرابع ثم العمر الخامس وهو أكثرها مقاومة، ووجد أن تركيز ٢ جزء فى المليون أكثرها فعالية ويسبب نسبة موت مرتفعة ضد دودة ورق القطن للعمرين الثانى والرابع بكفاءة كما وجد أن التركيزات الأقل بالرغم من أنها لا تسبب نتائج موت فورية على اليرقات فإن النتيجة الملموسة لها كانت فى منع تطور اليرقات وظهور جيل تالى للأفة. وجد أن العمر الخامس لا يتأثر ظاهريا حتى بأعلى تركيز مختبر (٨ جزء فى المليون) حيث لا تمتنع اليرقات عن التغذية وتستمر فى تطورها ولكنها أيضا تفشل فى تكرار دورة حياتها لما يسببه هذا المركب بتأثيره الكامن باليرقات.

شوهدت تشوهات فى أطوار الحشرة المختلفة والسابق تغذيتها على الأوراق المعاملة بالمركب علاوة على الفشل فى التعذير (موت طور ما قبل العذراء والعذراء) وكذلك الفشل فى تطور العذارى وظهور الفراشات المشوهة، وحتى عند ظهور بعض العذارى و الفراشات الكاملة ظاهريا والسابق التغذية وهى فى طور اليرقة على أقل تركيز وجد أن هذه الفراشات كانت عقيمة لجميع الأعمار حيث فشلت جميع لطح البيض الموضوعة عن جميع الأعمار فى النفوس حتى بأقل تركيز مستخدم فى المعاملات (٠,١ جزء فى المليون).