SPINOSAD AS A NEW BIOINSECTICIDE AGAINST THE GREASY CUTWORM *Agrotis ipsilon* (Hnuf.)

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ABSTRACT

Spinosad, a new bioinsecticide which is derived from a species of Actinomycetes bacteria, Saccharopolyspora spinosa was evaluated under laboratory and semifield conditions against larvae of the greasy cutworm, Agrotis ipsilon (Hnuf.). The effects of spinosad on pupation and emergence of moths were also studied. Three concentrations levels (0.0625, 0.125 and 0.250 ml/L water) were bioassayed versus 1st, 2nd, 3rd, and 4th instar larvae.

Results showed that the 1st and 2nd instar larvae were more sensitive to spinosad, at different tested concentrations, than the 3rd and 4th instar larvae. When the recommended rate (0.125 ml/L) was tested, total mortality reached 100, 92, 79 and 54 % for 1st, 2nd, 3rd, and 4th instar larvae, respectively.

Spinosad affected percentages of pupation and emergence of moths. Results indicated that percentage of deformed pupae and moths increased with increasing of the tested concentration. Percentage of pupation reached 0.0, 16, 30 and 60 % when 1st, 2nd, 3rd and 4th instar larvae were tested using a rate of 0.125 ml/L. When the tested concentration increased to 0.250 ml/L it reached 0.0, 0.0, 29, and 48 %, respectively.

Treated 2nd instar larvae suffered total mortality of 92 % when fed on cotton leaves sampled one hour after spraying (0-time) while mortality reached 48 % and 40 % when larvae fed on leaves sampled 6 and 7 days post-spraying.

Key words: Spinosad, *Agrotis ipsilon.*

INTRODUCTION

Chemical insecticides have several disadvantages on either one or more of the following phenomenon: a) creation of chemical pesticide resistance strains of the insect, b) modulate the equilibrium balance between the insect and its natural enemies. Spinosad, which used during the course of this study is a new bioinsecticide that is derived from a species of Actinomycetes bacteria, *Saccharopolyspora spinosa*, discovered in soil sample. It is a fermented product, much like the more familiar *Bacillus thuringiensis* materials. Spinosad is both a nerve poison and stomach poison, so it kill pests that it contacts and those consume it on the foliage they eat **(Anonymous, 1996)**. It has a novel mode of action which will help prevent cross-resistance with organophosphates and carbamates (which are acetylcholinesterase inhibitors), and even B.t. products which are also stomach poison, but work differently **(Salgado, 1998)**. Spinosad is relatively fast acting. The insect dies within 1 to 2 days after ingesting the active ingredient and there appears to be no recovery **(Bret *et al.*, 1997 and Salgado *et al.*, 1998).** The activity spectrum of spinosad is limited to Lepidoptera, Thysanoptera, certain Diptera, Cleoptera but spares most beneficials. Targeted Lepidoptera include army worms (*Spodoptera* spp.), cutworms (*Agrotis* spp.), fruitworms (*Heliothis* spp.) and leafrollers (*Tortricidae*) **(EPA, 1997; Dow AgroScience, 1997 and Thompson *et al.*, 2000)**. Spinosad is relatively fast acting.

Spinosad has relatively low activity against predaceous beetles, sucking insects, lace wings and mites **(Schoonover and Larson, 1995 and Mayer and Lunden, 1998).**

The present investigation aims to evaluate the role of spinosad as a microbial insecticide against larvae of the greasy cutworm, *Agrotis ipsilon* (Hufn.) (Lepidoptera: Noctuidae) under laboratory conditions, and its persistence on cotton plant under natural environmental conditions. It is to be noted that, spinosad was not tested against larvae of the greasy cut worm *A. ipsilon* befor

MATERIALS AND METHODS

Commercial formulation:

Spinosad, a product of Dow AgroSciences Limited, is a secondary metabolite from the aerobic fermentation of *S. spinaosa.* It is a mixture of the two most active naturally occurring metabolites (spinosyns A and D). Spinosad is an emulsive liquid, at a recommended rate of 50 ml/feddan.

Laboratory experiments:

Larvae of *A. ipsilon* were obtained from a laboratory culture reared on cotton leaves for several generations. Uniform age and size larvae of the 1st, 2nd, 3rd and 4th larval instars were used. Hundred larvae were taken from each larva instar and

distributed in hundred plastic cups 3.5 x 7 cm. It divided into ten replicates 10 larvae/each replicate were used for evalluting different concentrations. Concentrations of (0.0625, 0.125 and 0.250 ml/L) were used. Fresh aqueous suspensions of spinosad were individually prepared in distilled water. All dilutions of various concentrations were tested using dipping method of application against the larval instars of *A. ipsilon*. The fresh leaves of cotton plants were dipped for 5 seconds in prepared dilutions and left to dry airy. In the control experiments, the larvae were fed on untreated leaves. For bioassay, *A. ipsilon* larvae were starved for 4 hours, then fed for 24 h. on treated leaves. After that, the surviving larvae were allowed to feed on fresh untreated leaves until pupation and adults emergence. Mortality percentages were recorded daily. The percentages of mortality were corrected according to Abbott's formula **(Abbott, 1925)**. To study the latent effect of spinosad formulation on some biological aspects of *A. ipsilon*, surviving larvae were examined daily until pupation or moths emergence. The percentages of pupation and adults emergence were also calculated. After moth emergence, pairs of moths were introduced into oviposition glass cages provided with cotton wool soaked in 10% sucrose solution for feeding and fresh branches of *Nerium oleander* to serve as oviposition site. The experiments were done under laboratory conditions of 25 ± 2°C and 62 ± 5% RH.

Persistence of spinosad on plant leaves under field conditions:

Potted cotton plants were sprayed with spinosad at the rate of 50ml/400 liter water/feddan (0.125ml was diluted with one liter of water), using knapsack sprayer (5 liter). Ten pots were treated with spinosad and similar 10 pots were sprayed with water as untreated control. All potted plants were left under natural environmental conditions (mean 30.3 °C and 78 % R.H.). Cotton leaves were collected at the same day (zero time), and daily for 7 days after spraying to evaluate the potentiality of spinosad using 2nd instar larvae of *A. ipsilon*. The larvae were left to feed for 48 hours on the treated leaves in plastic cups, then transferred to new clean cups with untreated leaves. Number of dead larvae until pupal formations as well as number of dead deformed pupae was counted. Larvae in control groups were fed on untreated cotton leaves, their natural mortalities were calculated as well.

REULTS AND DISCUSSION

Laboratory experiments:

Data presented in Table (1) indicated that the 1st and 2nd instar larvae of A. ipsilon were more susceptible for spinosad than the 3rd and 4th instar. When the recommended rates was tested, mortality percent reached 100 % and 60 % for the 1st and 2nd larvae instars two days post-treatment versus 38 % and 18 % for the 3rd and 4th instar larvae respectively. In the three tested concentrations, all larvae of the 1st instar failed to pupate. It is obvious that, there is positive correlation between concentrations and percentages of mortality of the tested larval instars, even the 4th instar larvae, respective total mortality reached 67 %, 54 % and 36 % when concentrations of 0.250 ml/l, 0.125 ml/l and 0.0625 ml/l were tested.

Data in Table (1) show also that mortality percent increased as time elapsed from the onset of larval treatment, especially during the two days after which treatment took place and negligible during the 6th and 8th day.

According to results given in Table (1) spinosad affected percentage of pupation and causes malformation of pupae. When the concentrations were increased from 0.0625 ml/l to 0.250 ml/l percentage of deformed pupae increased from 11.5 % to 24.1 % and from 12.3 % to 14.5 % for tested 3rd and 4th instar larvae, respectively. Also percentage of emerged and deformed moths increased with increasing the concentrations.

Laboratory evaluations showed also that, the eggs deposited by female moths resulted from treated larvae did not hatch. This may be due to interference with oogensis damage of ovarian tissues. The obtained results are in agreement with those mentioned by **(Abdallah and Abul-Nasr, 1970)**, **Hafez *et al.* (1993)** on a study on the effectiveness of *B. thuringiensis* against the eggs, prepupal stages and moths of *A. ipsilon*.

Statistical analysis of the data (ANOVA) show that, the differences between percentages of mortality in treated larval instars and between the different concentrations were significant ($p < 0.05$).

Persistence of spinosad on plant leaves under field conditions:

Saunders and Bret, 1997 mentioned that the degradation of spinosad in the environment occurs through a combination of rules, primarily photodegradation and microbial degradation to its natural components of carbon, hydrogen, oxygen and nitrogen. They added that the half-life of spinosad degraded on leaf surface photolysis is 1.6 to 16 days. Therefore, it is important from a practical point of view to evaluate biocide virulence few days after application. Table (2) shows the periodical percentage of mortality after 2, 4, 6 and 8 days, percentage of pupation and adult's emergence when *A. ipsilon* 2nd instar larvae ingested cotton plant leaves sprayed with spinosad at a rate of 0.125 ml/L on the 0-time (1 hour after treatment) then daily for a week post-treatment. Mortality percent among tested 2nd instar larvae reached 40 % after 2 days post-ingestion of treated leaves of the 0-time, that increased to 52, 60 and 64 % after 4, 6 and 8 days, respectively. Treated larvae suffered total

Table (1): Effect of different concentrations of spinosad on 1st, 2nd, 3rd and 4th instar larvae of *Agortis ipsilon* (Hnuf.) under laboratory conditions.

Larval instar	Conc. (ml/L)	% Mortality after treatment				No. of pupae	% of deformed pupae	No. of emerged moths	% of deformed moths	% Total mortality
		2 days	4 days	6 days	8 days					
Treated 1st larval instar	0.250	100.0				0	0.0	0	0.0	100
	0.125	100.0				0	0.0	0	0.0	100
	0.0625	90.0	98.0	100.0	100.0	0	0.0	0	0.0	100
	Control	0.0	0.0	0.0	2.0	98	3.0	95	1.0	6.0
Treated 2nd larval instar	0.250	100.0				0	0.0	0	0.0	100
	0.125	60.0	72.0	78.0	82.0	16	31.0	11	27.2	92.0
	0.0625	36.0	46.0	48.0	48.0	50	16.0	42	14.2	64.0
	Control	0.0	0.0	1.0	2.0	98	2.0	96	3.0	7.0
Treated 3rd larval instar	0.250	51.0	64.0	69.0	71.0	29	24.1	22	22.7	83.0
	0.125	38.0	60.0	64.0	66.0	30	20.0	24	12.5	79.0
	0.0625	18.0	34.0	40.0	44.0	52	11.5	46	8.6	58.0
	Control	0.0	1.0	1.0	2.0	97	3.0	94	2.0	8.0
Treated 4th larval instar	0.250	24.0	40.0	48.0	52.0	48	14.5	41	19.5	67.0
	0.125	18.0	28.0	34.0	36.0	60	13.3	52	11.5	54.0
	0.0625	8.0	14.0	16.0	16.0	81	12.3	71	9.8	36.0
	Control	0.0	0.0	0.0	0.0	100	2.0	98	3.0	5.0

Calculated F= 6.435311 Tabulated F= 5.143249

Table (2): Persistence of spinosad sprayed on cotton plants at a rate of 50ml/fed. and assayed against *Agortis ipsilon* 2nd instar larvae.

Post spraying (days)	Mortality% Post-feeding (days)				No. of pupae	% of deformed pupae	No. of emerged moths	% of deformed moths	% Total mortality
	2 days	4 days	6 days	8 days					
0	40	52	60	64	24	41.6	14	42.8	92
1	36	50	58	68	29	41.3	17	29.4	88
2	32	46	56	65	31	25.8	23	17.3	81
3	30	42	54	60	37	16.2	31	12.9	73
4	26	32	42	50	48	12.5	42	7.1	61
5	22	30	40	47	50	10.0	45	4.4	57
6	18	24	32	39	58	6.8	54	3.7	48
7	14	18	24	32	64	4.6	61	1.6	40
Control	0	0	2	4	96	2	94	1	7

mortality of 92 % when fed on cotton leaves sampled one hour after spraying (0-time). Then mortality rates decreased with increasing the time elapsed after spraying, i.e. the lowest mortality occurred on the 7th day post-spraying reached 40 %.

It is noteworthy that this is a high persistence in the field so far in comparison with other formulations that persisted much less time and in some cases for only one day **(Hosney *et al.*, 1993)** and for 3 days **(Pinnock *et al.*, 1971)** and for 7 days **(Abou-Bakr, 1977 and Mahmoud, 2000)**.

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Spinosad كمبيد حيوي ميكروبي ضد الدودة القارضة (Hnuf.) *Agrotis ipsilon*

بسمة عبد العزيز محمود

قسم بحوث المكافحة الحيوية – معهد بحوث وقاية النباتات – مركز البحوث الزراعية

قدرت فعالية المبيد الميكروبي Spinosad (مبيد جديد ناتج عن نوع من أنواع البكتيريا Actinomycetes و هو النوع *Saccharopolyspora spinosa*) لمقاومة يرقات الدودة القارضة السوداء كما تمت دراسة تأثير هذا المبيد علي التعذير و خروج الفراشات.

درس تأثير الثلاثة تركيزات التالية : ٢٥٠ , ١٢٥ (الموصي به) ، ٠٦٢٥ , مللتر / لتر ماء علي الأعمار اليرقية من العمر الأول و حتي العمر الرابع حيث أظهرت النتائج ما يلي:

- يرقات العمر الأول و الثاني كانت أكثر حساسية من العمر الثالث و الرابع. عند استخدام التركيز الموصي به (١٢٥ , مللتر / لتر ماء) كانت النسبة الكلية لموت اليرقات ١٠٠ ، ٩٢ ، ٧٩ ، ٥٤ ، % في كل من العمر الأول و الثاني و الثالث و الرابع علي التوالي .
- يؤثر Spinosad علي نسبة التعذير و خروج الفراشات .
- تزداد نسبة العذارى بزيادة التركيز المستخدم .
- بلغت نسبة التعذير صفر ، ١٦ ، ٣٠ ، ٦٠ % عند معاملة يرقات العمر الأول و الثاني و الثالث و الرابع علي التوالي و عند زيادة التركيز المستخدم إلي الضعف (٢٥٠ , مللتر / لتر ماء) بلغت نسبة التعذير صفر ، صفر ، ٢٩ ، ٤٨ %
- عند دراسة فترة بقاء المبيد تحت الظروف الحقلية و جد أن نسبة الموت في يرقات العمر الثاني بلغت ٩٢% عند تغذيتها علي أوراق نبات القطن بعد المعاملة بساعة بينما بلغت هذه النسبة ٤٨ % و ٤٠ % عندما تغذت اليرقات علي أوراق النبات بعد المعاملة بستة أيام و سبعة أيام علي التوالي .