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EVALUATING THE TOXIC AND LATENT EFFICACY OF SPINOSAD AGAINST THE COTTON LEAFWORM, *Spodoptera littoralis* (BOISD.)

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ABSTRACT

Spinosad is an insect control agent. Spinosad is derived from the metabolites of the naturally occurring bacteria, *Saccharopolyspora spinosa.* The objective of this study is to evaluate the toxicity as well as the latent efficacy of spinosad against the 4th instar larvae of the cotton leafworm, *Spodoptera littoralis* (Boisd.). Different bioassays namely; dipping, contact to a surface film and immersion techniques were used in this investigation. The biocide was bioassayed after 24, 48, 72 and 96 hours. The highest toxicity of spinosad was noticed after 96 hours. Results illustrated the superiority of dipping technique for the 4th instar larvae to the biocidal action of spinosad. The efficiency of the bioassay techniques reflecting the larval susceptibility could be descendingly arranged as follows: dipping, immersion and contact to a surface film. The corresponding LC_{50} and LC_{90} values against the tested larvae after 96 hours were 122.85 & 518.61; 129.90 & 608.78 and 141.34 & 978.38 ppm, respectively. The 4th instar larvae of *S. littoralis* showed moderately susceptibility to the biocidal action of spinosad as demonstrated by the toxicity ratios. The toxicity ratios of all bioassay techniques were less than 1.00. The susceptibility index as well as the potency level expressed as numbers of folds at both LC_{50} and LC_{90} values increased with increasing the period of determination. The latent effects of spinosad on the pupation as well as the adult emergence were determined. The corresponding EC_{50} and EC_{90} values associated to quintals scoring of pupation due to dipping, immersion and contact to a surface film of bioassays were 36.24& 251.95; 50.02 & 549.34 and 63.57 & 711.97 ppm, respectively. Whereas the corresponding IC_{50} and IC_{90} values associated to inhibition of the adult mergence were 7.28 & 215.44; 43.52 & 291.42 and 54.12 & 386.07 ppm, respectively.

INTRODUCTION

Spinosad is the first active ingredient proposed for a new class of insect control products (Sparks *et al.,* 1995 and Thompson *et al.,* 1996). The active ingredient of spinosad is derived from the metabolites of the naturally occurring bacteria, *Saccharopolyspora spinosa,* it is a mixture of two macrocyclic lactones, spinosyn A and spinosyn D and it has been shown to be active on insects including species from the orders; Lepidoptera, Diptera, Hymenoptera, Thysanoptera and a few Cleoptera (Thompson *et al.,* 1997). The mode of action of spinosad is associated with excitation of the insect nervous system and acts at the nicotinic acetylcholine receptor (nACHRs) and exhibits activity on the gamma-aminobutyric acid receptor GABA (Salgado, 1998). Spinosad degrades rapidly in the soil environment and is non-persistent whereas in natural water spinosad rapidly dissipates (West, 1997).

The objective of this study is to evaluate the susceptibility of the 4th larval instars of the cotton leafworm, *Spodoptera littoralis* (Boisd.) to the biological activity of spinosad as well as to evaluate the latent effects of spinosad on pupation and the adult emergence resulted from treated larvae.

MATERIALS AND METHODS

1. Product used:

Spinosad is derived from the metabolites of the naturally occurring bacteria, *Saccharopolyspora spinosa.*

Common name: Spinosad (ISO 1750 provisional).

Trade named: Spentor 24% SC (Dow Agrow Sciences).

Chemical structure: Spinosad is a mixture of spinosyn A and spinosyn D.

Empirical formula: Spinosyn A : C_{41} H_{65} NO_{10}.

Spinosyn D : C_{42} H_{67} NO_{10}.

Mode of action: Spinosad is associated with excitation of the insect nervous system (Salgado, 1998).

Activity: Microbial insecticides (macrocyclic lactone insecticides).

Systemic activity: Foliar applications are not highly systemic; however, translaminar activity is evident in applications on certain vegetable and ornamental crops.

Rate of application: 50 ml/feddan.

2. Toxicological studies:

2.1. Rearing of the cotton leafworm, *Spodoptera littoralis* (Boisd.):

A laboratory strain of the cotton leafworm, *Spodoptera littoralis* (Boisd.) was maintained under constant conditions of 27±1°C and 70±3% RH and kept away from any contamination with insecticides to obtain a susceptible as well as homogenous strain. The insect was reared in the laboratory as described by El-Defrawi *et al.* (1964).

2.2. Bioassay tests:

2.2.1. Dipping technique:

Serial concentrations based on ppm of commercial formulated spinosad (spentor) in water were prepared. Castor bean leaves were dipped in each concentration for 30 seconds then left to dry in the room for one hour. The 4th instar larvae were confined with the treated leaves in glass jars covered with muslin for four days. The treated leaves were then removed and fresh untreated leaves were provided for another days till pupation. Bioassay included untreated check in which leaves was dipped in water only.

2.2.2. Surface film technique:

Same serial concentrations of commercial formulated spinosad in water as ppm were prepared. Five ml of each concentration were poured in glass Petri-dish (15 cm in diameter), shacked and left till air dryness. Batches of the 4th larval instars were exposed to each concentration, for the untreated check, batches of the 4th larval instars were exposed to the water surface film. The treated as well as the untreated larvae were fed daily on fresh untreated castor bean leaves till pupation.

2.2.3. Immersion technique:

Batches of the 4th larval instars were immersed in each concentration of spinosad for 15 seconds, and then transferred and confined daily with fresh untreated castor bean leaves in glass jars covered with muslin till pupation.

For each experiment mentioned previously three replicates (each of 20 larvae) were tested for each concentration. Daily inspection was carried out for all treatments and mortality percentages were recorded till the fourth day after treatments. The average of mortality percentages were corrected using Abbott's formula (1925). The corrected mortality percentage was statistically computed according to Finny (1971). From which the corresponding concentration probit lines (LC-p lines) were estimated. The slope, LC_{50} and LC_{90} values were estimated. Toxicity ratio was calculated by dividing the recommended field rate in ppm by LC_{50} values of each test.

3. Evaluation of the latent effect:

With the objective of evaluating the latent effect of spinosad against the 4th instar larvae tested with different techniques, the pupation and adult emergence percentages as well as the abnormalities of the pupae and adults resulted from each test were estimated and recorded. Quantal scoring of pupation expressed as EC_{50} and EC_{90} as well as inhibition of the adult emergence expressed as IC_{50} and IC_{90} were assessed.

The results of the present study were statistically analyzed using the analysis of variance.

RESULTS AND DISCUSSION

1. Susceptibility of the 4th larval instars of *S. littoralis* to spinosad:

The susceptibility of the 4th larval instars of the laboratory strain of *S. littoralis* to spinosad was evaluated by using different techniques; i.e., dipping, surface film and immersion methods of technique. Spinosad was bioassayed after 24, 48, 72 and 96 hours from treatment for each technique. Results represented in Table (1) illustrated that the susceptibility of the laboratory strain of *S. littoralis* fed continuously on various concentrations of the product until four days from treatment. The obtained data revealed that the order of the efficiency of the product used against the tested larvae was the same at both LC_{50} and LC_{90} levels. The highest efficiency of spinosad was attained 96 hours. The corresponding LC_{50} and LC_{90} values were 122.85 and 518.61 ppm, respectively. On the other hand, the lowest efficacy of spinosad was pronounced at 24 hours where the LC_{50} and LC_{90} values were 255.51 and 1215.22 ppm, respectively. Whereas, the biological activity of the compound against the 4th larval instars fed on the treated leaves for 48 and 72 hours occupied middle situation among its efficiency at 24 and 96 hours. The corresponding LC_{50} and LC_{90} values of the tested biocide after 48 hours were 173.28 and 653.60 ppm, while these values after 72 hours of feeding were 163.99 and 637.48 ppm, respectively. Regarding the slope values, the steepest value was attained 72 hours post-treatment giving 2.56; whereas the

flattest one was noticed at 96 hours from the biocidal application where the corresponding value recorded 1.79.

Table (1): Susceptibility of the 4th larval instars of the laboratory strain of the cotton leafworm, *Spodoptera littoralis* fed continuously on castor bean leaves treated with spinosad formulation for 4 days.

Periods after treatment (hours)	Slope	LC_{50} (ppm)	LC_{90} (ppm)	Toxicity ratio*	Susceptibility index at		Potency level at	
					LC_{50}	LC_{90}	LC_{50}	LC_{90}
24	1.89	255.51	1215.22	0.47	48.08	42.68	1.00	1.00
48	2.22	173.28	653.60	0.69	70.90	79.35	1.48	1.86
72	2.56	163.99	637.48	0.73	74.91	81.35	1.56	1.91
96	1.79	122.85	518.61	0.98	100	100	2.08	2.34
F	-	92.65 ***	111.49 ***	13.11 **	1809.56 ***	2301.29 ***	49.79 ***	106.74 ***
LSD 0.05	-	18.83	95.54	0.188	1.63	1.63	0.19	0.17

*Toxicity ratio is expressed as the recommended field rate in ppm/ LC_{50} value in ppm

Concerning the biocidal efficacy, against the 4th larval instars of the pest; the toxicity index method of Sun (1950) is used to determine the degree of toxicity of different insecticides by comparing them with a standard compound.

In this study, the equation of Sun was adopted to find out the degree of susceptibility of the instar larvae exposed to or fed on the compound action for different periods as follows:

$$\text{Susceptibility index} = \frac{LC_{50} \text{ or } LC_{90} \text{ of the highest susceptible larval instar attained period}}{LC_{50} \text{ or } LC_{90} \text{ of the treated larval instar at each period}} \times 100$$

The potency levels (number of folds) were obtained by dividing the LC_{50} or LC_{90} for less susceptible larval instar at the period by the corresponding figure for each period.

Data represented in (Table, 1) showed general similarity of the trend of both susceptibility index and number of folds at the two mentioned levels of toxicity.

On the ground of the larval susceptibility index at LC_{50} level, the susceptibility of the 4th instar larvae to spinosad action for 24, 48 and 72 hours were 48.08, 70.90 and 74.91% as susceptible to the biocide action for 96 hours, respectively; whereas these values of susceptibility index at LC_{90} level were 42.68, 79.35 and 81.35%, respectively. Concerning the potency level at LC_{50} and LC_{90} values, the potency levels of the 4th larval instars exposed continuously to the biocide action for 48, 72 and 96 hours at LC_{50} level were 1.48, 1.56 and 2.08; whereas these values at LC_{90} level were 1.96, 1.91 and 2.34 times as the larval susceptible exposed to the product for 24 hours, respectively. The toxicity ratios of the spinosad against the 4th larval instars fed on the treated castor bean leaves for 24, 48, 72 and 96 hours were 0.47, 0.69, 0.73 and 0.98, respectively.

Concerning the biological activity of spinosad via surface film to act as toxic contact against the 4th larval instars of *S. littoralis;* the obtained results are represented in Table (2). The data indicated that there was similarity in the trend of the susceptibility in the pest exposed four days to the contact action of the product used at both LC_{50} and LC_{90} values. The highest toxicity of spinosad was noticed after 96 hours of exposure. The corresponding LC_{50} and LC_{90} levels were 141.34 and 978.38 ppm, respectively; whereas the lowest biological activity of spinosad was attained 24 hours of exposure where the LC_{50} and LC_{90} values recorded 467.74 and 8912.51 ppm, respectively. On the other hand, the efficiency of the product used against the 4th larval instar exposed to the surface film of the product for 48 and 72 hours occupied the middle situation among its toxicity at 24 and 96 hours. The LC_{50} and LC_{90} values of the biocid used after 48 hours were 195.73 and 2282.09 ppm, respectively; whereas these values were 174.59 and 2199.59 ppm after 72 hours, respectively.

Concerning the larval susceptibility index exposed to the contact action of spinosad for 24, 48 and 72 hours at LC_{50} level were 30.22, 72.21 and 80.96% as susceptible to the biocide action for 96 hours, respectively; whereas theses values of susceptibility index at LC_{90} level were 10.97, 42.87 and 44.48%, respectively. On the other hand, the potency levels of the larval susceptibility that exposed continuously to the contact action of spinosad for 48 & 72 and 96 hours at LC_{50} levels were 2.39, 2.68 and 3.13; whereas these values at LC_{90} were 3.91, 4.05 and 9.11 times as the larval susceptibility to the contact action of the biocide for 24 hours, respectively. The toxicity ratios of spinosad against the 4th instar larvae exposed continuously for 24, 48, 72 and 96 hours were 0.26, 0.61, 0.69 and 0.85, respectively.

Through the light on the 4th instar larvae susceptibility immersed in serial concentrations of spinosad for 15 seconds, the obtained results are illustrated in Table (3). At LC_{50} level the order of the biocide toxicity against the 4th instar larvae for different periods of exposure could be descendingly arranged as follows: 96, 72, 48 and 24 hours from the larval treatment. The corresponding Median Lethal Concentrations were: 129.90, 169.27, 194.65 and 379.27 ppm, respectively. At LC_{90} level the descending order of toxicity was the same. The corresponding LC_{90} values were 608.78, 1041.77, 2203.86 and 3478.19 ppm, respectively. The toxicity ratios of spinosad against the 4th instar larvae after 24, 48, 72 and 96 hours from larval immersion were 0.32, 0.62, 0.71 and 0.92, respectively.

According to the susceptibility index; at LC_{50} levels, the susceptibility of the 4th instar larvae to spinosad action after 24, 48 and 72 hours from larval immersion were 34.25, 66.74 and 76.74% as susceptibility of the 4th instar larvae to spinosad action after 96 hours, respectively. The susceptibility index values at LC_{90} levels were lower than that of LC_{50}. The larval susceptibility index at LC_{90} for 24, 48 and 72 hours from larval immersion were 17.50, 27.62 and 58.44% as the larval susceptibility to spinosad after 24 hours from larval immersion, respectively.

Table (2): Susceptibility of the 4th larval instars of the laboratory strain of the cotton leafworm, *Spodoptera littoralis* exposed continuously to the surface film of spinosad formulation for 4 days.

Periods after treatment (hours)	Slope	LC_{50} (ppm)	LC_{90} (ppm)	Toxicity ratio*	Susceptibility index at		Potency level at	
					LC_{50}	LC_{90}	LC_{50}	LC_{90}
24	1.00	467.74	8912.51	0.26	30.22	10.97	1.00	1.00
48	1.20	195.73	2282.09	0.61	72.21	42.87	2.39	3.91
72	1.11	174.59	2199.59	0.69	80.96	44.48	2.68	4.05
96	1.78	141.34	978.38	0.85	100	100	3.31	9.11
F	-	677.44 ***	5130.24 ***	18.63 ***	2403.47 ***	5471.92 ***	417.48 ***	4966.05 ***
LSD 0.05	-	18.83	97.44	0.188	1.96	1.63	0.15	0.155

*Toxicity ratio is expressed as the recommended field rate in ppm/ LC_{50} value in ppm.

Table (3): Susceptibility of the 4th larval instars of the laboratory strain of the cotton leafworm, *Spodoptera littoralis* immersed in serial concentrations of the spinosad formulation.

Periods after treatment (hours)	Slope	LC_{50} (ppm)	LC_{90} (ppm)	Toxicity ratio*	Susceptibility index at		Potency level at	
					LC_{50}	LC_{90}	LC_{50}	LC_{90}
24	1.33	379.27	3478.19	0.32	34.25	17.50	1.00	1.00
48	1.22	194.65	2203.86	0.62	66.74	27.62	1.95	1.58
72	1.57	169.27	1041.77	0.71	58.44	58.44	2.24	3.34
96	3.17	129.90	608.78	0.92	1.00	1.00	2.92	5.71
F	-	366.89 ***	1965.61 ***	18.61 **	2977.11 ***	5500.24 ***	50.17 ***	1581.97 ***
LSD 0.05	-	18.83	94.75	0.19	1.63	1.63	0.36	0.17

*Toxicity ratio is expressed as the recommended field rate in ppm/ LC_{50} value in ppm

As shown in Table (3), it is noticed that compared with the biocidal action after 24 hours at LC_{50}, the susceptibility of the tested larvae after 48, 72 and 96 hours were 1.95, 2.24 and 2.92 times as the larval susceptibility to the product after 24 hours from the larval immersion in comparison with 1.58, 3.34 and 5.71 times as the larval susceptibility after 24 hours at LC_{90} levels, respectively.

2. Evaluation of the latent effect of spinosad on basis of quantal scoring of pupation and the inhibition of the adult emergence:

According to this method of assessment the quantal scoring of pupation included the larval mortality percentages during the larval stage and the percent of deformed pupae. On the other hand, the inhibition of the adult emergence percentages is based on recorded larval mortality, deformed pupae and remaining number of pupae that failed to produce normal emerged adults. Both quantal scoring as well as inhibition of the adult emergence percentages were was assessed as related to the original number of treated larvae. These percentages were corrected for natural

mortality and abnormality in the control by the use of Abbott's formula (1925). It is obvious that the EC_{50} and EC_{90} (effective concentrations causes 50 and 90% quantal scoring) as well as IC_{50} and IC_{90} (concentrations cause 50 and 90% inhibition of the adult emergence) are equivalent to LC_{50} and LC_{90} determined by means of the usual toxicity. The obtained results are summarized in Table (4). The EC_{50} and EC_{90} values of the quantal scoring as well as the IC_{50} and IC_{90} values of the inhibition of the adult emergence very clearly illustrate the superiority of dipping technique for the 4th instar larvae to the biological action of spinosad on both pupae and adults of *S. littoralis*. However, on basis of these values the efficacy of the bioassay techniques could be descendingly arranged as follows: dipping, immersion and surface film.

The corresponding EC_{50} values of the biocide associated to these techniques were 36.24, 50.02 and 63.57 ppm; whereas the corresponding EC_{90} values were 251.95, 549.34 and 711.97 ppm; whereas the corresponding IC_{50} values associated with the inhibition of the adult emergence were 7.28, 43.52 and 54.12 ppm and the IC_{90} values were 215.44, 291.42 and 386.07 ppm, respectively.

It could be concluded that spinosad had moderately biological activity against the 4th instar larvae of *S. littoralis*. Spinosad could be used only at very low population and infestation. Spinosad had slight efficiency against the pest when was used as contact surface film, therefore it could advice that no application of spinosad against bollworms.

Table (4): Latent effect of spinosad on the quantal scoring of pupae as well as the inhibition of the adult emergence of the laboratory strain of the cotton leafworm, *Spodoptera littoralis* resulted from the larval treatments at indicated techniques.

Methods of treatment	Quantal scoring of resulted pupae			Inhibition of the adult emergence		
	Slope	EC_{50}* (ppm)	EC_{90}* (ppm)	Slope	IC_{50}** (ppm)	IC_{90}** (ppm)
Dipping	1.52	36.24	251.95	0.87	7.28	215.44
Immersion	1.23	50.02	549.34	1.55	43.52	291.42
Surface film	1.22	63.57	711.97	1.50	54.12	386.07
F	-	560.21 ***	163.53 ***	4309.00 ***	180.90 ***	219.21 ***
LSD 0.05	-	1.99	1.99	0.019	0.199	1.99

*Effective concentration response tests associated to quantal scoring of pupation
**Concentration response tests associated to the inhibition of the adult emergence.

In this field of study, investigators considered that spinosad was relatively slow acting, with the maximum toxicity noted at 72 hours to house fly (Scott, 1998). Mascarenhas and Boethel (1997) cited that spinosad had lower LC_{50} at 72 hours against the field strain of the soybean, *Pseudoplusia includens* collected from Hamburg, Louisiana than that of the susceptible USDA strain. Mascarenhas *et al.* (1998) indicated that all field strains of *Spodoptera exigua* responded similarly to the laboratory strain for spinosad bioassays, except the strain which collected from Tallulah and Louisiana that had significant higher LC_{50}.

El-Aw (2003) found that the toxicity of spinosad persisted for 5 days on the 2nd and the 4th instar larvae of *Spodoptera littoralis* under laboratory conditions. Schmandke (2001) stated that spinosad is environmentally degraded by photolysis, oxidation and bacteria. Its half-life in sunlight is <1 day in soil and water and about 2 days on plants. Abdel-Mageed (2005) reported that the highest efficiency of spinosad against *Spodoptera littoralis* was obtained after 72 hours from treatment. He added that degradation of spinosad in the environment occurs mainly by photodegradation.

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تقييم التأثير السام والمتأخر للمبيد الحيوي سبينوساد ضد دودة ورق القطن

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يهدف هذا البحث الي تقييم أحد المبيدات الميكروبية الجديدة الناتجة من تخمر بكتريا *Saccharopolyspora spinosa* ويسمي مبيد سبينوساد. وقد درست حساسية العمر اليرقي الرابع للسلالة المعملية لدودة ورق القطن للمبيد الحيوي سبينوساد Spinosad ، كما درس التأثير المتأخر للمبيد المختبر علي كل من العذاري وخروج الفراشات الناتجة من معاملة اليرقات. استخدم في هذه الدراسة ثلاث طرق من الإختبارات البيولوجية. الطريقة الأولي غمر أوراق الخروع في تركيزات مختلفة من المبيد الحيوي وتغذية العمر اليرقي الرابع علي الأوراق المعاملة لمدة أربعة أيام متتالية وتسمي بطريقة Dipping technique ، والطريقة الثانية تم غمر اليرقات في تركيزات مختلفة من المركب الحيوي لمدة ١٥ ثانية وتسمي بطريقة Immersion technique ، والطريقة الثالثة تشمل تعريض يرقات العمر الرابع لفيلم من تركيزات مختلفة من المبيد لمدة أربعة أيام وتسمي Surface film technique وقدرت حساسية اليرقات المعاملة بعد ٢٤، ٤٨، ٧٢، ٩٦ ساعة.

أظهرت النتائج أن أعلي تأثير للمركب الحيوي في جميع الإختبارات البيولوجية الثلاث كان بعد ٩٦ ساعة. كما أشارت النتائج الي أن طريقة Dipping technique أعطت أعلي تأثير علي العمر اليرقي الرابع. وبصفة عامة أمكن ترتيب فعالية طرق الإختبارات البيولوجية الثلاث من حيث درجة سميتها ضد العمر اليرقي الرابع كما يلي: طريقة Dipping technique ثم طريقة Immersion technique ثم طريقة Surface film technique. وكانت قيم LC_{50} ، LC_{90} المطابقة لهذه الطريق ضد اليرقات المختبرة بعد ٩٦ ساعة هي ١٢٢,٨٥& ٥١٨,٦١ ، ١٢٩,٩٠& ٦٠٨,٧٨، ١٤١,٣٤& ٩٧٨,٣٨ جزء في المليون علي الترتيب. أيضا أشارت النتائج أن حساسية العمر اليرقي الرابع للمركب الحيوي سبينوساد عند إجراء الإختبارات البيولوجية سالفة الذكر كانت متوسطة إستنادا الي قيم معدلات السمية Toxicity ratios والتي بلغت أقل من واحد صحيح في جميع مراحل الإختبارات. وعند إلقاء الضوء علي التأثير المتأخر للمركب علي عمليات التطور لتكوين عذاري وخروج الفراشات الناتجة من معاملة اليرقات، أظهرت النتائج أن قيم EC_{50} وكذلك EC_{90} المطابقة عند إجراء الإختبارات البيولوجية Dipping technique و Immersion وكذلك Surface film بلغت ٣٦,٢٤& ٢١٥,٩٥ ، ٥٠,٠٢& ٥٤٩,٣٤ ، ٦٣,٥٧ & ٧١١,٩٧ جزء في المليون علي الترتيب، بينما كانت قيم IC_{50} وكذلك IC_{90} المطابقة لمعدلات تثبيط خروج الفراشات عند إجراء الإختبارات البيولوجية. السابقة الذكر بلغت ٧,٢٨& ٢١٥,٤٤ ، ٤٣,٥٢& ٢٩١,٤٢ ، ٥٤,١٢& ٣٨٦,٠٧ جزء في المليون علي الترتيب.