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**LABORATORY AND FIELD EVALUATION OF SPINOSAD AND SOME
BIO-INSECTICIDES FOR CONTROLLING THE COTTON LEAFWORM,
SPODOPTERA LITTORALIS (BOISD.) (LEP.: NOCTUIDAE) ON COTTON
BY**

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ABSTRACT

Four commercial formulations Spinosad (a new bioinsecticide which derived from a species of actinomycetes bacteria *Sacharopolyspora spinosa*), Biogard, Agerin and Biotect which based on *Bacillus thuringiensis* were evaluated under laboratory, semifield and field conditions against larval instars of *Spodoptera littoralis* at recommended rates.

Results showed that the 2nd instar larvae was more sensitive to all tested bioinsecticides than the 4th instar.

The data also indicated that the Spinosad was more effective than Biogard, Agerin and Biotect, respectively.

In the semifield experiments, Spinosad was the most effective one, it caused 92% end-mortality for *S. littoralis* 2nd instar larvae, when it fed on cotton leaves sampled one hour after spraying, while when Agerin, Biogard and Biotect were used, percentage of mortality were 75, 71, and 70, respectively.

Under field conditions, the data also showed that, Spinosad was the most effective against larvae of *S. littoralis* than the other tested bioinsecticides the percentage of reduction reached to 97.3 compared to 78.1, 72.8 and 68.5 in case of Agerin, Biogard and Biotect, respectively after 8 days of spraying.

INTRODUCTION

The widespread use of chemical insecticides to control agricultural pests has created severe problems, principally ecological ones which emerge various health hazards. Non-degradable chemical residues, as well as degradable toxic products, have gradually accumulated to harmful levels in the environment. Moreover, synthetic pesticides oftenly disrupted the balance of insect communities and destroyed the populations of resident natural enemies. Repeated application, through years, of chemical pesticides lead to those the secondary pests become firstly pests and the development of resistance to chemical pesticides among many target insect population.

One of these severe pests is the Egyptian cotton leafworm, *Spodoptera littoralis* (Boisd.) results big economic losses in cotton crop, hence all possible ways are tried to control it. Recently microbial control agents such as bacteria and virus were evaluated against the cotton leafworm, and some other lepidopterous pests by many authors (Dunbar and Kaya, 1972; Hamed *et al.*, 1990; El-Husseinei *et al.*, 1997 and Romeilah and Abdel Megiud, 2000).

The aim of the present study is to evaluate the efficacy of four commercial preparations; Biotect, Biogard, Agerin and Spinosad 24SC on *S. littoralis* larvae under laboratory and field conditions. Also, a study has been done on persistence of each bioinsecticide on cotton leaves under field conditions of Beni-Suif Governorate.

MATERIALS AND METHODS

Tested bio-insecticides:

- a - Spinosad 24SC, is a product of Dow Agrosiences. It is an emulsive liquid based on a mixture of the two active naturally occurring metabolites spinosyns A and D. (Spinosad, the first active ingredient in the Naturalyte class of insect control products, is a mixture of spinosyn A and spinosyn D. Thompson *et al* 1997) . produced as the metabolite of the actinomycetes *Saccharopolyspora spinosa* (Thompson *et al.*, 2000). It has been used during the course of these studies at the recommended rate (50 ml/fed. 0.125ml/l).
- b- Biotect. Based on *B. thuringiensis* var *kurstaki*, Wettable Powder 9.4%, 32000 Iu/mg it is a product of Chemical Insecticides of Kafr el Zayat Company and used at a recommended rate of (300 gm/fed 0.75gm/l).
- c- Biogard. Based on *B. thuringiensis* var. *kurstaki*, Wettable Powder 32000 Iu/mg it is a product of Al Nasr Company for Fertilizers and Bioinsecticides and used at a recommended rate of (500 gm/fed. 1.25 gm/l).
- d- Agerin. WP based on a genetically engineered of *B. thuringiensis* produced by Agric, Genetic Engineering Res. Inst., Agric. Res. Center, Egypt, used at a recommended rate of (500 g/fed 1.25gm/l.)

Bioassay:

Spodoptera littoralis larvae were obtained from a laboratory culture reared on cotton leaves for several generations. Uniform age and size larvae of the 2nd and 4th instars were used in both laboratory and semifield experiments.

I. Laboratory experiments:

In case of laboratory experiments, fresh aqueous suspension of Spinosad, Biotect, Biogard and Agerin were prepared in distilled water. Cotton leaves were dipped for five seconds in the bioinsecticide and left to dry. For bioassay, *S. littoralis* larvae were starved for 4 hours, then fed for 48 h. on the treated leaves (100 larvae for each bioinsecticide). After that, the surviving larvae were allowed to feed on fresh untreated cotton leaves till pupation and adults emergence. Mortality percentages were recorded 2, 4, 6 and 8 days after feeding on the treated leaves. Percentages of pupation and adults emergence were recorded. In the control experiments, larvae were fed on cotton leaves dipped in water only.

The experiments were done under laboratory conditions of $25 \pm 2^{\circ}\text{C}$ and 65 % RH.

II. Impact of Bioinsecticides and Spinosad on *Spodoptera littoralis* larvae:

An experiment was conducted at Sids Agric. Res. Station farm, Beni-Suif Governorate during 2005 season. Four microbial insecticides were used, Biotect, Biogard, Agerin and Spinosad.

For conducting the experiment, a cotton field of less than half feddan was divided into 16 plots, the size of each plot was 45 square meters. The variety of cotton grown was Giza 80. The field was rather heavily infested artificially with *Spodoptera littoralis* egg-masses. The complete randomized blocks was followed and the experiment included four replicates for each treatment (3 replicates/ bioinsecticid +1 replicate as control). After 6 days, the bioinsecticides were applied at the recommended dose by means of a Knapsack motor sprayer with of 150 liters water per feddan. Spraying was conducted on 15 June, 2005. To evaluate the effect of spraying, larval counts were made on different dates. The first count was made just before spraying then on 2nd, 4th, 6th and 8th day after spraying. Each count represented the average number of larvae counted from each plot, i.e. 12 square meters per each treatment and 12 m per control. For determining the effectiveness of the tested compounds, percentage of reduction in the larval population of *S. littoralis* was calculated according to Henderson and Tilton (1955).

$$\text{Reduction percentage} = \left(\frac{cb \cdot Ta}{ca \cdot Tb} - 1 \right) \times 100$$

Where, Tb and Ta are counts before and after treatment, cb and ca are counts of untreated checks.

III. Persistence of bioinsecticides activity on plant leaves:

Cotton plants of 30-35 cm height, grown in large pots (40 cm diam.) were treated with either of the 4 bioinsecticides and the 5th was sprayed with water only to be considered as a control.

From control and all treatments plant leaves were collected at intervals of zero-time, 1, 2, 3 and even 7 days to be assayed against 2nd and 4th instar larvae of *S. littoralis*. Larvae were fed for 48 h. on the treated leaves. Larval mortality was recorded daily till pupation. Also, percentages of pupation and adults emergence were calculated.

RESULTS AND DISCUSSION

1. Laboratory experiments:

2. Mortality values among *S. littoralis* 2nd and 4th instar larvae are recorded in Table (1):

- a- **Biotect:** The percentage of end-mortality (till the emergence of moths) of 2nd larval instar fed on cotton leaves treated with Biotect (300 gm/fed.) reached 62%. Only 48 pupae were obtained from 100 treated larvae from which 38 moths were emerged. In case of treated 4th instar larvae, less end-mortality was recorded, 42%, 64 pupae and 58 moths were obtained, the total mortality reached 42%.
- b- **Biogard:** The obtained results indicate that, effective control of 59% could be achieved after 8 days against 2nd instar larvae when concentration of 1.25 g/L was used. The total mortalities among the 2nd instar larvae was 72%. While treated 4th instar larvae, with Biogard it caused mortalities of 5, 5, 28 and 28%, 2, 4, 6 and 8 days after treatment, respectively. Total mortality reached 47%
- c- **Agerin:** The obtained results indicated that, when *S. littoralis* 2nd instar larvae were fed on cotton leaves treated with recommended rate of 1.25 g/L and 8 days after treatment, a moderate mortality percentage 57% had been recorded. It increased to 75% in the end-mortality. Percentage of total mortality was 56% when the recommended rate of Agerin was tested against *S. littoralis* 4th instar larvae.

- d- Spinosad 24SC: The percentage of mortality reached 92%, when larvae of the 2nd instar were fed on cotton leaves treated with Spinosad 24SC, only 10 pupae were obtained from 100 treated larvae and 8 moths were emerged. In treated 4th instar larvae, Spinosad 24SC caused mortalities ranged between 16-58%, 2 and 8 days after treatment, respectively.

Table (1): Percentage of mortality among 2nd and 4th instar larvae of *Spodoptera littoralis* fed on cotton leaves treated with recommended doses of Bioinsecticides and Spinosad 24SC.

Bioinsecticide Con .		Days after treatments				Pupation %	Adults %	Total mortality %
		2	4	6	8			
2nd instar larvae								
Biotect	0.75gm/l	0	24	36	52	48	38	62
Biogard	1.25gm/l	8	19	33	59	32	28	72
Agerin	1.25gm/l	7	21	38	57	38	25	75
Spinosad	0.125ml/l	82	83	88	88	10	8	92
Control	-	0	0	3	6	93	93	7
4th instar larvae								
Biotect	0.75gm/l	0	2	6	18	64	58	42
Biogard	1.25gm.l	5	5	28	28	67	53	47
Agerin	1.25gm/l	0	5	23	34	54	44	56
Spinosad	0.125ml/l	16	23	33	58	33	21	79
Control	-	0	1	2	4	95	94	6

2. Impact of bioinsecticides and Spinosad on larval stages of *S. littoralis* .

From practical point of view, it is important to evaluate Biotect, Biogard, Agerin and Spinosad virulence and potentiality on different stages of *S. littoralis* on successive days after spraying cotton plant in the outdoor, Table (2 & 3) summarized the obtained results when larvae of 2nd and 4th instars fed on cotton plants sprayed with the forementioned bioinsecticides at the recommended rates on 0-time (1 hour after spraying) and then daily for a week post spraying. Data in table 2 indicate that Spinosad was the most effective one, followed by Agerin, Biogard and Biotect, on the 2nd instar *S. littoralis* larvae whereas the total mortalities were 94%, 75%, 71% and 70% when larvae fed on treated cotton leaves, one hour after spraying with Spinosad, Agerin, Biogard and Biotect, respectively .

In all cases, mortality rates decreased with increasing the time elapsed after spraying *i.e.*, the lowest values occurred on the 7th day post spraying reached 65, 28, 20 and 10%, after spraying with Spinosad, Agerin, Biogard and Biotect, respectively.

Degradation of virulence and potentiality of the aforementioned bioinsecticides under field conditions were also found in case of treated 4th instar larvae (Table 3). The end-mortality recorded among treated larvae of 0-time post spraying treatment reached 79, 56, 4 and 44% for Spinosad, Agerin, Biotect and Biogard, respectively, then it decreased gradually to reach 38, 34, 12 and 8%, respectively on the 7th post spraying.

Table (2): Persistence of Bioinsecticides and Spinosad at the recommended rates against *Spodoptera littoralis* 2nd instar larvae.

Bio-insecticide	Time after spraying	Periodical mortality% (days post treatment)				% of Pupaetion	% of Adults emergence	Total mortality %
		2	4	6	8			
Biotect	0	0	24	26	39	52	30	70
	1	2	18	22	38	48	38	62
	2	0	15	24	32	64	56	44
	3	2	10	16	22	72	68	32
	4	0	4	10	16	80	74	26
	5	2	6	6	10	86	78	22
	6	1	4	8	8	88	84	16
Biogard	0	15	37	51	58	35	29	71
	1	9	33	49	56	40	33	67
	2	4	34	46	50	41	34	60
	3	0	32	36	48	45	40	57
	4	0	16	34	32	48	43	53
	5	0	7	18	32	50	60	40
	6	0	0	18	15	63	80	20
Ageria	0	13	24	55	59	37	25	75
	1	9	19	52	57	40	32	68
	2	7	13	46	52	340	40	60
	3	0	15	42	50	45	41	59
	4	0	14	34	50	45	41	59
	5	0	15	23	47	50	42	58
	6	0	0	20	37	60	60	40
Spinosad	0	87	93	93	94	8	6	92
	1	85	88	90	90	10	10	90
	2	80	85	85	85	14	12	88
	3	75	80	83	88	12	12	88
	4	56	75	82	82	12	18	82
	5	43	55	75	75	25	25	75
	6	25	53	69	69	30	30	65
7	19	41	65	65	30	30	70	
Control	0	2	5	7	93	91	9	

These results are in agreement with those obtained by Fast and Regniere (1984), Abd El-Haleem, Sawzan (1997), Mahmoud Basma A.(2004), Morsi *et al.* (2004) and Mahmoud Basma A. *et al.* (2005). It is clear that the percentage of mortality decreased gradually after treatment because of degradation of the used bioinsecticides as a result of the different environmental effects.

It is to be noted that Saunders and Bret (1997) indicated that degradation of Spinosad happened through primarily photodegradation and microbial degradation to its natural components of carbon, hydrogen.

Table (3): Persistence of Bioinsecticides and Spinosad at the recommended rates against *Spodoptera littoralis* 4th instar larvae.

Bio-insecticide	Time after spraying	Periodical mortality% (days post treatment)				% of Pupation	% of Adults emergence	Total mortality %
		2	4	6	8			
Biotect	0	0	8	16	24	62	56	44
	1	1	6	14	20	68	60	40
	2	0	3	12	20	78	67	33
	3	0	4	8	12	84	78	22
	4	1	3	6	8	88	82	18
	5	0	2	2	4	92	88	12
	6	0	1	3	5	94	90	10
	7	0	0	2	4	92	92	8
Iogard	0	0	7	15	27	65	54	46
	1	0	5	13	26	65	56	44
	2	0	4	16	25	67	57	43
	3	0	2	15	25	70	65	35
	4	0	3	14	24	70	68	32
	5	0	2	9	24	76	79	21
	6	0	2	8	22	85	87	13
	7	0	1	5	22	88	88	12
Agerin	0	3	17	21	33	54	44	56
	1	0	16	26	31	55	45	55
	2	0	13	25	30	57	49	51
	3	0	4	21	28	59	50	50
	4	0	2	13	25	65	56	44
	5	0	5	7	25	66	57	43
	6	0	3	9	23	70	66	34
	7	0	2	8	23	75	66	34
Spinosad	0	5	21	32	50	38	26	79
	1	4	19	31	49	39	32	68
	2	0	15	25	47	50	35	65
	3	0	13	21	45	50	38	62
	4	0	12	20	41	52	50	50
	5	0	13	20	40	52	52	48
	6	0	12	20	38	62	62	38
	7	0	12	20	38	62	62	38
Control	0	2	4	5	93	92	8	

3. Field experiments:

Results obtained from field experiments of cotton season 2005 are summarized in Table (4). It shows clearly that there was an appreciable reduction in the efficacy of the tested bio-insecticides. Two days after application, the percentages of reduction in the larval population were 4.5, 4.5, 4.8 and 88.6% for Biotect, Biogard, Agerin and Spinosad, respectively.

After four days from application these percentages increased to be 8.5, 8.7, 6.9 and 91.1% for the above-mentioned bioinsecticides, respectively, while, after

six days from application, the respective values reached 67.9, 71.4, 72.8 and 96.9. Eight days after application, the maximum reduction in the leafworm numbers amounted to 97.3 for Spinosad and 68.5, 72.8 and 78.1 for Biotect, Biogard and Agerin, respectively.

It is noticed that the maximum of reduction in larval population was obtained after 8 days from treatment with all tested bioinsecticides . Also, Spinosad gave the highest reduction 97.3 %in larval population .

Table (4): Effect of Bioinsecticides and Spinosad on cotton leafworm, *Spodoptera littoralis* (Boisd.) larvae after spraying on cotton fields at Sids Agric. Res. St. farm, Beni-Suif Governorate.

Treatment	*No. of larvae before spraying	No. of larvae and reduction% in larval population at indicated days after spraying							
		2 days		4 days		6 days		8 days	
		No. of larvae	% Red.	No. of larvae	% Red.	No. of larvae	% Red.	No. of larvae	% Red.
Biotect	142	132	4.5	122	8.5	41	67.89	36	68.5
Biogard	140	130	4.5	126	8.7	36	71.4	31	72.8
Agerin	135	125	4.8	118	6.9	33	72.8	25	78.1
Spinosad	181	20	88.6	15	91.1	5	96.9	3	97.3
Check	149	145		140		134		120	

* No . of larvae / 12m² before spraying

It can be concluded that the microbial control of insect pests is of crucial importance to developing countries (Salama *et al.*, 1993). The overuse or misuse of chemical pesticides and their negative impacts on soil and water quality, human health, wildlife and the ecological balance within agroecosystems are increasingly becoming causes for concern, underlining the need for development of alternative pest control methods. Although *B.t.* has proved to be highly successful weapon for fighting some agricultural pests and some vectors of diseases, its use is still limited in developing countries. The obtained results indicated that, the tested bioinsecticides, Biogard, Biotect, Agerin, Spiosad can be used against the 2nd instar larvae of the cotton leafworm . Spinosad proved to be the most effective one against 2nd and 4th larval instar .

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التقييم المعملی والحقلی لفاعلية السبينوساد وبعض المبيدات الحيوية الأخرى على دودة ورق القطن .

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تم تقييم فاعلية أربع مستحضرات حيوية على العمر البرقسي الثاني والرابع لدودة ورق القطن باجراء تجارب معملية نصف حقلية وحقلية وهذه المستحضرات هي سبينوساد وهو من المبيدات الحيوية التي تم الحصول عليها من مصادر طبيعية من نواتج التمثيل الغذائي لتنمية الميكروب ساكاروبوليسپورا اسبينوزا وبعض المبيدات الحيوية التي تعتمد على الميكروب باسيلس ثورنيجينسيس في إنتاجها وتمت هذه التجارب بالجرعات الموصى بها لكل مبيد.

وقد أشارت النتائج أن العمر اليرقى الثانى أكثر حساسية من العمر اليرقى الرابع وذلك للمستحضرات التى قيمت.

كما أشارت النتائج أيضا إلى أن سبينوساد أكثر فاعلية على يرقات دودة ورق القطن بالمقارنة بـ بيوجارد، الأجرين، بيوتكت على التوالي فى التجارب النصف حقلية والمعملية.

أيضا أشارت النتائج أن السبينوساد فى التجارب النصف حقلية كان أكثرهم فاعلية حيث سبب نسبة موت وصلت الي ٩٢% ليرقات العمر الثانى بعد الرش مباشرة بينما الأجرين، البيوجارد وبيوتكت سببت نسبة موت أقل وهى ٧٥ و ٧١ و ٧٠ على التوالي وذلك بعد ٨ ايام من المعاملة.

اما تحت الظروف الحقلية كان السبينوساد أكثر فاعلية حيث أحدث نسبة خفض فى أعداد اليرقات وصلت إلى ٩٧,٣ بالمقارنة بـ ٧٢,٨ و ٧٨,١ و ٦٨,٥ فى حالة المبيدات بيوجارد والأجرين وبيوتكت وذلك بعد ٨ أيام من الرش.