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METABOLITES DERIVED FROM FERMENTATION OF *Saccharopolyspora spinosa* AS INSECTICIDE FOR THE COTTON LEAFWORM *Spodoptera littoralis* (BOISD.)(Noctuidae – lepidoptera).

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ABSTRACT

Spintor 24 sc is a new class of insecticides. It is a mixture of two active naturally occurring metabolites spinosyns A and D produced by the actinomycetes *Saccharopolysposa spinosa*. Spintor has a broad spectrum of activity against many insect pests of vegetables and field crops but spares beneficials. The obtained results of laboratory and field experiments showed that. Spintor 24 Sc at a rate of 0.125 ml/L caused high mortality reached to 84% and 71.5% to 2nd and 4th instars of *S. littoralis* larvae, respectively. It causes also malformation of pupae and moths. 38.5% of pupae obtained from 2nd instar treated larvae, and 43.8% of emerged moths were malformed. 75 pupae and 57 moths were obtained from rearing of 200 treated 4th instar larvae. 24% and 15.8% of them were malformed respectively. The hatchability of eggs deposited by emerged moths decreased to be 50.1% compared to 93.8% of control. The eggmasses laid by moths, which exposed during 2nd larval instar to Spintor, have less number of eggs than those laid by untreated ones. It ranged between 22 and 62 eggs/eggmass with an average of 43.9 eggs/eggmass compared to that of control treatment which has an average of 70.2 eggs/eggmass.

Degradation of impact of Spintor on different stages of *S. Littoralis* has been observed under field conditions. The endmortality of 2nd instar larvae, which fed on cotton leaves 1 hour after spraying in the field, reached 80%. The mortality decreased with increasing the time elapsed after spraying, till it reached its lowest percent of (33.3%) on the 7th day after spraying. The effect of Spintor on larvae, pupae, moths and hatchability of deposited eggs proved that Spintor 24 sc may prevent building up of high populations of the cotton leaf worm in the treated fields year after year.

INTRODUCTION

The discovery and characterization of soil actinomycetes *Saccharopolyspora spinosa* represented a novel opportunity to develop an insect management tools. Spintor 24sc, which is used during the course of these studies, is a secondary metabolite from the aerobic fermentation of *S. spinosa* on nutrient media. It is a mixture of the two most active naturally occurring metabolites spinosyns A and D produced by *S. spinosa* (Thompson *et al.* 2000). This insecticide has extremely low toxicity to mammals (LD_{50} oral and dermal >5,000 mg/kg), birds and many aquatic invertebrates. It is moderately to slightly toxic to fish, but is highly toxic to marine mollusks (shellfish) (EPA, 1997).

Spintor has a broad spectrum of activity against many of insect pests of vegetables and field crops but spares most beneficials. Targeted Lepidopterous insects include *Spodoptera* spp., *Agrotis* spp., *Heliothis* spp., and *Tortricid* spp. In addition, diamond back moth, cabbage lopper, European

corn borer and Colorado potato beetle larvae can be controlled by this insecticide. It has been applied to over 200 different crops including cotton. (Shoonover and Larson, 1995; Carson and Trumble, 1997; Linduska *et al.,* 1998, Walgenbach and Palmer, 1997; Dow, 1997 and Thompson *et al.*, 2000).

The larvae die after ingesting Spintor treated plants with no recovery. Symptoms are paralysis, cessation of feeding and mortality within few days (Bret *et al.*, 1997).

The aim of the present studies is to evaluate the effect of this new natural insecticide on the different larval stages of *Spodoptera littoralis* (Boised.) and on pupation, moth emergence, malformation of pupae and moths, depositing of egg masses and hatchability of deposited eggs.

In addition to these groups of laboratory experiments, persistence of Spintor on cotton plants under field conditions has been evaluated. Its effect on *S. littoralis* larvae has been studied on the same day of spraying and also daily for one week after spraying.

MATERIALS AND METHODS

Tested insecticide:

Spintor 24 sc, is a product of Dow Agro Sciences. It is an emulsive liquid and based on a mixture of the two active naturally occurring metabolites spinosyns, A and D 22-23.6%. It has been used during the course of these studies at the recommended rate (50 ml/fed.).

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 the 4th instars were used in both of laboratory and semifield experiments.

In case of laboratory experiments, fresh aqueous suspension of Spintor was prepared in distilled water. Cotton leaves were dipped for seconds in the prepared dilution of Spintor (0.125 ml/L =recommended rate) and left for air-drying. Two hundred larvae in ten replicates of 2nd instar and of 4th instar were used for bioassay. Amounts of treated cotton leaves were taken and offered to the larvae, which kept under laboratory conditions of 25±2^{o}C and 62±5% R.H. In control treatment, larvae were fed on cotton leaves treated with water only. Larvae were fed on treated leaves for 2 days. The survivors were transferred to new plastic jars with untreated leaves until pupation and adult emergence. Mortality records were taken on the 2, 4, 6 and 8th day post treatment. Percentages of pupation ,adult emergence and egg masses hatchability were estimated. All laboratory experiments were undertaken in Biological Control Res. Department, Plant Protec. Res., Institute, Giza.

Impact of Spintor on *S. littoralis* larvae on successive days after spraying in cotton fields:

Two cotton fields at Sakha Res. Station, Kafer El Sheikh Governorate, each about one kerat, were used for studying the effects of Spintor on 2nd and 4th instar larvae of *S. littoralis*. The first field was sprayed with Spintor at the recommended rate of 50 ml/400 L water/fed. using Knapsak sprayer. The

other field was sprayed with water only and used as control. Amounts of treated cotton leaves were taken on intervals of zero time (just before spraying) and daily for 7 days after spraying. Then transported to the lab to feed 100 larvae of 2nd and other 100 of 4th instar each in 5 replicates for 48 hours to evaluate the potentiality of Spintor on *S. littoralis* larvae on successive days after spraying. Larval mortality were recorded on the 2nd, 4th, 6th and 8th day after the start of feeding on Spintor or water sprayed cotton leaves. In each case percentages of pupation, deformed pupae, emergence of moths, deformed moths and hatchability of eggs were calculated. Percentages of mortality were corrected according to Abbotts formula (1925).

RESULTS AND DISCUSSION

Laboratory experiments:

As shown in Table (1), the percentage of endmortality (till the emergence of moths) of 2nd larval instar fed on cotton leaves treated with Spintor 24sc (0.125 ml/L) reached 84%. Only, 52 pupae were obtained from 200 treated larvae and 38.5% of them were malformed. 32 moths were emerged and 43.8% of them were malformed. The percentage of hatched eggs (of the eggs laid by the emerged moths) reached 50.1%. The percentage of endomortality among the untreated 2nd instar larvae reached 7%. 190 pupae and 186 moths were obtained most of pupae and moths were good formed, only 2.1% and 4.3% of pupae and moths were malformed respectively.

In case of treated 4th instar larvae, less endmortality was recorded (71.5%). 75 pupae and 57 moths were obtained from rearing of treated 4th instar larvae. 24% and 15.8% of them were malformed respectively.

The hatchability of eggs deposited by moths resulted from treated 4th instar larvae increased to be 77.6% compared to 50.1% in case of treated 2nd instar larvae.

Among the untreated larvae, the endmortality reached 5% only. The percentage of hatched eggs laid by resulted moths reached 93.8%. It was also observed that, the eggmasses laid by moths exposed during their larval stage to Spintor (L_2 or L_4) have less number of eggs than those of control treatment. In case of treated 2nd instar larvae. The moths laid eggmasses, which have number of eggs ranged between 22 and 62 eggs with an average of 43.9 eggs/eggmass. In control treatment it reached 70.2 eggs/eggmasses in the average. While in case of treated 4th instar larvae, the resulted moths laid eggmasses more larger and have a number of eggs ranged between 26 and 83 with an average of 56.9 eggs/eggmass, compared to untreated control which has an average of 63.2 eggs/eggmass.

Impact of spintor on larval stages of *S. littoralis* on successive days after spraying:

From practical point of view, it is important to evaluate Spintor virulence and potentiality on different stages of *S. littoralis* on successive days after spraying in the field. The Tables (2) and (3) summarized the obtained results, when larvae of 2nd and 4th instars fed on cotton plants sprayed with Spintor at

T1.2

t3

rate of 50 ml/fed. on 0-time (1 hour after spraying) and then daily for a week post spraying.

Mortality among tested 2nd instar larvae reached 39% after 2 days post-ingestion of treated cotton leaves of the 0-time, then it increased to 46.9, 55.2 and 64.2 after 4, 6 and 8 days respectively. The endmortality (till emergence of moths, which resulted from treated larvae) reached 80%. After 2 days post spraying the endmortality reached 68.9%. The mortality percentages decreased with increasing the time elapsed after spraying. The lowest percentage of endmortality (33.3%) has been recorded on the 7th day post spraying.

The number of emerged moths, which obtained after rearing 100 treated 2nd instar larvae. Increased from 18 moths (resulted from 0 time post spraying treated larvae) to reach 60 moths (resulted from 7 days post spraying treated larvae). The highest percentage of malformed moths (22.2%) occurred among the moths, which resulted from larvae fed on treated cotton leaves of 0 time; and the lowest (3.3%) was of 7 days post spraying treatment (Table 2). Spintor affect also hatchability of deposited eggs, which laid by moths resulted from treated larvae. Hatchability of eggs was 48.5% in case of 0 time treatment and then it increased gradually till it reached 94% in case of 7 days post spraying treatment.

Degradation of virulence and potentiality of Spintor under field condition was also found in case of 4th instar treated larvae Table (3). The endmortality recorded among treated larvae of 0-time post spraying treatment reached 64%. Then it decreased gradually to reach 15% only on 7 days post spraying treatment. When larvae of different treatments (0-time to 7 days post spraying) reared till moths emergence, the numbers of emerged moths increased gradually from 36 (0-time post spraying) to 85 moths (7 days post spraying treatment).

The percentage of hatchability of deposited eggs ranged between 70.2% and 95.2% on 0-time and 7 days post-spraying treatments respectively.

It is to be noted that, Saunders and Bret (1997) indicated that degradation of spinosad (based on the same two active naturally occurring metabolites spinosyns A and D, on which Spintor based) happened through primarily photodegration and microbial degradation to its natural components of carbon, hydrogen, oxygen and nitrogen. They added that half-life of spinosad is 1.6 to 16 days. The obtained results which prove degradation of Spintor virulence and its impact on *S. littoralis* larvae under field conditions are in agreement with those obtained by Saunders and Bret (1997) and Mahmoud (2004).

Since Spintor has a strong impact on the different stages of the target insect pest *S. littoralis* spares most beneficial insects, caused malformation of pupae and moths, decreased numbers and hatchability of deposited eggs, it may be prevent building up of high populations of the cotton leaf worm in the treated fields year after year.

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نواتج التمثيل الغذائى لتنمية الميكروب ساكاروبوليسبورا اسبينوزا كمبيد حشري لمكافحة دودة ورق القطن سبودوبترا ليتورالز

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ينتمى سبنتور ٢٤ sc لمجموعة جديدة من المبيدات الحشرية والتي يتم الحصول عليها من مصادر طبيعية، وهو عبارة عن معلق مركز يحتوى على خليط من اثنين من نواتج التمثيل الغذائي المتوفرة بصورة طبيعية نتيجة لتنمية الميكروب ساكاروبوليسبورا اسبينوزا وهما سبيتوزين A, D.
يتميز سبنتور٢٤ sc بأنه له تأثير واسع على عدد كبير من آفات الخضر ومحاصيل الحقل ودون أن يسبب ضرراً للحشرات النافعة.

أثبتت نتائج التجارب المعملية والحقلية المتحصل عليها ، أن عند استخدامه رشاً بمعدل ٥٠سم٣/فدان أو بما يعادل ذلك بالمعمل ٠,١٢٥ ملليلتر/لتر. تحت الظروف المعملية بلغت نسبة موت يرقات العمر الثاني المعاملة ٨٤٪ وبالعمر اليرقى الرابع ٧١,٥٪ . كما تشوهت العذارى والفراشات الناتجة مما تبقى من اليرقات المعاملة حيث بلغت النسبة المئوية لتشوه العذارى والفراشات الناشئة من يرقات العمر الثاني المعاملة ٣٨,٥٪ و ٤٣,٨٪ على التوالي وتلك التي تم الحصول عليها من يرقات العمر الرابع المعاملة ٢٤٪ و ١٥,٨ ٪ على التوالي.

انخفضت نسبة الفقس التي تضعه الفراشات والتي تعرضت في طورها اليرقى الثاني لفعل الـ Spintor إلى ٥٠,١ ٪ بالمقارنة إلى ٩٣,٨٪ في الغير معاملة. كما أن الفراشات تضع عددا من البيض أقل من مثيلاتها غير المعاملة. حيث تراوحت أعداد البيض بين ٢٢ و ٦٢ بيضة/لطعة بمتوسط ٤٣,٩ بيضة/لطعة بينما بلغت في لطع الفراشات في تجربة المقارنة ٧٠,٢ بيضة/لطعة في المتوسط.

تمت متابعة فعالية المستحضر سبنتور ضد الأطوار المختلفة لدودة ورق القطن تحت الظروف الحقلية ولمدة أسبوع ويومياً عقب الرش. حيث بلغت النسبة الكلية للموت بيرقات العمر الثاني إلى ٨٠٪ عند تغذية اليرقات على النباتات المعاملة بعد الرش مباشرة و أنخفضت هذه النسبة إلى ٣٣,٣٪ في اليوم السابع بعد الرش . وحدث نفس الانخفاض التدريجي في تأثيره على تكون العذاربو تشوهها و كذلك خروج وتشوه الفراشات وفقس البيض الذي وضعته.

أوضحت النتائج المتحصل عليها من التجارب المعملية والحقلية أن سبنتور يمكن أن يمنع تكون تعداد عالي من دودة ورق القطن فى حقول القطن المعاملة عاما بعد عام نظراً لأنه لا يضر بالحشرات النافعة ولإحداثه نسبة موت عالية باليرقات، وتشوه العذارى والفراشات وانخفاض بنسبة فقس البيض.

Table (1): Mortalities of 2nd 4th *S. littoralis* larvae fed on cotton leaves treated with Spintor, numbers of resulted pupae and moths, percentages of deformed pupae and moths, and hatchability of deposited eggs. (No. = 200 larvae/treatment)

Treatment	Larval stage	% of mortality after … days				No. of resulted pupae	No. of malformed pupae	No. of emerged moths	No. of malformed moths	% of end mortality	Hatchability of deposited eggs		
		2	4	6	8						No. of examined eggs	No. of hatched eggs	% of hatched eggs
0.125 ml Spintor/L	L_2	60	64	68	71	52	38.5	32	43.8	84	439	220	50.1
Untreated	L_2	0	2	4	5	190	2.1	186	4.3	7	702	668	95.2
0.125 ml Spintor/L	L_4	11.5	26	45	57.5	75	24	57	15.8	71.5	569	442	77.6
Untreated	L_4	0	2	3	4	192	1	190	1	5	632	594	93.8

Table (2): Impact of Spintor on 2nd instar larvae of *S. littoralis* fed on treated cotton leaves sampled on successive days after spraying in the field and its latent effects on pupation, moth emergence, and hatchability of deposited eggs. (No. = 100 larvae/treatment/day)

Treatment	Days after spraying	% of mortality after … days				No. of Resulted Pupae	No. of emerged moths	% of malformed moths	% of endmortality	Hatchability of deposited eggs		
		2	4	6	8					No. of examined eggs	No. of hatched eggs	% of hatched eggs
Spintor 50 ml/fed	0	39	46.9	55.2	64.2	26	18	22.2	80	177	86	48.5
	1	26	33.7	42.7	53.7	34	21	28.5	76.7	260	176	67.7
	2	23	38.8	50.0	61.1	26	28	25	68.9	246	190	77.2
	3	18	22.4	31.3	41.1	52	35	14.2	61.1	286	242	84.6
	4	17	29.6	34.4	47.4	50	43	11.6	52.2	334	279	83.5
	5	15	17.3	25.0	33.7	54	48	8.3	46.7	330	286	86.6
	6	17	21.4	34.4	37.9	57	50	4	44.4	290	266	91.7
	7	12	16.3	17.7	22.1	65	60	3.3	33.3	338	318	94.0
Untreated		0	2	4	5	93	90	0	10	316	304	96.2

Table (3): Impact of Spintor on 4nd instar larvae of *S. littoralis* fed on treated cotton leaves sampled on successive days after spraying in the field and its latent effects on pupation, moth emergence, and hatchability of deposited eggs. (No. = 100 larvae/treatment/day)

Treatment	Days after spraying	% of mortality after … days				No. of resulted pupae	No. of emerged moths	% of malformed moths	% of endmortality	Hatchability of deposited eggs		
		2	4	6	8					No. of examined eggs	No. of hatched eggs	% of hatched eggs
Spintor 50 ml/fed	0	13	33	44	56	36	36	11.1	64	630	442	70.2
	1	13	24	45	57	42	41	4	59	882	695	78.8
	2	17	22	23	40	51	50	4	50	945	696	73.5
	3	10	19	23	38	54	52	3.8	48	1137	884	77.7
	4	10	21	21	34	54	52	3.8	48	1140	948	83.1
	5	3	16	20	30	67	65	0	35	-	-	-
	6	0	0	4	15	73	73	0	27	660	596	90.3
	7	0	3	4	9	85	85	0	15	702	669	95.2
Untreated		1	2	3	3	97	97	0	3	739	705	95.5