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OVICIDAL ACTIVITY AND LATENT EFFECTS OF LUFENURON AND SPINOSAD ON THE COTTON LEAFWORM, *Spodoptera littoralis*

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ABSTRACT

The ovicidal activity and latent toxicicity of certain actinomycetes namely Spinosad and chitin synthesis inhibitor Lufenuron were studied in the laboratory against the cotton leafwrom *Spodoptera littoralis*. The summarized results show that both toxicants possess a moderate ovicidal activity and toxicicty to 2nd and 4th instars as a result to direct treatment. On the other hand both toxicants induced drastic effect on fecundity and fertility of adult moths.Also caused sever histological aberration of the ovarioles and reduced the total lipid, protein and carbohydrate of the ovaries of females resulted from treatment of pretreated as 4th instar larvae with the two toxicants.

INTRODUCTION

Since the egg-masses of the cotton leafworm, *Spodoptera littoralis* (Boisd.) represented one of the most important measurements of its infestation levels (the higher the number of egg-masses, the heavier infestation levels and vice versa). So, it is becoming increasingly important to find and support a way that reduce the egg-mass numbers in cotton fields either by hand picking collection (which are commonly used in Egypt for a long time ago), or by using natural and/or synthetic compounds that act as ovicides and exhibited inhibition of egg hatchability. This situation is confirming the necessity to find an alternative method to reduce the egg-mass numbers in cotton fields through foliar application. The ovicidal action of different pesticides against *S. littoralis* has been investigated during the four decades by several authors; i.e El-Guindy *et al.* ., 1983, Ascher and Nemny *et al.*., 1990, Moawad *et al.*. 1996, Charmillot *et al.*.,2001 and El-Sweerky,2002 etc. Most of these studies covered the direct ovicidal action on egg-masses. However, the using of such agents (as ovicides) against egg-masses of *S. littoralis* is still limited and needs more studies to clarify their role as a component of its IPM programme. On the other hand, several investigations had been carried out in attempts to disclose the effects of the benzoylphenylureas, that are looked as a group of promising insecticides, known as insect growth inhibitors (IGI) which interfere with the formation of the new cuticle (Ishaaya and Casida, 1974), or inhibit ecdysone metabolism as reported by Yu and Terrier (1975).Since; the ovi –larvicidal compounds are very rare , trails are undertaken to test the ovi-larvicidal activity of the new natural product "Spinosad" on the cotton leafworm.

MATERIALS AND METHODS

1- Insect Rearing Technique: Egg masses of the cotton leafworm, *Spodoptera littoralis* were obtained from Plant Protection Research Institute without any insecticidal pressure. Newly hatched larvae were transferred to clean glass jars covered with muslin held in position with rubber bands. They were fed on castor bean leaves, *Ricinus communis,* L. at 27 ± 2 ^{O}C and 65 ± 5% RH and examined daily. As larvae reached the 2ndand 4th instars, they were used in the experiments described below, El-defrawi *et al.* (1964).

2-Tested insecticides: two untraditional compounds ,the first was a new one named Spinosad ,formulated as Spinitor 24 % SC.Spinosad was obtained from Dow Agroscience Co. The product is admixture of two active components, spinosyn A and D which produced by fermentation of the soil actinomycetes,*Sacharopolyspora spinosa* .The second compound was a compound belonging to chitin synthesis inhibitor ,named Lufenuron formulated as Match 5% EC obtained from Syngenta Agro S.A.E. to be use for comparison.

3-Bioassay tests: The dipping technique was applied to evaluate the ovicidal action of Spinosad and Lufenuron. Different ages of *S. littoralis* egg-masses (0-24;24-48 and 48 -72 hrs old)which deposited on *Nerium oleander* leaves were immersed in different water dilution of the tested compounds for 5 seconds .The tested concentrations were (50,25, 12.5 6.125 and3.62ppm for Spinosad and 10,5,2.5.1.25and0.65ppm for Lufenuron. Five replicates of 5 egg masses /each concentration. The same number of egg-masses were dipped in plain water to be used as untreated check .Treated egg-masses were left to natural dry, placed in petri-dishes and incubated at 27 ± 2 C^{o} and 70 ± 5 % RH.Unhatchability was recorded daily until 3days after the time needed for untreated egg-masses to hatch ,and the data obtained was corrected according to daily inspection for all concentrations.

4-Relative susceptibility of 2nd and 4th instars of *S. littoralis* to tested insecticides :- From the maintained insect culture, the 2nd and 4th instar larvae were obtained. Both instars were allowed to feed on castor oil leaves treated with different concentrations of the tested compounds. The percentages of mortality in untreated and treated larvae were recorded and calculated per each concentration,corrected using Abbott's formula (1925) if necessary. The corrected percentage mortality of the compound was statistically computed according to Finney (1971) to determine LC_{50} , LC_{90} and slope values of the tested compounds.

5- The latent effect on adult:- The pupae resulted from fourth instars treated with $LC_{50}$of both toxicants were sexed and then placed in pairs in the glass globes in one of the following combinations : treated male x treated female, treated male x untreated female and treated female x untreated male, in addition to untreated male x untreated female as control ,in these cases the adults were confined in a glass jar containing a cotton pad soaked with 20% sugar solution as a food and stripes of filter paper as an ovipositional

substrate. The eggs deposited per each mated were collected daily and counted to give an estimate of fecundity the deterrent index was calculated according to lundgren (1975)as follows:

deterrent index ={(A- B) / (A+B)}x 100

where A : the total number of eggs per female in control .B : the total number of eggs per female in treatment.

The percentage of egg hatch or fertility was determined and subsequently ,the percentage of sterility was calculated according to Crystal(1968)as follows:

sterility = (1-fh)x100, where "f" is the corrected decimal fractions of the percentage of fecundity."h" is the corrected decimal fractions of the percentage of fertility.

The corrected percentage of either fecundity or fertility was then calculated as (A/ B)x100,where A and B are the treatment and control effect of either fecundity or fertility, respectively.

6-Histopathologicl studies of the ovaries:-The surviving virgin treated and untreated females were dissected in ringer's solution on the first day of emergence. The ovaries were fixed in carnoy's solution, embedded in paraffin wax, and stained with heamatoxylin and aeosin .

7-Biochemical effects on the females ovaries:- the effect on the ovaries of virgin females obtained from larval treatment with LC_{50}values of Spinosad and Lufenuron was studied.The total protein , carbohydrate and lipid of the ovaries of both treated and normal females were determined according to lowery *et al.*.(1951), Singh and Sinha (1977), Baronos and Blackstck (1973),respectively.

RESULTS AND DISCUSSION

The effect of Spinosad and Lufenuron on egg hatchability:- Data presented in table (1) show that the three day old eggs are more affected than that of one or two days old in case of Spinosad while the reverse was in case of Lufenuron where the younger treated eggs the induced a higher unhatchability percentage.Result may be attributed to that Spinosad affected the newly hatched larvae which feed on the treated chorine of the eggs in case of older eggs Accordingly insecticidal activity is more potent through ingestion than direct contact with egg treatment with Spinosad . It is of interest to note that after treating eggs of different ages with Lufenuron, normal development of the embryo took place and the failure of the egg hatch could be explained by the known mode of action of the chitin synthesis inhibitors, where the chitin synthesis was blocked and the larvae probably cannot use its muscles to free itself from the egg wall (Watson ,*et al.* 1986).

Table (1) Inhibition of egg hatching by Spinosad and Lufenuron to *S.littoralis* eggs using dipping technique.

Compound	concentration	% of unhatching eggs		
		0-24 hrs old eggs	24-48 hrs old eggs	48- 72 hrs old eggs
Spinosad	100	3	25	68
	50	2	16	56.6
	25	2.2	9.5	48
	12.5	0	8	38
	6.125	0	6.5	22
Lufenuron	10	8.6	0	0
	5	48	42	33
	2.5	38	35	29
	1.25	33	26	22
	0.65	25	20	16
	0.325	19	15	12
control		0	0	0

Susceptibility of different larval instars :-

Data in table (2) shows the susceptibility of the 2nd and 4th instar *S. littoralis* larvae towards the tested insecticides. Based on LC_{50} lufenuron was more toxic than spinosad to both 2nd and 4th instar *S. littoralis* larvae . The LC_{50} values were 3.956 and 0.012 ppm, for 2nd instars of Spinosad and Lufenuron respectively , while the LC_{50} values were 7.61 and 0.303 ppm, for 4th instars of Spinosad and Lufenuron respectively. Wanner *et al.* .,2000 reported that paralysis was the primary effect of spinosad, with mortality as a secondary result .This explanation was reported by(Adan *et al.*., 1996;Scott, 1998; Wanner *et al.* .,2000).The bioefficiency of the Lufenuron act as chitin synthesis inhibitors and cause a slow detoxification in the insect body, as reported by others Rao *et al..,* (1994); Shaurub *et al.*., (1999); Retnakaran, *et al.*., (1985) and Abdel-Al (2003).

Table(2): Susceptibility of *Spodoptera littoralis* 4th instars to Spinosad and Lufenuron.

Insecticides	LC_{50}		95 % fiducially limits		Slope	LC_{90}
			upper	lower		
spinosad	2nd	3.956	7.087	0.889	0.93	94.573
	4th	7.61	10.52	4.99	1.925	50.039
Lufenuron	2nd	0.012	0.0067	0.017	1.46	0.088
	4th	0.303	0.534	0.176	1.09	0.258

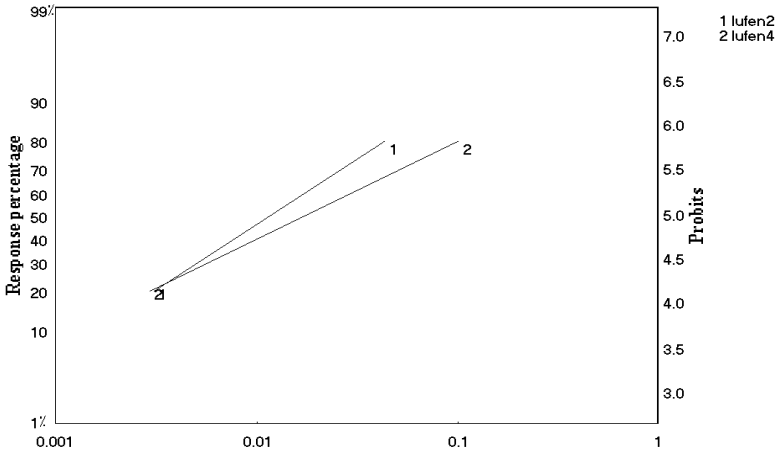

Fig. (1): Toxicity regression lines post 48-h of feeding 2nd and 4th instars larvae of *S. littoralis* on castor oil leaves treated with lufenuron.

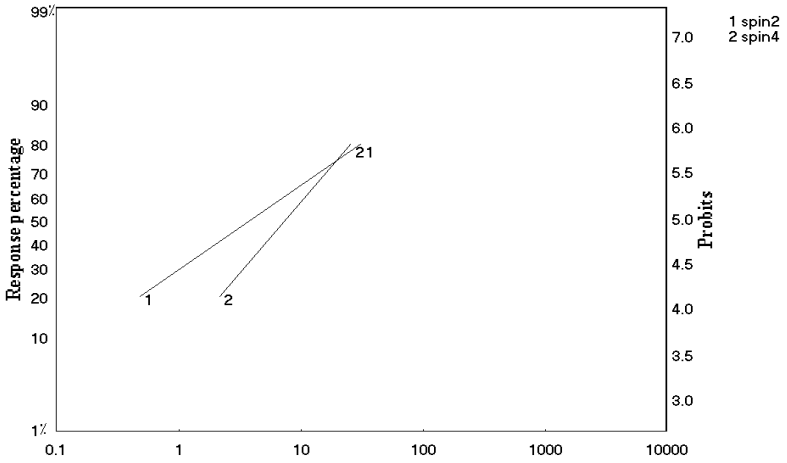

Fig. (2): Toxicity regression lines post 48-h of feeding 2nd and4th instars larvae of *S.Littoralis*.

The latent effect on adult fecundity and fertility:-

Table (3) shows latent effect on the fecundity of *S. littoralis* female moths surviving treatment of 4th instars with spinosad, lufenoron. The results obtained show that the lowest number of eggs laid per female and consequently the highest deterrent index were obtained for treated females mated with treated males, followed by treated females mated with normal males, as compared with control. On the other hand, the highest number of eggs laid per female and consequently the lowest deterrent index were

occurred when the males were treated only. This may indicate that the females were more sensitive to toxicants than the males. In all mating combinations, lufenuron was the most effective in reduction of both fecundity and fertility than spinosad.

Histological Effects : The normal female of *S. littoralis* have well developed ovaries with "8" polytrophic ovarioles . Each ovariole consists of a chain of devolping ova. The histological deformities to the ovarioles as the result of treatments are recorded in Fig3 (3 B & C).

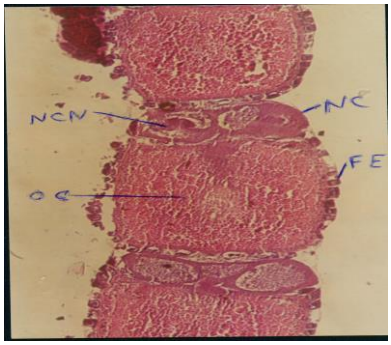

Fig 3 (A)): L.S through normal female ovarioles of S*. littoralis* NCN: nucleus of nurs cell .NC:nurs cell . FE:follicular epithillum .OC: oocyt

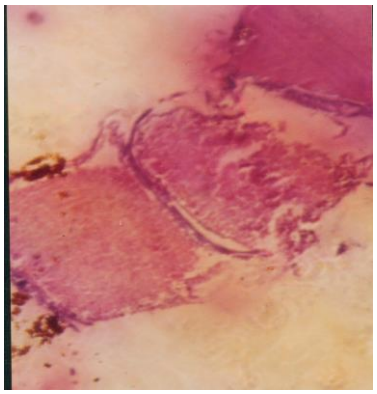

Fig 3 (B): L.S through female ovarioles of S*. littoralis* following feeding of 4th instars larvae for 48 hours on castor oil leaves treated with lufenuron

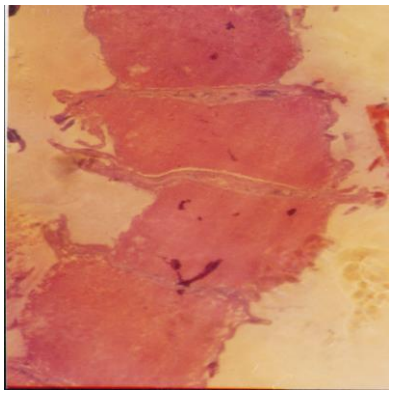

Fig 3 (C)): L.S through female ovarioles of S*. littoralis* following feeding of 4th instars larvae for 48 hours on castor oil leaves treated with Spinosad

T3

Absence of follicular epithelium for both treatment the egg follicle had masses of cells so mixed that it was very difficult to differentiate between the nurse cells and oocyte .The follicular epithelium cells had syncytial form (Fig .3 –A). Comparatively, Lufenuron (3B) caused histological damages more than those caused by spinosad (3C) and the follicular epithelium was greatly damaged in many parts particularly around the oocyte compared to control Fig (3A) ,. Shaurub *et al.* (1999)reported completely damage for A.ipsilon female ovariolar cells when treated as fourth instar with chlorofluazuron.

Biochemical Effects : From data in Table (4) ,it is clear that both treatments decreased the biothynthesis of total protein , carbohydrate and lipid contents of the overioles of *S. littoralis* females as compared wih normal females This reduction was more obvious in case of treatment with Lufenuron than in case of treatment with Spinosad . Decreased total ovarian protien of *S. littoralis* following treatment is similar to the the results of Soltani and Mazouni(1992) they found that diflubenzuron reduced the total protein per ovary in *Cydia Pomonella* Also the same result recorded by Shurab *et al.* (1999) they found that chlorofluazuron and flufenoxuron reduced the total (protein , carbohydrate and lipid) in ovary in *A.ipsilon* female .The decreaseed ovarian protein obtained in this study may be due to decreased larval haemolymph , protein as a result of treatment .This suggestion is confirmed by the data of many workers that larval haemlymph protein contributed in developing ova in lepidoptera (Raja *et al.* ., 1986 and kong and kim, (1988).Decreased in total carbohydrate in the ovariole of *S. littoralis* treated with Lufenuron and Spinosad may be accounted for the histological damages of both the oocyte and follicular epithelium caused by these two compounds as shown in this study.Decreased in total lipid in the overioles of *S. littoralis* treated with Lufenuron and Spinosad may be interperted by the damage of both the nurse cells and follicular epithelium as shown in this study ,where these tissues were found to contributed in lipid deposition to the devolping oocyte (Tiripathi and kumar ,1982)Moreover, it is probably that these compounds affected the fat bodies of *S. littoralis* during the period following larval treatment with them which ultimately led to decreased lipid deposition in the devolping oocyte , .In general ,the devolpment of oocytes in most insects is under hormonal control and involves the neurosecretory cells and corpora allata.

Table 4):Effect of Spinosad and Lufenuron on Total protein, Carbohydrat and lipid contents in the ovarioles of *S. littoralis* post 48-h of feeding 4th instar larvae.

Compound	LC_{50}	Total protein (mg/g) fresh tissues±S.E.	Total carbohydrat (mg/g)fresh tissues±S.E.	Total lipid (mg/g) fresh tissues±S.E.
Spinosad	7.61	28.6*±.8	40.4***±0.9	82.3***±0.7
Lufenuron	0.303	22.6**±0 .5	56.3***±0.6	65.2***±1.3
control	0.0	31.7±o.2	86.9±1.2	95.3±0.9

*** Significant at p< 0.05.** ****: Highly significant at P< 0.01.**
***** : Very highly significant at P< 0.001.**

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دراسة تأثير النشاط الإبادي للبيض لكلاً من المركب الحيوي (سبينوساد) ومركب مثبط للكيتين (الماتش) على دودة ورق القطن سبودوبترا ليتوراليس

عزيزة عبد العال و إدريس سالم عبد الوهاب

معهد بحوث أمراض النباتات- مركز البحوث الزراعية- وزارة الزراعة – الجيزة – مصر

تم دراسة تأثير النشاط الإبادي للبيض لكلاً من المركب الحيوي (سبينوساد) ومركب مثبط للكيتين (الماتش) على دودة ورق القطن سبودوبترا ليتوراليس وقد أوضحت النتائج أن كلا المركبين لهما تأثير إبادي متوسط علي البيض ولهما أيضاً تأثير سمى على كلا العمريين الثانى والرابع كنتيجه للمعامله المباشرة وعلى الجانب الآخر كلا المبيدين أثر على كفاءة وضع البيض للأنثى وخصوبتها وقد كان لهما أيضاً تأثيرات هستولوجيه بالغه في مبيض الأنثى كما أثرة على المحتوى الكلى للبروتين والكربوهيدرات والدهون لهذا المبيض وقد كانت هذه النتائج كتأثيرات متأخره نتيجة معاملة العمر اليرقي الرابع بهذين المركبين.

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Table(3): Effect of LC_{50} of Spinosad and lufenuron on fecundity of *S littoralis* larvae reated as 4th instar.

compounds	T♂X N♀				T♀X N♂				T♀X T♂	
	fecundity		fertility		fecundity		fertility		fecundity	
	No. of eggs ±S.E.	Deterrent index	Egg hatch %	Sterility %	No. of eggs ±S.E.	Deterrent index	Egg hatch %	Sterility %	No. of eggs ±S.E.	Deterrent index
Spinosad	784.7*** ±48.5	31	76.3	59.2	627*** ±47.9	22.9	62.1	61	578.7.7** ±5.7	7.6
lufenuron	181*** ±2.6	84.46	25.0	97.9	172*** ±3.6	85.17	20.0	98.4	163*** ± 3.5	85.89
control	1476±71		98							

****: Highly significant at P< 0.01 ***: Very highly significant at P< 0.001.**

5035 5036 5037 5038 5039 5040 5041 5042 5043 5044 5045 5046 5047
5035 5036 5037 5038 5039 5040 5041 5042 5043 5044 5045 5046 5047

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