EFFECTS OF CERTAIN INSECTICIDES ON EGGS OF *SPODOPTERA LITTORALIS*

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Abstract

Seven chemical insecticides were tested for their ovicidal activity against *Spodoptera littoralis* eggs, using two methods, namely dipping of tafla leaves containing 0-1 day old eggs and o-day old eggs were deposited on insecticide residues on tafla leaves. Rynaxypyr was the most promising. It was taken as the standard insecticide and given an arbitrary index value of 100 units, when 0-1 day old eggs were treated directly by dipping in insecticide solution. As for the indirect ovicidal activity, emamectin benzoate came first and was given arbitrary index value of 100 units. On the other hand spinetoram, in comparison showed the least indirect ovicidal effect.

Regarding the inhibitory action, it was obvious that rynaxypyr and indoxacarb exhibited high inhibitory activity (100%) in suppressing the number of deposited eggs when moths were subjected to 100 ppm and it was followed closely by pyridalyl, emamectin benzoate and spinetoram being 97.8, 97.0 and 95.3%, respectively. However, all insecticides showed variable degrees of inhibition at the least concentration tested (0.1 ppm), except spinosad and spinetoram having no inhibition activity for deposited eggs.

Key words: *Spodoptera littoralis*, ovicidal activity, egg-laying inhibition, novel insecticides.

INTRODUCTION

The cotton leafworm *Spodoptera littoralis* (Boisd.) is considered the most serious and destructive cotton pests and other agronomic as well as vegetable crops in Egypt (Magd El-Din & El-Gengaihi, 2000). It is also proved to be an important pest of cotton in Africa, Middle East and Southern Europe.

Pest management programs for combating such pest up till year 2000 was directed mainly for hand picking egg-masses early in the season by children and to implement the application of insecticides purposely for their larvicidal activities laterly.

However, due to the recent universal announcement of childhood rights, using of children in collecting egg-masses is forbidden and restricted by law. Therefore, it was felt necessary find out newly developed compounds using as ovicidal action in addition to their well known larvicidal activity seemed to have some potential as a solution to the lack of labors collecting *S. littoralis* egg-masses.

Identification of pesticides possessing ovicidal activity, belonging, to organophosphorus, carbamate and synthetic pyrethroids had received extensive attention either against *S. littoralis* (Mitri and Kamel, 1970, Ascher and Nemny, 1974, Abo Elghar *et al.,* 1976, Abo-Elghar *et al.,* 1980, El-Guindy *et al.,* 1976, Radwan *et al.,* 1985)., and other investigators working against other insect pests (Abd El-Megeed *et al.,* 1987, Joginder *et al.,* 1993, Charmillot *et al.,* 2001, Baiteau and Noronha, 2007, Charmillot *et al.,* 2007, El-Barkey *et al.,* 2009, El-Saeed *et al.,* 2009).

There are few studies regarding the ovicidal effect of novel insecticides on eggs of lepidopteran pests and most reports have been focused on the control of larval stage, therefore little information is available about the effect of novel groups of insecticides on eggs of *S. littoralis*, particularly those of low risk.

Therefore, it was important to test the effectiveness of these novel insecticides, either registered those under investigation, at different concentrations against egg masses and moths of *S. littoralis* laboratory strain.

MATERIALS AND METHODS

Test insect

The test insect, cotton leafworm *Spodoptera littoralis* (Bosid.) was reared in the laboratory at 25$\pm$ 2 °C and 60 $\pm$ 5% R.H. according to El-Defrawi *et al.,* (1964) technique, feeding on castor bean *Ricinus communis* (L.) leaves . Egg stage (0-1 day old) and newly emerged adult stage were used in the present study for estimating the direct and indirect ovicidal activity of seven novel insecticides.

Insecticides tested

The seven novel insecticides tested includes: pyridalyl (5-1812, 50% EC) Sumitomo Chemical Co., Ltd, rynaxypyr (Coragen 20% SC) Du Pont, indoxacarb (Avaunt 15% SC) Du Pont, emamectin benzoate (Radical 1.9% EC) Agromen Chemical Co. Ltd, spinosad (Tracer 24% SC) Dow Agro Science, methoxyfenozide (Runner 24% SL) Rohm and Haas Co., spinetoram (Radiant 12% SC) Dow Agro Science.

A stock solution (W/V) of each tested formulated insecticide was freshly prepared as aqueous solution and diluted serially by water to obtain seven progressive concentrations ranging from 100 to 0.1 ppm.

Ovicidal experiments

a- Direct ovicidal effectiveness (curative)

Egg-masses of uniform age (0-1 day old) were obtained by copulating females and males together in big glass jar 2 kg equiped with tafla branches as ovipositional site and cotton pad soaked in 10% sugar solution for feeding.

Newly deposited egg-masses of uniform age (0-1 day old) were obtained after starting oviposition in the rearing colony. The upper layers of egg-masses were removed gently under the binocular in order to count the number of eggs in the remaining lower layer, which was divided into patches, each contained about100 eggs.

In all cases, three replicates each of 5 egg-masses were used for each concentration. The leaves of tafla with eggs (0-1 day old) were immersed for 5 seconds in each concentration. The treated egg-masses were allowed to dry and then placed in 15 cm diam. petri-dishes (5 egg- mass/dishes) and held at 25 $\pm$ 2 °C for hatching. Similar number of water-treated glass jars provided with water-treated tafla leaves were prepared to provide 0-1 day old egg-masses used as a control. Daily inspection was made till 2 days after the water treated eggs of check hatch.

Once all eggs in control experiment had hatched out, the eggs in treatments were observed under binocular and the rate of hatching were recorded.

b- Indirect ovicidal effectiveness (preventive)

In the laboratory, branches of tafla, *Nerium oleander* were used as site for eggs deposition, they were dipped in aqueous solution of the tested insecticides at different concentrations and then left to dry under laboratory conditions. Also 1 Lb. glass jars were internally coated with the same concentrations. Once both tafla branches and treated glass jars dry, three replicates, each of 2 males and 3 females moths were allowed to mate in each pre-coated jar, provided with treated tafla as a site for eggs laying. The egg-masses were collected daily till death of moths and transferred to clean Petri-dish.

c- Calculations and statistical analysis

Daily inspection for petri dishes was carried out till 2 days after the water-treated check hatch. Unhatchability percentages were recorded for each tested conc. /insecticide. Unhatched percentages of eggs representing eggs mortalities were corrected according to natural mortality in check treatment using Abbott's(1925) formula and the corrected unhatchability percentages were subjected to statistical analysis according to Finney (1971) and consequently LC_{50} values, were computed and used for calculating the toxicity index (Sun, 1950) which was used for comparing the relative toxicity of insecticides used.

d- Inhibitory activity for egg-laying

For studying the inhibitory activity for each insecticide, the total number of eggs deposited in each concentration/ insecticide were calculated and compared as relative to untreated check to obtain the percent of reduction in number than check (inhibitory action).

RESULTS AND DISCUSSION

1- The curative ovicidal activity

The curative ovicidal action of seven insecticides belonging to novel groups against 0-24 hrs old eggs of the laboratory strain of *Spodoptera. littoralis* is represented in Table (1). According to toxicity index values calculated on the basis of LC_{50} values obtained on 0-24 hr old eggs Rynaxypyr the most effective compound was taken the standard insecticideand give the arbitrary index value of 100 followed by relatively lower toxicity index value (13.52%) for indoxacarb and then methoxyfenozid (5.31%). By comparison, the other four insecticides produced extremely low activity recording toxicity index values of 1.21, 1.0, 0.84 and 0.36% for spinosad, emamectin benzoate, spinetoram and pyridalyl, respectively as the effectiveness of rynaxypyr against the 1-day old eggs deposited by *S.littoralis*. Furthermore, the relative potency level can be used as a convenient method in comparing the degree of toxicity of the different compounds in this study. The potency levels of the tested insecticides are expressed as number of folds, at the required toxicity level, compared with the least efficient toxicant included in the evaluation in the study.

Concerning the relative potency levels based on the LC_{50} values as represented in Table (1), the ovicidal activity values of Rynaxypyr, indoxacarb, emamectin benzoate, spinosad, methoxyfenozid and spinetoram were 271.4, 36.71, 2.74, 3.29, 14.42 and 2.28 times as toxic as the ovicidal action of pyridalyl, respectively against the egg masses deposited by *S. littoralis*.

Table 1. Direct (curative) ovicidal effectiveness of seven novel insecticides applied by dipping technique on 0-24 hr old egg-masses of *S. littoralis* (Bosid.).

Insecticide	Conc. tested range(ppm)	Slope ± SE	LC_{50} ppm	95% CL	Toxicity index	Relative Potency
Pyridalyl	0.1-100	0.763±0.08	7.60	3.94-15.52	0.36	1
Rynaxypyr	0.1-100	0.316±0.05	0.028	0.001-0.127	100	271.4
Indoxacarb	0.1-100	0.767±0.11	0.207	0.041-0.530	13.52	36.71
Emamectin b.	0.1-100	0.990±0.18	2.776	0.903-7.925	1.00	2.74
Spinosad	0.1-100	0.851±0.14	2.310	0.602-6.493	1.21	3.29
Methoxyfenozid	0.1-100	0.626±0.05	0.527	0.269-0.895	5.31	14.42
Spinetoram	0.1-100	0.581±0.05	3.335	1.793-5.942	0.84	2.28

CL: confidential limit

Sun's toxicity index= LC_{50} or LC_{90} of the most toxic compound/ LC_{50} or LC_{90} of the tested compound x 100

Relative Potency = LC_{50} of the least toxic compound/ LC_{50} of the tested compound

The results are accordance with those published Bassi *et al.,* (2009) they found that rynaxypyr (Coragen) exhibited strong ovicidal and larvicidal activity. However, the most promising findings were obtained when Coragen (rynaxypyr) was applied before egg-hatch, during embryonic stage of codling moth. The ovicidal timing (egg-laying to black-head stage) provides the highest overall performance against codling moth. This is explained by the long lasting biological availability of the molecule, the partial ovicidal effects, the potent ovicidal effects and the strong larvicidal activity on codling moth neonates, either by contact or ingestion.

In this field of study Mahmoud *et al.,* (2011) found that all eggs of *Plutella xylostella* were killed by dose of 1000 mg/L of pyridalyl, spinosad and hexaflumuron and the mortality of eggs was enhanced as the concentrations increased.

As for spinosad compounds the obtained results are going in line with those obtained by Temerak (2005) who found that spinosad (Spintor 24 SC) produced 100% initial mortality of the fresh natural egg masses of *S. littoralis* during and after hatching.Temerak (2007) reported that spinetoram (Radiant 12% SC) was 5 and 7 times stronger than spinosad for control the cotton leafworn in the field and laboratory respectively. However, the high toxicity of spinosad on lepidopterous eggs was also supported by Baiteau and Noronha (2007) findings against the European corn borer, *Ostrinia nubilalis*.

As for pyridalyl the results are inagreement with Ishayama *et al.,* (2005) who reported that the efficacy of pyridalyl at 100 mg/ml on eggs of *Orius stringicollis* was lower than 20%. Recent results of Venkateswari *et al.,* (2008) revealed that the LC_{50} values of abamectin and emamectin benzoate for ovicidal action against 1 day old egg batches of *S. littoralis* by dip method were 2.0 and 0.1 µg ml^{-1}.

2. Preventive ovicidal activity

Results of the preventive (indirect) ovicidal activity when *S. littoralis* eggs were laid directly on residues of the chosen concentrations of the tested insecticides are shown in Table (2). Toxicity index calculated on the basis of LC_{50} for each insecticide revealed that emamectin benzoate exhibited the highest preventive ovicidal action and given arbitrary value of 100 units. The effectiveness of other insecticides varied considerably, where indoxacarb, pyridalyl and methoxyfenozide exhibited moderate preventive ovicidal activity recording toxicity index of 68.85, 37.38 and 33.42%, respectively. The other three insecticides gave relatively less LC_{50} values that were spread out over a fairly wide range of concentration from 0.445 ppm for rynaxypyr to 1.299 ppm for spinetoram, resulted in toxicity index of 28.31 to 9.29%, respectively.

Table 2. Preventive (indirect) ovicidal effectiveness of seven novel insecticides for egg-masses deposited after exposure of *S. littoralis* moths to surfaces (tafla and glass) treated with the tested insecticides.

Insecticide	Conc. Tested range (ppm)	Slope ± SE	LC_{50} (ppm)	95% CL	Toxicity index	RelativePotency
Pyridalyl	0.1-100	0.669±0.18	0.337	0.002-2.072	37.38	3.85
Rynaxypyr	0.1-100	0.850±0.10	0.445	0.187-0.798	28.31	2.92
Indoxacarb	0.1-100	0.904±0.15	0.183	0.031-0.421	68.85	7.1
Emamectin b.	0.1-100	0.964±0.08	0.126	0.093-0.163	100.00	10.31
Spinosad	0.1-100	0.725±0.11	0.537	0.138-1347	23.46	2.42
Methoxyfenozid	0.1-100	1.155±0.27	0.377	0.038-1.195	33.42	3.45
Spinetoram	0.1-100	1.781±0.10	1.299	1.137-1.486	9.69	1

CL: confidential limit

As shown in Table (2), the relative potency based on the LC_{50} values, the ovicidal effectiveness values of pyridalyl, rynaxypyr , indoxacarb, emamectin benzoate, spinosad, and methoxyfenozid were 3.85, 2.92, 7.10, 10.31, 2.42 and 3.45 folds as the ovicidal efficacy of spinetoram, respectively against egg masses produced from moths and deposited on treated tafla with different concentrations of the aforementioned insecticides

On the contrary, Bassi *et al.,* (2009) indicated that Coragen demonstrated highly activity on the different leafminers species affecting the pome fruits when applied during the egg-laying before the mines are visible on leaves. Also, they indicated that the ovicidal effects are enhanced when eggs of *Cydia pomonella* are laid on treated surfaces.

Recently Amer *et al.,* (2012) found that emamectin benzoate was more effective on 1-day old eggs of *Tuta absoluta* than pyridalyl. Also, similar results was obtained when both compound were tested against 1-day old eggs *Pectinophora gossypiella* (saund.).

Data in Table (3) showed the inhibiting activity of egg-laying of the tested insecticides when eggs were laid directly on insecticidal residues, expressed as reduction percentages in the number of eggs deposited on treated surfaces of tafla plant or/and glass vials. However it is clearly evident that the most pronounced percent of reduction in eggs deposition at 100 ppm has been achieved in indoxacarb, rynaxypyr recording 100% for both and was followed closely by pyridalyl, emamectin benzoate and spinetoram, recording 97.85, 97.02 and 95.38% inhibition, respectively. In the meantime three insecticides, i.e., pyridalyl, emamectin benzoate and indoxacarb are still effective lately at the lower concentration tested (0.1 ppm) and recording relatively higher recognized inhibitory activity of 80.74, 74.71 and 72.39% reduction, respectively. In contrast, spinosad seemed to be the least active in its indirect ovicidal activity at all concentrations tested, recording 45.28-Zero% inhibition for concentrations ranged between 100 and 0.1 ppm.

Table 3. The inhibitory activity of seven novel insecticides on *Spodoptera littoralis* egg-laying after exposure of moths to treated surfaces of tafla leaves and internally coated glass jars.

Treatment	% Inhibition of deposited eggs at indicated cone. (ppm)						
	100	50	10	5	1	0.5	0.1
Pyridalyl	97.85	95.53	94.38	93.22	90.82	83.38	80.74
Rynaxypyr	100	91.32	82.32	62.52	61.62	44.18	31.26
Indoxacarb	100	90.66	84.87	82.39	82.33	75.95	72.39
Emamectin b.	97.02	92.89	91.32	90.66	88.51	78.67	74.71
Spinosad	45.28	36.36	25.45	20.33	16.85	9.91	--
Methoxyfenozid	86.14	82.94	81.34	71.22	65.18	62.87	59.85
Spinetoram	95.38	78.86	76.37	73.17	67.14	44.05	--

The results are accordance with those published by Mohamed *et al.,* (2009) who found that avermectin (methylamine-avermectin) exhibited potential effect on the fecundity of *Collosobruchus maculatus* females and resulted in reduction (inhibition) of the deposited eggs number. Also they found that hatching rate of eggs decreased dramatically as the concentration of avermectin increased.

However, our results elucidate in general that the application of the ovicides must take into consideration not only the direct effect (curative) on eggs but also the indirect (preventive) influence on eggs deposition later, specially when these insecticides were used too early at starting of egg laying period.

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دراسات لبعض المبيدات على بيض دودة ورق القطن

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تم اختبار كفاءة سبع مبيدات لتقيمها كمبيدات ضد بيض دودة ورق القطن بطريقتين احدهما مباشرة واخري غير مباشرة:

اولا الطريقة المباشرة: تم غمر بيض من عمر ٠– ٢٤ ساعة فى التركيزات المختلفة للمبيدات السبع المستخدمة.

***ثانيا* الطريقة غير المباشرة:** وذلك بمعامله سطح البرطمانات من الداخل وايضا اوراق التفله (اماكن وضع البيض) بالتركيزات المختلفة للمبيدات السبع المستخدمة (التأثير المتبقى).

وقد اسفرت النتائج عن ان المركب ريناكسيباير كان اكثر كفاءة (واعطى ١٠٠ وحدة اعتبارية) على البيض والذى تم معاملته مباشرة عن طريق الغمر ثم تبعه اندوكساكارب ثم ــ ميثوكسى فينوزايد (١٣.٥٢ و ٥.٣١% من تأثير ريناكسيباير) على التوالى.

–امابالنسبة للتأثير غير المباشر على البيض فقد سجل ايمامكتين بنزوات اعلى تأثير وقد اعطى ١٠٠ وحدة اعتبارية ثم اندوكساكارب ثم بايريداليل ثم ميثوكسى فينوزايد ليسجل دليل سمية (٦٨.٨ و٣٧.٣٨ و ٣٣.٤٢%) على التوالى مقارنة بمركب ايماميكتين بينما سبينتورام كان الاقل كفاءة ليسجل دليل سمية (٩.٦٩ %)

–امابالنسبة للتأثير المثبط على وضع البيض الناتج من وضعه على تفلة معاملة فقد كان ريناكسيباير و اندوكساكارب الاكثر تثبيطا حيث كانت نسبة تثبيط وضع وفقس البيض ١٠٠% عند تركيز ١٠٠جزء فى المليون يليه بايريداليل ثم ايمامكتين بنزوات ثم سبينتورام وكانت النسب المطابقة هى ٩٧.٨ و٩٧.٠ و ٩٥.٣ على التوالى.

وقد سجلت المبيدات المستخدمة درجات متفاوتة عند المعاملة بتركيز ٠.١ جزء فى المليون ماعدا سبينوساد وسبينتورام لم يكن لهما تأثيرا على وضع البيض.

ويمكن الاستنتاج من هذه الدراسة انه عند معاملة البيض بهذه المركبات يفضل الا يكون فقط بالرش المباشر للبيض ولكن ايضا اماكن وضع البيض وكذلك توقيت الرش فى بداية ظهور اللطع وحتى الفقس.