LABORATORY TOXICOLOGICAL STUDIES ON SEVEN REDUCED-RISK SELECTED NOVEL INSECTICIDES AGAINST *Spodoptera littoralis* LARVAE

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ABSTRACT: *Laboratory bioassays were performed to determine the efficacy of seven novel selected insecticides (Indoxacarb, Pyridalyl, Rynaxypyr, Methoxyfenozide, Emamectin benzoate, Spinosad and Spinetoram) against the 4th instar larvae of Spodoptera littoralis, to generate base line concentration and time-mortality response. The tested bioassays includes (1) leaf dip (ingestion) bioassay, (2) residue film on glass (contact) bioassay, (3) time-mortality bioassay.*

Significant variation was revealed in lethal concentration (LC_{50}) and lethal time (LT_{50}) values. Among all tested insecticides emamectin benzoate gave the lowest LC_{50} value i.e., 1.29 ppm (ingestion biossay) and was followed by pyridalyl, recording LC_{50} of 11.97 ppm, while in contact bioassay pyridalyl being the most effective, recording LC_{50} of 2.22 ppm and was followed by indoxacarb (LC_{50} = 14.05 ppm). However, methoxyfenozide and rynaxypyr exhibited the least contact toxicity, recording LC_{50} of 1259.4 and 3859.2 ppm, respectively, at 24 h post treatment. Time-mortality bioassay showed that emamectin benzoate was the most effective (faster) at ingestion, recording LT_{50} of 4.57 h and was followed by indoxacarb (37.76 h), whereas pyridalyl was the most effective as contact, recording LT_{50} of 20.71 h to kill 50% population of the 4th instar larvae.

Spinosad, however as ingestion require more time (171.58 h) whereas methoxyfenozide, rynaxypyr and spinosad as contact were the weakest (slowest) recording LT_{50} of 51.29, 46.7 and 41.72 h, respectively.

Key words: *Spodoptera littoralis , lethal ingestion and contact concentration, Lethal time, Indoxacarb, Pyridalyl, rynaxypyr, Methoxyfenozide, Emamect benzoate, Spinosad, Spinetoram.*

INTRODUCTION

The cotton leafworm, *Spodoptera littoralis* (Boisd.) is one of the most destructive polyphagous insect pests in Egypt. It is serious pest of cotton *Gossypium hirsutum* (L.), *Zea mays* (L.) and various field crops and vegetable plants (Willcocks, 1937). The cotton leafworm larvae feed on vegetative as well as reproductive structures in these crops.

Owing to its polyvoltine characteristics and serious overlap of generations it was easy for the cotton leafworm to develop resistance to various kinds of insecticides (El-Guindy, *et al.*, 1982, Keddis, *et al.*, 1988, El-Guindy *et al.*, 1989; El-Bermawy *et al.*, 1991-92; Rashwan *et al.*, 1991-92; Temerak, 2002 and Ghoneim, 2002). Insecticide resistance in key insect pests like cotton leafworm become a significant problem in crop production due to extensive use of synthetic chemical insectirides, where chemical control remain the most practical way to reduce cotton leafworm population.

Recently pest management strategies have evolved over the years from broad-spectrum to target specific narrow-spectrum pesticides (Retnakaran *et al.*, 2003). Though, there has been a continuing need for investigating new compounds particularly those that act on novel biochemical pathways, due to the propensity of target pest population to develop resistance.

However, surveying insect population for changing in susceptibility to insecticides is an integral component of insecticide resistance management.

The development of dose-mortality responses to insecticides is necessary to

provide baseline data for future resistance monitoring efforts for pests (Cook *et al.*, 2004). In addition several of these new insecticides have bean developed in recent years and exhibit activity against Lepidopteran pests. In most instances the most appropriate time in the life of an insecticide to establish base-line reponses is prior to the wide spread use of these products in crops.

Many of there compounds exhibit novel modes of action to which the insect has not yet been exposed. One such group of the tested insecticides is the diamides class which include rynaxypyr (chlorantraniliprole, coragen). These molecule featuring a new mode of action and described as ryanodine receptor modulator by activating the insect ryanodine receptors (R_yR_s). It stimulate the release and depletion of interacellar calcium stores from the sarcoplasmic reticulum of muscle cells, causing impaired muscle regulation, paralysis and ultimately death of sensitive species (Cordova, *et al.* 2006).

Spinosad is an older member in the new chemical class known as spinosyns. (Naturalyte) that has two unique mode of action, acting primarily on the insect nervous system at the nicotine acetylcholine receptor and exhibiting activity at the GABA receptor (Sparkes *et al.*, 1995). Spinetoram (Crouse and Sparks, 1998) is new generation of spinosyn group. It causes excitation of the insect nervous system by altering the function of nicotin acetylcheline receptors and GABA-gated ion channels.

Indoxacarb represents another new class of insecticides (the oxidiazines), it blocks the movement of sodium ions into certain nerve cell ion channels, resulting in paralysis and death.

The fifth investigated insecticide is emamectin benzoate (methylamine avermectin) which represent a second generation of abamectin in avermectin family which acts as nerve poisons, stimulate the gama-aminobutyric acid (GABA) system, a chemical transmitter produced at nerve endings (Fritz *et al.*, 1979), and block the post-synaptic potential of neuromuscular junction, leading to paralysis and death.

The six tested compound is methoxyfenozide which belongs to dibenzoyhydrazine, developed as non-steroidal agonist of the insect moulting hormone (20 E) and acts via binding to the ECR/USP (ecdyson receptor protein/ ultraspiracle protein). It kill insect larvae by induction of premature lethal moulting (Wing *et al.*, 1988). Another tested compound is Pyridalyl, its action requires cytochrome P 450 activity, possibily for production of a bioactive derivative, pyrodalyl metabolite, which results in production of reactive oxygen species (ROS), that lead to damage to cellular macromolecules (e.g., proteins) and enhanced proteasome activity leds to increased protein degeneration and necrotic cell death (Moriya *et al.*, 2008, Powell *et al.*, 2011).

Generally, it have to be considered that time-dose (or concentration) relationships to mortality are of practical and theoretical importance in study of pesticide activity. For some insect species, the primary criteria for selection of a pesticide are speed of kill and residual activity that persists for the period during which the insect pest will attack the host plant.

In theoretical studies, time trends in mortality may be useful preliminary indicators of chemical mode of action and detoxification mechanisms.

However, early establishment of susceptibility base-line for these novel compounds are critical step for successful and practical implementation of these compounds. Accordingly the objective of the present study was to generate insecticide mortality responses for cotton on leafworm larvae in laboratory through three bioassay methods, i.e., (1) insecticide treated leaves, (2) insecticide residue film on glass, (3) short time residuality.

MATERIALS AND METHODS

1. Insects :

The susceptible strain of *Spodoptera littoralis* (Boisd.) used in the present study has maintained under laboratory conditions of 27 ± 2°C and 65 ± 5% RH (El-Defrawi *et al.*, 1964), for more than 3 years without

contamination with insecticides. The strain was established in the cotton leafworm Department, Plant Protection Insitiute, Dokki, Giza.

2. Insecticides :
Seven new chemical insecticides includes:

Pyridalyl (5-1812, 50% EC), sumitomo chemical co., Ltd; Spinetoram (Radiant 12% SC), Dow Agro Sciences; Rynaxypyr (Coragen 20% SC), DuPont; Indoxacarb (Avaunt 15% SC), DuPont, Methoxyfenzide (Runner 24% SK), Rohm & Haas Co. Spinosad (Tracer 24% SC), Dow Agro Science, Emamectin Benzuate (Radical 1.9% EC), Agro men chemical Co. Ltd.

3. Laboratory bioassays

Three methods of bioassays were adopted to determine (1) the contact (tarsal contact with residus film on glass), (2) ingestion (feeding on treated plant leaves), (3) time-oriented mortality bioassay.

3.1. Leaf-dip bioassay (insecticide treated plant leaves)

stock solutions of insecticides were prepared freshly and diluted using water. At least six concentration exhibiting 20-80% larval mortality were tested for each insecticides. The leaf dipping technique was adopted where freshly castor bean leaves were dipped for 5 seconds in one of the prepared conc./ insecticide. The treated leaves were left to natural dryness at room temperature. Befor being offer to 4th instar larvae. Four replicates contained 10 larvae/jar were used for each concentration /insecticide and also for control experiment. The mortality percentages of treated larvae were scored at 24 and 48 h and 72 h after feeding on treated leaves. On the other hand treated leaves were replaced by untreated ones at 48 h post-treatment. Data were corrected against those of control by using Abbott formula (Abbott, 1925). Control larvae feeding water-treated leaves showed <10% mortality in all bioassays. LC_{50} values were calculated according to Finney (1971), through software computer program. Also, the insecticides were arranged on the basis of toxicity index (Sun, 1950).

3.2. Insecticide residue film on glass (internally treated Petri-dish)

Cotton leafworm 4th instar larvae were subjected to a modified larval bioassay similar to those used by Plapp *et al.*, (1987) for determining the susceptibility of selected lepidopteran adults. Formulated insecticides were dissolved in water to prepare stock solutions of insecticide which were diluted to yield 5-7 desired insecticide concentrations. The interior surface of glass Petri dish (cover and bottom) was coated with 2-0 ml of insecticide solution and were left to dry under natural laboratory conditions. Twenty 4th instar larvae were placed into insecticide-treated and nontreated (control) Petri – dishes. Three replicates were used for each concentration. Mortality were determined at 24 and 48 h post treatment. Larvae were considered dead if they were incapable of originated the thereselves up side down.

Data were corrected and analyzed as previously described to obtain dose mortality LC_{50} values. Non-overlapping confidence limits (95%) were used to indicate the significant differences among insecticides.

3.3. Time-oriented mortality:

To determine the speed of lethal action through either contact and/or ingestion bioassay methods, the larval mortality percentages at a fixed concentration, i.e., 10 ppm, were scored at 24, 48, 72 and 96 h post treatment.

Time-mortality response data was analyzed according to Finney (1971), with time replacing concentration and accordingly LT_{50}'s values (time required to record 50% mortality) were computed .

RESULTS AND DISCUSSION

1. Laboratory larval ingestion bioassay

LC_{50} values, slope and their 95% CL of the seven investigated insecticides are listed in Table (1). The LC_{50}'s for the seven insecticides in larval ingestion bioassay ranged from 1.292 to 107.77 ppm after 24 h

feeding on treated leaves. Emamectin benzuate exhibited the highest activity against 4th instar larvae of *S. littoralis* , recording the mimimum LC_{50} of 1.292 ppm as ingestion (oral) bioassay after 24 h feeding period and was followed by pryridalyl (11.97 ppm), indoxacarb (22.03 ppm), rynaxypyr (32.40 ppm), methoxyfenozidc (39.10 ppm) spinetoram (44.04 ppm), while spinosad was the least effective one (107.77 ppm).

Concerning the efficiency of the tested insecticides against the 4th instar larvae after longer feeding period of 48 h on insecticide treated leaves, additional mortality did occur and the performance was moderately improved, almostly showing similar arranging order, where emamectin benzoate still recorded the highest toxicity expressed as the least LC_{50} value, (0.54 ppm) while Spinosad being the least toxic one (52.98 ppm). On the other hand, other tested compounds could be arranged according to ingestion LC_{50} as follow: pyridalyl (3.94 ppm), indoxacarb (9.7 ppm) methoxyfenozid (16.44 ppm), rynaxypyr (22.79 ppm) and spinetoram (22.84 ppm). This indicated that the larvae sould be exposed to treated foliage at least 72 h for accurate bioassay results in loboratory.

Several insecticides representing various classes of chemistries have been evaluated against lepidopterous larvae with bioassays of diet surface-treated (ingestion).

Adamezyk *et al.*, (1999) exposed 3rd instar fall armyworm and recorded LC_{50} values of 197.9 ppm for methoxyfenozide and 4.4 ppm for spinosad. Cock *et al.*, (2001) using first instars on indoxacarb-treated diet recorded LC_{50} 0.59 ppm which agree with results of Hardke *et al.*, (2011) against fall armyworm. In agreement with our findings, Argentine *et al.*, (2002) found that emanectin benzuate showed high activity as surface-treated diet, (ingestion) recording LC_{50} of 0.0029 ppm.

Table (1): Probit analysis of concentration-mortality data for different insecticides against 4th instar larvae of *S. littoralis* via ingestion route (feeding on treated leaves).

Insecticide	LC_{50} ppm	Slope±SE	C L 95%	T.I
24 hr Ingestion toxicity				
Indoxacard	22.03	1.117±0.143	13.907-35.805	5.86
Pyridalyl	11.97	1.784±0.294	7.008-18.272	10.79
Rynaxypyr	32.404	0.9/3±0.091	19.941-56.734	3.98
Methoxyfenozide	39.106	0.393±0.090	13.872-281.22	3.30
Emamectin benzoate	1.292	1.242±0.135	0.853-1.93	100
Spinosad	107.73	1.301±0.313	49.61-502.41	1.19
Spinetoram	44.04	1.266±0.210	28.294-79.779	2.93
48 hr Ingestion toxicity				
Indoxacarb	9.7	1.922±0.240	6.869-13.595	5.56
Pyridalyl	3.94	1.509±0.271	1.618-7.607	13.70
Rynaxypyr	22.794	0.669±0.103	12.335-51.720	2.37
Methoxyfenozide	16.446	0.454±0.906	7.086-54.812	3.28
Emamectin benzoate	0.54	1.130±0.140	0.333-0.836	100
Spinosad	52.98	1.010±0.205	22.126-197.65	1.02
Spinetoram	22.84	1.035±0.138	14.196-40.856	2.36

2. Laboratory larval contact bioassay

Data in Table (2) summarized the contact toxicity via residue film on glass Petri dish. It was obvious that pyridalyl was the most effective one against the 4th instar larvae recording the least LC_{50} value (2.22 ppm) and was followed by indoxacarb (LC_{50} : 14.05 ppm). These results indicate that pyridalyl bioassay under laboratory was effective against *S. littoralis* . These results agree with Satio *et al.,* (2002), who reported that pyridalyl possesses excellent insecticidal activity against numerous lepidopterous pests. The present data consistent also with results reported by Nair *et al.,* (2008), who indicate that pyridalyl provide excellent control of the two bollworm species of cotton and Satio *et al.,* (2005) who reported that pyridalyl caused 100% mortality in the 4th instar of *S. littoralis* at concentration of 500 mg/L.

The results regarding the contact toxicity of the indoxacarb in our study (Table 2) were comparable to those Hammes *et al.,* (1998) who reported it was very effective against *S. littoralis* . The results can also be compared with those of Ahmad and Saleem (2004) who reported that amongst new chemistry insecticides, emamectin benzuate resulted in maximum mortality of *S. littoralis* Bret *et al.,* (1997) reported spinosad oral toxicity to be 5-10 time greater than contact toxicity which disagree with our results, where ingestion LC_{50} was 107.77 versus 67.55 ppm for contact, which agree with finding of Wanner *et al.,* (2002) but the magnitude of difference was moderate.

Andaloro *et al.,* (2000) reported LC_{50}>100 ppm for bollworm, tobacco budworm and beet armyworm larvae exposed to glass surfaces treated with indoxacarb indicating that contact exposure to residues is not a primary route of intoxication for indoxacarb. As rynaxypyr the compound was more effective at 24 h as ingestion (32.4 ppm) than contact (1289.49 ppm) which improved later on at 48 h to reach 33.48 ppm. However, recently Temple *et al.,* (2009) indicated that rynaxypyr have contact (residue film on glass) and ingestion (feeding on insecticide-treated leaves).

Table (2): Probit analysis of concentration-mortality data for different insecticides against 4th instar larvae of *S. littoralis* via contact route (contact with residue film on glass of petri dish).

Insecticide	LC_{50} ppm	Slope ±SE	C L 95%	T.I
24 hr contact toxicity				
Indoxacarb	14.05	2.856±0.357	11.190-17.298	15.80
Pyridalyl	2.222	1.143±0.133	1.377-3.412	100
Rynaxypyr	1289.49	0.571±0.164	449.82-25257.6	0.17
Methoxyfenozide	3859.2	0.722±0.152	1230.04-44807.7	0.057
Emamectin benzoate	49.34	0.914±0.303	12.25-50106.3	4.50
Spinosad	67.55	1.042±0.187	38.59-164.54	3.29
Spinetoram	119.57	0.609±0.124	45.028-1808.2	1.86
48 hr contact toxicity				
Indoxacarb	2.05	1.624±0.173	1.393-2.909	33.70
Pyridalyl	0.691	1.342±0.193	0.405-1.054	100
Rynaxypyr	33.48	0.705±0.094	18.146-62.07	2.06
Methoxyfenozide	271.5	0.746+-0.102	144.6-635.43	0.25
Emamectin benzoate	1.84	0.709±0.208	0.007-15.605	37.55
Spinosad	13.62	1.018±0.146	8.36-23.14	5.07
Spinetoram	7.83	0.802±0.12	4.50-14.36	8.82

3.Time-oriented mortality bioassay:

The time-mortality studies for the seven tested insecticides (ingestion, oral) at a fixed concentration of 10 ppm were performed and expressed as LT_{50} (Table 3). Emamectin benzuate required the least time (4.57 h) to kill 50% population followed by indoxacarb (37.76 h), pyridalyl, methoxyfenazide and rynaxypyr came next recording almost, similar LT_{50} of 61.03, 61.49 and 61.76 h, respectively. However both of spinetoram and spinosad required the maximum time of 83.87 h and 171.58 h to kill 50% exposed insect to treated leaves (ingestion).

On the other hand when time-mortality results were obtained for contact-toxicity (treated glass), it was obvious that pyridalyl was faster in action and required the least time 20.71 h to kill 50% population. However, indoxacarb, spinetoram, and emamectin-benzoate came next recording 29.04, 34.41 and 37.47 h for them, respectively. The rest, spinosad rynaxypyr and methoxyfemozide required maximum time (LT_{50} value) of 41.72, 46.70 and 51.29 h to kill 50% population, respectively.

It is worthy mentioning that abamectin and emamectin benzuate are very susceptible to photodegrodation. MacConnell *et al.*, (1989) showed that the half-life of abamectin was <10 h in simulated sunlight and there were marked differences in the half-life of abamectin an petri dishes (contact) and on leaves in light and dark environments. The half-life of emamectin benzuate on celery has been estimated to be 0.66 days (15.48 h) and on cole crop expected to be even shorter. Numerous photodegradations of emamectin benzuate have been identified (Feely *et al.*, 1992). However, translaminar movement of abamection has demonstrated in numerous studies (Dybas, 1989). Therefore presence of abamectin and emamectin benzoate reservoirs in parenchyma tissue accounts for their long residual activity on certain crops under field conditions (Jansson and Dybas, 1996).

Table (3): Probit analysis of time-mortality data for different insecticides against 4th instar larvae of *S. littoralis* via ingestion and contact route.

Insecticide	LT_{50} hr	Slope ±SE	C L 95%
Ingestion toxicity (treated leaves)			
Indoxacarb	37.76	2.412±0.484	26.51-48.14
Pyridalyl	61.03	1.527±0.403	40.58-92.24
Rynaxypyr	61.76	1.108±0.349	32.81-93.49
Methoxyfenozide	61.49	0.949±0.369	25.18-119.22
Emamectin benzoate	4.57	2.020±1.473	
Spinosad	171.58	1.730±0.453	122.59-404.07
Spinetoram	83.87	1.767±0.394	64.85-113.86
Contact toxicity (treated glass)			
Indoxacarb	29.04	4.868±0.929	23.26-33.99
Pyridalyl	20.71	5.221±1.491	12.851-25.071
Rynaxypyr	46.70	5.226±1.594	
Methoxyfenozide	51.29	7.656±2.299	
Emamectin benzoate	37.47	5.506±0.811	31.99-42.71
Spinosad	41.72	5.503±0.799	35.87-47.33
Spinetoram	34.41	3.950±0.658	27.37-40.60

Overall-result provided emamectin benzuate as the best management tool in respect of concentration and time providing along with other new chemistry insecticides tested. Ryridalyl and indoxacarb proved to be the second most effective insecticides either as ingestion (oral) or contact. Incorporation of new chemistry insecticides specilly for the pests like *Spodoptera littoralis* (Boisd.) of vegetable need safer insecticides like emamectin benzuate with least phtotoxic effects with efficient control of the insect pests (Clarke and Fleischer, 2003).

Generally, data generated from the present study comprise initial efforts in establishing baseline susceptibility of the tested insecticides that can be used as reference points for future monitoring program associated with field population of the cotton leafworm, and other economically lepidopterous pests attacking cotton. Also these data will serve for detection of changes in insect susceptibility to insecticide. However, additional field work is also needed to compliment these laboratory studies to determine the most effective rates of compound given their respective residual properties.

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دراسات معملية تكسيكولوجية باستخدام سبعة من المبيدات الجديدة قليلة المخاطر على يرقات دودة ورق القطن

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الملخص العربى

تم عمل تقييم حيوى معملى لتقدير كفاءة سبعة من المبيدات الحشرية التى تصنف كمبيدات قليلة المخاطر وهى (اندوكساكارب . بايريداليل . ريناكسيباير . ميثوكسى فينوزايد . ايمامكتين بنزويت . سبينوساد وسبينتوران) وذلك باستخدام العمر الرابع اليرقى لدودة ورق القطن . وذلك لتقدير العلاقة بين التركيز ونسبة الموت . وكذلك العلاقة بين الوقت ونسبة الموت في اليرقات .

وقد استخدم في التقييم الحيوى طريقتين : غمر الأوراق . ومتبقى المبيد على السطح الزجاجى .

وقد سجلت النتائج اختلافات معنوية بين الجرعة النصفية المميتة LC_{50} والوقت النصفى المميت LT_{50} . ففى التقييم الحيوى عن طريق الابتلاع سجل مركب ايمامكتين بنزويت أقل قيمة للجرعة القاتلة النصفية LC_{50} حيث سجل ١.٢٩ ppm يليه مباشرة مركب بيريداليل حيث سجل ١١.٩٧ ppm ، بينما كان مركب بيريداليل أعلى كفاءة كسمية بالملامسة حيث سجل ٢.٢٢ ppm يليه مركب اندوكساكارب حيث سجل ١٤.٠٥ ppm .

ومن ناحية أخرى فقد سجل كل من مركب ميثوكسى فينوزايد ومركب ريناكس باير أقل سمية بالملامسة حيث كانت قيمة LC_{50} لكل منهما ١٢٥٩.٤ ، ٣٨٥٩.٢ ppm على الترتيب وذلك بعد ٢٤ ساعة من المعاملة .

أما عن الوقت النصفى المميت فقد وجد أن مركب إيمامكتين بنزويت كان أكثر المركبات كفاءة وأسرعها بالنسبة للسمية عن طريق الابتلاع حيث سجل LT_{50} = ٤.٥٧ ساعة . يليه مركب اندوكساكارب ٣٧.٧٦ ساعة ، بينما سجل مركب بيريداليل أعلى كفاءة للسمية طريق الملامسة LT_{50} ٢٠.٧١ ساعة لقتل ٥٠% من يرقات العمر الرابع لدودة ورق القطن .

بينما سجل مركب سبينوساد وقتاً أطول لاحداث السمية عن طريق الابتلاع حيث سجل ١٧١.٥٨ ساعة ، بينما كانت السمية عن طريق الملامسة ٥١.٢٩ ، ٤٦.٧ ، ٤١.٧٢ ساعة لكل من ميثوكس فينوزايد . ريناكس باير . وسبينرساد على الترتيب .