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Joint Action of Two Novel Insecticides Mixtures with Insect Growth Regulators, Synergistic Compounds and Conventional Insecticides Against *Spodoptera littoralis* (Boisd.) Larvae

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ABSTRACT

In laboratory study the interaction between two recently developed insecticides (pyridalyl and spinetoram) were combined at different mixing ratios in binary mixtures with eight compounds including three conventional insecticides (chlorpyrifos-E, methomyl and deltamethrin), two IGRs (hexaflumuron and pyriproxyfen) and three synergists compounds (Pepronyl butoxide, sesame oil and oleic acid) and investigated against *S. littoralis*, 4th instar larvae. Based on co-toxicity coefficient, both insecticides (pyridalyl and spinetoram) response positively when mixed with chlorpyrifos-E and exhibited remarkable potentiation effect at most tested mixing ratios within 24 and 48 h post treatment. On the other hand, slight synergism was only recorded for pyridalyl/pyriproxyfen mixtures at 9:1 and 4:1 while similar trend was also recorded at 9:1, 4:1 and 1:1 of pyridalyl/hexaflumuron mixtures whereas no synergism was almostly recorded for spinetoram/IGRs mixtures. In contrast, the three compounds (pbo, sesame oil and oleic acid) exhibited highly considerable synergism at all tested mixing ratios with pyridalyl while sesame oil and oleic acid evoked remarkable synergism only in their binary mixtures with spinetoram.

Key words: Pyridalyl, spinetoram, joint action, cotton leafworm

INTRODUCTION

The Egyptian cotton leafworm *Spodoptera littoralis* (Boisd.) is one of the most destructive phytophagous insect pests in Egypt, not only to cotton but also to other field crops and vegetables (Kandil *et al.*, 2003). These caterpillars are major polyphagous key pests in Egypt, causing serious and considerable economic losses in both greenhouses and open fields.

During the last few decades, Egypt is suffering from the onset of resistance in cotton leafworm for the most conventional insecticides (Maher, 1975; Yehia *et al.*, 1985; El-Bermawy *et al.*, 1992; Rashwan *et al.*, 1992).

To overcome development of resistance, several studies have been made for investigating new and non-traditional control agents or/and approaches, effective against this pest. One of the new non-traditional control compounds is spinosyns, a novel family of insecticidal macrocyclic lactones which are active on wide variety of pests, especially lepidopterans and dipterans. Spinosad (Tracer, Success) belong to this family and possess a mode of action (Salgado *et al.*, 1997) that appears unique, with studies to date suggesting that nicotinic and gamma-aminobutyric acid receptors functions are altered in a novel manner (Salgado and Saar, 2004).

Another spinosyns, spinetoram (Radiant) is a recently developed and belong to IRAC mode of action class 5 (IRAC, 2009). Insecticides in this class primarily activate the neonicotinic acetylcholine receptors by acting on a unique site (Salgado *et al.*, 1998; Salgado, 1998). In Egypt, Temerak (2007) used spinosyn products, spinosad (Spintor 24% SC) and spinetoram (Radiant 12% SC) to combat egg masses of the cotton leafworm, he indicated that Radiant 12% SC was 5 and 7 time stronger than Spintor 24% SC in field and Laboratory, respectively.

Another new and non-traditional compound is pyridalyl, a novel insecticide that has a phenoxy-pyridaloxyl derivative structure introduced in 2002 by Sumitomo (Sakamoto *et al.*, 2004). The compound was reported effective generally on the pests of order Lepidoptera and Thysanoptera on cotton and vegetables. The compound was effective in particular against populations of tobacco budworm, *Heliothis virescens*, cotton bollworm *Helicoverpa zea* (Johnson *et al.*, 2000), *Plutella xylostella* (Umeda and Strickland, 1999) which are resistant to various currently used insecticides.

Searching for a new approach for use of each of these new compounds to be standing by for the onset of resistance problem in future had led to investigating it in mixtures, to improve their performance and elongate their life as effective control measures. Early studies of synergism (Busvine, 1971) indicated that mixing of chemicals of different mode of action can led to more potent use of toxicants which could theoretically prevent or delay the emergence of resistant strains. In this respect, it was recorded organophosphates synergize/pyrethroids against several pests, i.e., *S. littoralis* (Asher *et al.*, 1986), *Heliothis armigera* (Martin *et al.*, 2003). Also, synergism was obtained by combinations of IGRs and traditional insecticides (El-Guindy *et al.*, 1985; Radwan *et al.*, 1985, 1990; Kandil *et al.*, 2006).

Though, the present study aimed to characterize the interaction between each of pyridalyl and spinetoram when combined with, three traditional insecticides, two IGRs and three biorational compounds. when tested against the 4th instar larvae of the cotton leafworm *S. littoralis* (Boisd.).

MATERIALS AND METHODS

Test insects: The culture of cotton leafworm, *Spodoptera littoralis* (Boisd.) used in the present study has been reared in laboratory out of contamination with any insecticide for more than two years and was initiated from egg masses supplied from the Central Laboratory of Pesticides, Ministry of Agriculture, Dokki, Giza. All stages of *Spodoptera littoralis* were reared and tested at 27±2°C and 70±5% RH (El-Defrawi *et al.*, 1964).

Insecticides: Three groups of formulated insecticides were chosen for investigation either alone or in mixtures. These insecticides include:

- Newly developed insecticides, pyridalyl (S1812 50% EC) and spinetoram (Radiant 12% SC)
- The conventional insecticides: Deltamethrin (Decis 2.5% EC), methomyl (Lannate 90% SP) and chlorpyrifos-ethyl (Dursban 48% EC)
- Insect growth regulators: Hexaflumuron (Consult 10%) and Pyriproxyfen (Admiral 10% EC)
- Pepronyl butoxide (Pbo), sesam oil and oleic acid

Bioassay: For assessing the toxicity of each insecticide either separately or in binary mixtures, stock solution of each insecticide alone or mixture of two components were prepared fresh daily on the basis of active ingredient (w/v). The tested mixing ratios of each mixture were 9:1, 4:1, 1:1, 1:4

and 1:9 on the basis of active ingredient for both components. Stock solutions of either separate insecticide or mixture were serially diluted to 5-7 concentrations, resulting in 10-90% mortality which were used to calculate LC_{50} values.

Castor bean leaves were dipped in either water (control) or in one of the prepared insecticide (s) dilutions for 20 sec and were air dried under room temperature. Leaves treated with water alone were used for feeding control larvae. Larvae were placed in glass jars (1 Lb) and offered treated leaves for 24 h and then were replaced by untreated fresh leaves at the 2nd day post treatment. Five replicates, each of ten 4th instar larvae were used. Mortality% were recorded after 24 and 48 h and corrected according to Abbott formula (Abbott, 1925).

To calculate LC_{50} values for either mixtures or their components separately, probit analysis (Finney, 1971) were adopted, using the corrected mortality percentages. The co-toxicity coefficient based on LC_{50} values was calculated according to method of Sun and Johnson (1960), using toxicity index (Sun, 1950).

$$\text{Actual T.i of mixture M (using A as standard)} = \frac{LC50 \text{ of A}}{LC50 \text{ of M}} \times 100$$

$$\text{Theoretical T.i. of mixture M} = \text{T.i. of A} \times \% \text{ of A in M} + \text{T.i. of B} \times \% \text{ of B in M}$$

$$\text{Co-toxicity coefficient of mixture} = \frac{\text{Actual T.i. of a mixture}}{\text{Theoretical T.i. of mixture}} \times 100$$

where, T.i. = Toxicity index

When the co-toxicity coefficient of a mixture is 100, the effect of this mixture indicates probability of similar action. If the mixture gives a coefficient significantly greater than 100, it indicates synergistic action. An independent action usually should give a coefficient less than 100.

RESULTS AND DISCUSSION

The joint action data shown in Table 1 illustrate that pyridalyl in combinations with methomyl and deltamethrin at most of the tested mixing ratios exerted an antagonistic action based on co-toxicity coefficient calculated on the basis of the LC_{50} values at 24 and 48 h post treatment, except only pyridalyl+methomyl (9:1) exhibited moderate synergistic action reached 167.04 and 576.31 fold after 24 and 48 h, respectively and pyridalyl+deltamethrin (9:1 and 1:1) after 48 h only. On the other hand, pyridalyl+chlorpyrifos-ethyl combinations evoked remarkable synergistic action at all mixing ratios at 24 and 48 h post treatment, except at mixing ratio of 1:4, recording co-toxicity coefficient ranged between 121.68 and 5620.05, 105.84 and 90661.44 at 24 and 48 h post treatment, respectively.

As for combinations of spinetoram with methomyl, deltamethrin and chlorpyrifos-e when tested at the same mixing ratios (Table 2), it was obvious that spinetoram revealed synergistic action at 24 h only when combined with deltamethrin at 1:9 and chlorpyrifos at 4:1, 1:1 and 1:9, resulting in co toxicity coefficient values of 147.42 for deltamethrin and 265.41, 113.32 and 965.56 for chlorpyrifos at the prementioned mixing ratios, respectively. Regarding to the degree of synergism at 48 h (Table 2) it was evident that spinetoram revealed moderate synergistic action when combined with deltamethrin and chlorpyrifos-e recording co toxicity coefficient ranged between 101.34-347.93 and 119.87-2156.91 for spinetoram+deltamethrin and spinetoram+chlorpyrifos-e,

Table 1: Toxicity of different conventional insecticides separately and in binary mixtures with pyridalyl and their co-toxicity coefficient when mixed at different ratios against the 4th instar larvae of *S. littoralis* (Boisd.)

Mixing ratios Pyr.+ins.	Toxicity data for pyridalyl in mixtures with the indicated insecticide					
	Chlopyrifos-E		Deltamethrin		Methomyl	
	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)
24 h post treatment						
1:0	-	275.030	-	275.03	-	275.03
9:1	121.68	163.500	82.13	337.92	167.04	47.65
4:1	405.82	38.390	53.23	526.08	85.96	54.16
1:1	5620.05	1.680	45.43	633.92	13.45	153.96
1:4	70.89	95.540	38.80	763.55	8.06	165.49
1:9	907.60	6.820	35.87	833.90	18.33	65.01
0:1	-	56.990	-	302.09	-	10.77
48 h post treatment						
1:0	-	14.910	-	14.91	-	14.91
9:1	105.84	14.180	114.53	14.30	576.31	2.10
4:1	149.56	10.100	62.93	28.87	67.84	15.01
1:1	90661.44	0.017	137.35	19.66	32.97	20.93
1:4	79.86	19.700	41.14	127.83	17.86	29.23
1:9	11733.18	0.135	58.57	131.39	21.56	22.39
0:1	-	15.950	-	143.03	-	4.49

Table 2: Toxicity of different conventional insecticides separately and in binary mixture with spinetoram and their co toxicity coefficient when mixed at different ratio against the 4th instar larvae of *S. littoralis* (Boisd.)

Mixing ratios Pyr.+ins.	Toxicity data for pyridalyl in mixtures with the indicated insecticide					
	Chlopyrifos-E		Deltamethrin		Methomyl	
	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)
24 h post treatment						
1:0	-	344.750	-	344.75	-	344.75
9:1	47.19	485.300	64.69	525.43	28.81	292.17
4:1	265.41	64.640	62.85	533.49	19.47	246.17
1:1	113.32	86.310	58.39	551.43	99.52	20.99
1:4	19.57	349.540	12.32	2512.71	50.58	26.41
1:9	965.56	6.440	147.42	207.50	11.85	100.66
0:1	-	56.990	-	302.09	-	10.77
48 h post treatment						
1:0	-	55.020	-	55.02	-	55.20
9:1	119.87	36.870	101.34	121.66	177.10	14.62
4:1	312.43	11.820	146.49	73.96	25.80	52.65
1:1	218.45	11.320	156.11	50.90	917.41	0.905
1:4	157.27	11.820	65.01	96.51	24.83	22.15
1:9	2156.91	0.796	347.93	16.85	14.25	34.70
0:1	-	15.950	-	143.03	-	4.49

respectively. On the other hand, spinetoram+methomyl exhibited synergistic action only at two mixing ratios 9:1 and 1:1 recording co toxicity coefficient 177.10 and 917.41, respectively.

Considering the response of the 4th instar larvae to different tested mixtures of pyridalyl and two insect growth regulators (Table 3), it was obvious that pyridalyl exhibited synergistic action when combined with hexaflumuron at 9:1, 4:1 and 1:1 while when mixed with pyriproxyfen at 9:1 and 4:1, at 24 h post treatment, resulting in co toxicity coefficient values of 264.89, 180.77 and 194.74 for the former and 165.66, 119.68 for the later. respectively. Regarding the degree of synergism at 48 h it was obvious that pyridalyl revealed synergistic action when combined with hexaflumuronat (9:1 and 1:4) and with pyriproxyfen at (9:1 and 4:1), recording co toxicity coefficient (152.03 and 464.09) and (524.15 and 386.53), respectively.

As for combinations of spinetoram with the two insect growth regulators when tested at the same mixing ratios (Table 4), it was obvious that spinetoram exhibited antagonistic action at all mixing ratios at 24 h post treatment except at 1:1 mixing ratio with hexaflumuron which revealed synergistic action. On contrary at 48 h spinetoram/hexaflumuron exhibited remarkably considerable synergistic action at all mixing ratios studied and recording co toxicity coefficient ranged between 108.20-2.78, while spinetoram/pyriproxyfen exhibited synergistic action at only 9:1, 4:1 and 1:1 mixing ratios, recording co toxicity coefficient of 249.97, 218.21 and 114.81, respectively.

The response of the 4th instar larvae when exposed to pyridalyl binary mixtures of Pbo, sesam oil and oleic acid are shown in Table 5, the data of co toxicity coefficient values indicate highly remarkable synergistic action for all tested mixtures of pyridalyl/sesam oil and pyridalyl/oleic acid recording co toxicity coefficient ranged between 367.63 to 10745.84 and from 598.29 to 1736.73, respectively. Meanwhile, pyridalyl/pbo revealed synergistic action at 99:1 and 90:10 mixing ratios recording cotoxicity coefficient 338.08 and 650.31 at 24 h post treatment, respectively.

Table 3: Toxicity of different insect growth regulators separately and in binary mixture with pyridalyl and their co toxicity coefficient when mixed at different ratios against the 4th instar larvae of *S. littoralis* (Boisd.)

Toxicity data for pyridalyl in mixtures with the indicated insecticide				
Mixing ratios Pyr.+IGR	Pyriproxyfen		Hexaflumuron	
	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)
24 h post treatment				
1:0	-	275.03	-	275.0300
9:1	165.66	184.36	264.89	115.1100
4:1	119.63	286.96	180.77	189.2300
1:1	58.90	928.93	194.74	276.9800
1:4	75.82	1775.62	40.41	3154.3600
1:9	80.36	3262.28	9.34	24950.8300
0:1	-	50523.57	-	13906.600:1
48 h post treatment				
1:0	14.91	-	14.91	-
9:1	524.15	3.16	152.03	10.8800
4:1	386.53	4.82	35.16	52.8300
1:1	22.66	131.34	85.22	34.5200
1:4	20.35	363.97	464.09	15.2400
1:9	54.83	268.28	80.12	165.9400
0:1	-	9071.99	-	1107.0300

Table 4: Toxicity of different insect growth regulators separately and in binary mixtures with spinetoram and their co toxicity coefficient when mixed at different ratios against the 4th instar larvae of *S. littoralis* (Boisd.)

Toxicity data for pyridalyl in mixtures with the indicated insecticide				
Mixing ratios Pyr.+IGR	Pyriproxyfen		Hexaflumuron	
	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)
24 h post treatment				
1:0	-	344.75	-	344.75
9:1	66.97	571.51	97.78	390.70
4:1	91.50	470.16	34.07	1256.79
1:1	72.73	941.57	202.40	332.41
1:4	8.23	20429.00	18.38	8527.56
1:9	39.77	8173.22	34.51	8173.22
0:1	-	50523.57	-	13906.60
48 h post treatment				
1:0	-	55.02	-	55.02
9:1	249.97	24.44	252.78	24.05
4:1	218.21	31.47	108.20	62.79
1:1	114.81	95.45	134.73	77.80
1:4	7.76	3462.83	177.81	129.02
1:9	25.31	2061.19	233.45	162.86
0:1	-	9071.99	-	1107.03

Table 5: Toxicity of different biorational insecticides separately and in binary mixtures with pyridalyl and their co toxicity coefficient when mixed at different ratios against the 4th instar larvae of *S. littoralis* (Boisd.)

Toxicity data for pyridalyl in mixtures with the indicated insecticide						
Mixing ratios Pyr.+Bior.	Oleic acid		Sesam oil		Pbo	
	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)
24 h post treatment						
1:0	-	1571.440	-	23184.72	-	5917.07
99:1	598.29	46.350	367.63	75.56	338.08	82.13
95:5	1140.00	25.140	929.40	31.13	87.81	328.90
90:10	1736.73	17.260	10745.84	2.84	650.31	46.75
0:1	-	275.030	-	275.03	-	275.03
48 h post treatment						
1:0	-	98.500	-	34.41	-	41.03
99:1	9958.82	0.151	167.36	8.96	25010.06	0.06
95:5	1428.46	1.090	371.53	4.13	793.80	1.94
90:10	4792.14	0.340	2258.03	0.70	233.97	7.11
0:1	-	14.910	-	14.91	-	14.91

The results after 48 h post treatment revealed a remarkable increase in synergistic action for pyridalyl/pbo, pyridalyl/sesam oil and pyridalyl/oleic acid at all mixing ratios recording co toxicity ranged between 233.97 to 25010.06, 167.36 to 2258.03 and 1428.46 to 9958.82, respectively.

As for combinations of spinetoram with biorational compounds (Pbo, Sesam oil and Oleic acid) when tested at the same mixing ratios (Table 6), it was obvious that spinetoram revealed its

Table 6: Toxicity of different biorational insecticides separately and in binary mixtures with spinetoram and their co toxicity coefficient when mixed at different ratios against the 4th instar larvae of *S. littoralis* (Boisd.)

Mixing ratios Pyr.+Bior.	Toxicity data for spintoram in mixtures with the indicated insecticide					
	Oleic acid		Sesam oil		Pbo	
	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)	Cot. Coe.	LC ₅₀ (ppm)
24 h post treatment						
1:0	-	1571.44	-	23184.77	-	5917.07
99:1	749.65	46.35	237.55	146.58	14.82	2349.11
95:5	163.38	219.57	232.59	155.91	20.73	1745.45
90:10	122.82	304.49	252.17	151.65	106.75	356.57
0:1	-	344.75	-	344.75	-	344.75
48 h post treatment						
1:0	-	98.50	-	34.41	-	41.03
99:1	36845.81	0.15	153.35	35.67	99.13	55.32
95:5	1894.40	2.97	76.62	69.72	1405.21	3.85
90:10	505.79	11.38	118.29	43.88	107.44	49.52
0:1	-	55.02	-	55.02	-	55.02

synergistic action at 24 h when combined with sesam oil and oleic acid at all mixing ratios recording co toxicity coefficient ranged between 232.59 to 252.17 and 122.82 to 749.65, respectively. On the other hand, spinetoram/pbo exhibited antagonistic action at 99:1 and 95:5 mixing ratio while slight synergistic was recorded at 90:10 mixing ratio. Following the response of the 4th instar larvae to different tested mixtures at 48 h post treatment, it was clear that high increase in synergistic action was achieved for spinetoram/oleic acid at all mixing ratios recording co toxicity coefficient ranged between 505.79 to 36845.81. On the other hand combinations of spinetoram/pbo exhibited synergistic action at 95/5 and 90/10; spinetoram/sesam oil at 99:1 and 90:10 mixing ratios, recording co toxicity coefficient (1405.21 and 107.44) and (153.35 and 118.29), respectively.

In conclusion it is obvious that both new compounds pyridalyl and spinetoram when mixed with chlorpyrifos in particular, exhibited considerably high potentiation at all tested mixing ratios. Similar findings were achieved by Attique *et al.* (2006) when investigated the synergism between pyrethroids, organophosphates and new insecticide against diamondback moth *Plutella xylostella*. The toxicity of chlorpyrifos in combination with spinosad and emamectin against the field strain, increased significantly. In the same study, the toxicity of pyrethroid bifenthrin against the field population increase significantly ($p < 0.01$) when combined with spinosad or/and emamectin. Also, our data are in agreement with Abdallah and Kandil (1985) where they found that chlorpyrifos acts as potentiator for several insecticides belonging to different groups including antimoulting compounds (IGRs).

As for spinetoram it was obvious that slight synergistic action was achieved when mixed with hexaflumuron at 1:1 mixing ratio after 24 h and that synergistic action increased remarkably at most of mixing ratios of spinetoram/hexaflumuron and spinetoram/pyriproxyfen after 48 h, post treatment. This agree with Abdel-Rahman and Abou-Taleb (2007) where they found that the spinosad or spinetoram at LC₂₅/IGR compounds at LC₂₅ mixtures revealed potentiating effect higher than the mixtures of spinosad or spinetoram at LC₂₅/IGR compound at LC₁₀. Although the toxicity of tested IGR compounds was appeared after 48 and 72 h of exposure, the potentiating effect of these compounds in mixtures with spinosad and spinetoram appeared after 24 h. Generally, based

on their interaction it is preferred to use high concentrations of spinosad or spinetoram with low concentration of the IGR compounds and not the opposite.

Also, Abdel-Hafez and Mohamed (2009) found that, the interaction between spinetoram and chlorfluazuron or fenopropathrin revealed a potentiation effect in all treatments at different concentration of binary mixtures, except the mixture of spinetoram at (LC₅₀), with fenopropathrin (LC₁₀) which produced an additive effect (+19.1). The observed mortality caused by both agents together was higher than with each agent alone. The most promising synergism occurred when LC₂₅ of spinetoram was combined with LC₂₅ of fenopropathrin (+118.7), followed by chlorfluazuron (+77.91). Laboratory studies suggests that, the use of spinetoram in combinations with either chlorfluazuron or fenopropathrin at low doses may reduce the rate of the used insecticides.

Generally the present data suggests that the use of pyridalyl or and spinetoram in binary mixtures with conventional insecticides particularly chlorpyrifos, sesam oil and oleic acid may improve the performance and thus inhibit resistance development in *S. littoralis*, in addition to reduce the rate of used insecticides and consequently reduce the costs as well as reduce environmental pollution.

CONCLUSION

The synergistic compounds (pbo, sesam oil and olic acid) exhibited highly considerable synergism at all mixing ratios with pyridalyl while sesame oil and oleic acid exhibited remarkable synergism only in their binary mixture with spinetoram, whereas both insecticides (spinetoram and pyridalyl) evoked no considerable synergistic action with conventional insecticides and IGRs.

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