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TOXICITY OF PROFENOPHOS, LUFENURON AND *BACILLUS THURINGIENSIS* TO THE PARASITOID *MICROPLITIS RUFIVENTRIS* Kok. (HYMENOPTERA: BRACONIDAE)

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ABSTRACT: The acute and chronic toxicity of the conventional organophosphorus insecticide profenophos , the insect growth inhibitor lufenuron , and the bioinsecticide *Bacillus thurigiensis* against the *M. rufiventris* - parasitized in the second instar larvae of *S. littoralis* was investigated .

It was found that parasitism increased the susceptibility of *S. littoralis* larvae to the three tested insecticides. Thus parasitism added additional adverse effect to insecticide – exposed host larvae.

It was found that the parasitoid did suffer from severe deleterious effect during development in the survivors of host larvae exposed to two levels of toxicity (LC_{25} and LC_{50}) of the three preceding insecticides. The three insecticides exhibited acute toxicity when the egg and larval stages were directly exposed to the toxicant as well as chronic toxicity which took place later in the other two stages ,i.e., pupae and adults which are found outside the host larvae. The developmental insecticide lufenuron exhibited the highest deleterious effect followed by the OP compound profenophos . The bioinsecticide *B. th* . displayed the lowest bioactivity.

Key words: *Microplitis Rufiventris*, *Bacillus thurigiensis*, lufenuron, profenophos

INTRODUCTION

It is known that the cotton leafworm , *Spodoptera littoralis* (Boisd.) causes tremendous damage to different field and vegetable crops including tomatoes. During the last three decades the developmental and bioinsecticides are included in the schedule of the control of lepidoptran pests simultaneously with the

conventional insecticides. The unwise and intensive use of conventional and developmental insecticides resulted in violent deleterious effect on the beneficial natural enemies (e.g., parasitoids and predators).

The solitary endoparasitoid wasp *Microplitis rufiventris* Kok. (Hymenoptera: Braconidae) is considered a potential agent of some nuctuid pests including the cotton leafworm *S. littoralis* (Hammad *et al* ., 1965; Gerling, 1969 ; and El- Maghraby, 1993) .

The aim of this study is to throw some light on the acute and chronic toxicity of the conventional OP insecticide profenophos , the developmental (insect growth inhibitor) insecticide lufenuron, and the bioinsecticide *Bacillus thurigiensis* subsp. *kurstaki* against *M. rufiventris* – parasitized *S. littoralis* 2nd instar larvae - after different periods from the onset of parasitoidism.

MATERIALS AND METHODS

Tested Insecticides

Formulated samples of three known insecticides were used : Ecotech – Bio, (10%WP) , a wettable powder , based on *Bacillus thuringiensis* subsp . *kurstaki* strain EG 2371, the OP compound profenophos (Selecron, 72% EC) [O - (4 – bromo – 2 - chlorophenyl) – O – ethyl S – propyl phosphorothioate , and the insect growth inhibitor lufenuron (Match ,5% EC) [N-(2,5-dichloro-4-(1,1,2,3.3,3 - hexafluoropropoxy) - phenylaminocarbonyl]-2,6-difluorobenzamide] .

Insects

Egg and larval stages of the cotton leafworm *Spodoptera littoralis* (Boisd.) were collected from a tomato field and reared for several generations in the laboratory. The larvae and adults were fed on castor bean leaves and honey solution (10%), respectively The parasitoid *Microplitis rufiventris* Kok. was reared according to Tawfik *et al* (1977) and El – Maghraby (1993) . Batch of (50 to 100) second instar larvae of *S. littoralis* were individually subjected to appropriate number of males and females (2 ♂ and 1 ♀) of the parasitoid and observed until the last instar larvae exited the host for pupation . Both cultures , the host and the parasitoid , were held at 27 ± 2 °C and 80 ± 5 % R . H .

Bioassay

Acute toxicity

Parasitized and unparasitized *S. littoralis* 2nd instar larvae were confined , individually , with a piece of castor bean leaf pre – dipped in the test concentration of each compound . At least four concentrations were used for each insecticide . Four replicates were used each contained 25 larvae . Holding period ranged between 24 to 72 h depending on the test compound ; with all insecticides the length of the holding period was based on the settlement of mortality percentages . The two levels of toxicity (LC_{50} and LC_{90}) and their confidence limits as well as slope values were obtained by probit analysis (Finney , 1971) using the software package EPA probit.

Chronic toxicity

To detect the effect of the three test compounds on the development of the parasitoid , the survivors of the larvae at the LC_{25} and LC_{50} levels of toxicity were observed to inspect the different biological aspects of the parasitoid, namely , the egg- larval and pupal durations , number of cocoons and emerged adults simultaneously with sex ratio , and adult longevity . Some aspects were observed with another level of toxicity (LC_{80}).

RESULTS AND DISCUSSION

Acute Toxicity

The acute toxicity of the conventional organophosphorus insecticide profenophos (Selecron, 72% EC),the insect growth regulator inhibitor lufenuron (Match,5%EC) and the bioinsecticide *Bacillus thurigiensis* subsp . *kurstaki* (Ecotech -- Bio, 10% WP) against *M. rufiventris* – parasitized and unparasitoized second instar larvae of *S. littoralis* is presented in Table 1 . Data clearly show that parasitized larvae were more susceptible to the action of the three tested insecticides . LC_{50} values of profenophos, lufenuron, and *B. th* . were 81.86 , 56.29 and 65.58 ppm, respectively, in unparasitized larvae. The corresponding values with the

Table 1. Susceptibilty of M. rufiventris – parasitized and unparasitized second instar larvae of *S. littoralis* to profenophos, lufenuron , and B. thurigiensis .

Insecticide	LC 50 * (conf . limit)		LC 90 * (conf . limit)		Slope	
	unparasitoized	parasitoized	unparasitoized	parasitoized	unparasitoized	parasitoized
Profenophos	81.86	63.36	262.04	192.04	2.54	2.66
	(71.69-117.84)	(55.15-72.42)	(202.15- 375.75)	(149.81-286.15)	-	-
Lufenuron	56.29	42.69	196.98	143.43	2.36	2.43
	(49.17-65.270	(37.44- 49.29)	(151.99-284.13)	(111.84-205.13)	-	-
B. th.	65.58	45.49	227.26	149.26	2.38	2.48
	(57.60-67.24)	(39.96-51.91)	(175.86-326.37)	(119.43-202.82)	-	-

* in ppm

Initial and latent effects of profenophos, lufenuron and *B.th.* in the endoparasitoid *M. rufiventris* on the second instar larvae of *S. littoralis*.

To detect the toxicity of the three used insecticides, namely, the conventional OP compound profenophos and the developmental insecticide lufenuron, which interferes with chitin synthesis, and the bioinsecticide *B.th.* during direct exposure of the egg and larval stages of the endoparasitoid *M. rufiventris* to insecticides or later during being outside the host larvae (i.e., latent effect), the host second instar larval of *S. littoralis* were applied with the insecticide after 0 to 24, 24 to 48, and 48 to 72 hrs from the onset of parasitidism. With this way, the age of each immature stage will differ as time lapses between the onset of parasitism and insecticide treatment. During this period the physiological aspects of the immature stages due to their development, as well as the metabolism of the insecticide may, however, play a role in this respect. It is known that the OP compound profenophos is mainly an acetylcholinesterase inhibitor and the efficiency of the chitin synthesis inhibitor lufenuron is restricted during a very limited vulnerable period, i.e., during apolysis. Two levels of toxicity of each insecticide were used, namely, the LC_{25} and , LC_{50}, to ascertain the correlation between the side effect of the used insecticide, if present, and its concentration . The egg - larval duration, total developmental period, number of emerged adults and longevity of both sexes, sex ratio, were observed in the survivors of the preceding levels of toxicity. The obtained results are presented in tables (2,3 and 4).

Effect on egg – larval development

Data in Tables 2, 3, and 4 show that egg – larval duration was affected and resulted in elongation in this period which includes egg incubation as well as larval duration . The compound used as well as its concentration did affect this criterion of development . The duration period ranged between 12 to 13.35, 12.24 to 13.50, and 11.03 to 12.51 days at the high used level of toxicity. The corresponding ranges at the lower level of toxicity were 11.04 to 12.83, 11.78 to 11.97, and 10.99

to 11.39 days . The duration in the control was 10.02 days. These figures of egg – larval duration show that the level of toxicity plays a slight role in this respect . Inspection of all the figures of duration in the three tables refers to the highest activity of the developmental insecticide lufenuron compared with the bioinsecticide *B. th.* which exhibited the lowest bioactivity ; the OP compound occupied an intermediate position. It was found that another higher level of toxicity (LC_{90}) of profenophos and lufenuron displayed severe destructive effect ; there was no transformation from the larval to the pupal stage . This means that both compounds did inhibit either embrynic development or exhibited acute larvicidal action .

It is known that the first compound is an AchE inhibitor and the second one is a developmental insecticide affects both metamorphosis and growth . It seems that the insecticide – exposed parasitoid larvae which suffered from sublethal concentrations need additional time to recover from the toxic symptoms and thus gain their requirement to moult normally to the next instar or stage .

Pupal duration

As has been found with egg – larval duration , pupal duration was also retarded , in all insecticide treatments . During the three periods of parasitoid development, exposure to the developmental insecticide lufenuron rasulted in the longest pupal duration (7.09 to 9.35days) whereas the bioinsecticide resulted in shortest duration (5.28to 7.27); the OP compound occupied an intermediate position in this respect . This phenomenon was more noticeable with the high concentration of each toxicant .

Total developmental period

Since both egg – larval and pupal durations were increased , the total development of period , in turn , is expected to be longer than that of the control . The total developmantal period with the low level of toxicity ranged between 16.43 to 21.73, 18.19 to 19.41, and 17.04 to 18.48 days when the host larvae were exposed to the three insecticides after 0-24 , 24-48, and 48-72 hrs , respectively . The corresponding ranges at the high level were 18.14 to 22.70 , 19.61 to 21.84 ,and 17.99 to 20.59 . These figures show that the profenophos and lufenuron – exposed larvae seffered from the

toxicity of these two insecticides which need a longer time to develop to the next stage . It seems that the bioinsecticide *B. th.* exhibited lower bioctivity .

Number of cocoons

Regarding number of cocoons, which reflect the last larval instar which was capable to exit and pupate outside the host larvae , data show that the three insecticides did reduce the number of cocoons , i.e., the number of pupae . This deletrious effect was much more noticed with the higher level of toxicity ; number of cocoons were 25, 28, and 34 ; 23 , 24, and 29 ; and 28, 29, and 30 compared with 37 cocoon in the control treatment . These figures show the superiority of the devlopmental insecticide lufenuron

Number of emerged adults

Data cleary show that insecticidal treatments did reduce adult emergence. Number of emerged adults in insecticides treatments ranged between 13 to 28, 11 to 24, and 15 to 25 compared with 34 in the control . Reduction percentages in adult emergence ranged between 12.5 to 35.29 depending , however , on the used insecticide and its comcentration as well as the development of the internal stages of the parasitoid . It is worth to mention that the emerged adults are, however , not absolutlely normal but seem to be normal , which may add additional deletrious effect to those mentioned with the immature stages. However , percentages of adult emergence ranged between 64.71 to 87.5 compared with 91.89 in the control .

Sex ratio

The sex ratios show that the number of males were , in general , higher among adults resulted from the control . The sex ratios (female : males) ranged between 1:1.2 to 1:1.78 compared with 1:1.83 in the control. These figures reflect the higher susceptibility of the indviduals which are going to differentiate to males .

Adult longevity

Data show that the three insecticides exhibited latent effects took place later in the adult stage . The adults suffered from wide range of deleterious effect depending on the used insecticide and its level of toxicity simultaneously with the time lapse between the onset of parasitism and exposure to the tested insecticide. Percentages of shortening in male adult longevity ranged between 10.7 to 63.3 in

mals compared with 1.4 to 55.9 in the female adults .

In conclusion , the obtained data show that insecticides – exposed *S. littoralis* larvae suffered from additional detrimental effect (i.e. higher susceptibility) to the three used insecticides compared with the unparasitized ones . Also , retarding in the larval and pupal development and deleterious effects on the adult stage were found . Such alterations of parasitoid development might have been caused by the lack of sufficient nutrients (Vinsn and Iwantch , 1980) , an unfavorable hemolymph composition of their hosts (Beckage , 1985) , or the presence of the parents or metabolites of the used three toxicants ; *Spodoptera* host 2nd instar larvae were fed on insecticides – treated castor bean leaves . Hegazi and furhrer (1985) found that *M. rufiventris* is influenced by the physiological state of its *S. littoralis* host . In this respect , Salama *et al* . (1982) showed that certain aspects of the parasite *M. demolitor* is affected when the host *S. littoralis* were fed on diet containing *B. thuringiensis.* Lawrence (1981) found that topical application of the chitin synthesis inhibitor on the larvae of the Caribbean fruit fly , *Anastrepha Suspensa* Loew , parasitized by the braconid wasp *Biosteres longicaudatus* Ashmead caused deleterious effects on the development of the egg stage of the parasitoid . This result is in full agreement with the present results which showed that lufenuron exhibited the highest deleterious effect within egg – larval duration .

In this respect Harvey (1996) indicated that hymenopterous endoparasitoids are known to cause many physiological changes in their hosts in action known as host regulation and this regulation alters many aspects of host physiology and ontogony .

In contrary with the obtained results, Garasimovo and Polyakova (1988) found that parasitism of *Pieris brassicae* by *Apanteles glomeratus* reduced the effect of preparations containing *B. th.* but increased the susceptibility of the larvae to trichlorofon .

The above results indicate that the conventional OP compound , the chitin synthesis inhibitor lufenuron , and the bioinsecticides simultaneously with parasitism with *M. rufiventris* resulted in higher susceptibility of the host larvae compared with the unparasitized ones. Sublethal concentrations of these toxicants displayed severe deleterious effect on the parasitoid development .

Table 2. Effect of sublethal concentrations* of profenophos, lufenuron and *B .th.* on development of the endoparasitoid *M. rufiventris* after 0 - 24 hrs from parasitoidism on second instar larvae of *S. littoralis* .

Insecticides	Tested conc.	No of parasitized - larvae	Egg- larval duration (in days)	Pupal duration (in days)	Total developmental period (in days)	No. of cocoons	No. of emerged adults		% emergence	Sex ratio	Longevity(in days)	
							Female	Male		♀ : ♂	Female	Male
Profenophos	$L.C_{50}$	40	13.20	8.29	22.40	17.00	5.00	8.00	76.47	1:1.60	5.75	6.13
	$L.C_{25}$	40	11.04	7.35	18.49	25.00	7.00	11.00	72.00	1:1.57	6.80	8.71
Mean			12.11^{b}	7.81^{b}	20.44^{a}	21.00^{d}	6.00^{c}	9.50^{c}	74.23^{c}		6.27^{b}	7.42^{b}
Lufenuron	$L.C_{50}$	40	13.35	9.35	22.70	20.00	6.00	8.00	70.00	1:1.30	6.14	8.77
	$L.C_{25}$	40	12.83	8.90	21.73	28.00	8.00	12.00	71.43	1:1.50	7.30	9.75
Mean			13.09^{a}	9.12^{a}	22.21^{a}	24.00^{c}	7.00^{c}	10.00^{c}	70.71^{a}		6.72^{b}	9.26^{ab}
B .th	$L.C_{50}$	40	12.00	6.14	18.14	19.00	7.00	9.00	84.21	1:1.29	7.50	9.6
	$L.C_{25}$	40	11.15	5.28	16.43	34.00	12.00	16.00	82.35	1:1.30	9.17	10.0
Mean			11.57^{c}	5.71^{c}	17.45^{b}	26.50^{b}	9.50^{b}	12.50^{b}	83.28^{b}		7.33^{ab}	9.80^{ab}
Cont.		40	10.02^{b}	4.92^{d}	15.29^{c}	37.00^{a}	12.00^{a}	22.00^{a}	91.89^{a}	1:1.83	9.3^{a}	10.92^{a}
Means of concentrations.		50	12.14^{a}	7.17^{a}	19.65^{a}	23.25^{b}	7.50^{b}	11.75^{b}	81.14^{a}		7.17^{a}	8.85^{a}
		25	11.26^{b}	6.61^{b}	18 44^{b}	31.00a	9.75^{a}	10.25^{a}	79.41^{a}		7.64^{a}	9.84^{a}
L.S.D ...• for pesticides =			0.542	0.411	2.119	2.347	2.547	2.447	2.247		2.447	NS
L.S.D ...• for concentrations =			0.383	0.290	1.498	1.530	1.830	1.730	NS		1.730	NS
L.S.D ...• for (P XC.)-			0.794	NS	NS	3.494	NS	NS	3.026		NS	NS

- in ppm

Table. 3 . Effect of sublethal concentrations* of profenophos, lufenuron and *B .th.* on development of the endoparasitoid *M. rufiventris* after 24-48 hrs from parasitoidism on second instar larvae of *S. littoralis* .

Insecticides	Tested conc.	No of parasitized – larvae	Egg- larval duration (in days)	Pupal duration (in days)	Total developmental period (in days)	No. of cocoons	No. of emerged adults . Female	Male	% emergence	ratio ♀ : ♂	Longevity(in days) Female	Male
Profenophos	$L.C_{50}$	40	12.46	7.71	20.17	15.00	5.00	6.00	73.33	1 : 1.20	4.10	4.0
	$L.C_{25}$	40	11.78	7.01	18.79	23.00	6.00	10.00	69.57	1 : 1.67	5.63	5.87
Mean			12.12[a]	7.27[b]	19.48a	19.00[c]	5.50[b]	8.00[c]	71.45[c]		4.86[b]	4.93[b]
Lufenuron	$L.C_{50}$	40	13.50	8.34	21.84	17.00	4.00	7.00	64.71	1 : 1.75	4.88	5.5
	$L.C_{25}$	40	11.85	7.56	19.41	24.00	7.00	10.00	70.83	1 : 1.43	6.54	6.80
Mean			12.67[a]	7.95[a]	20.23a	20.50[bc]	5.50[b]	8.50[c]	67.77[d]		5.71[b]	6.15[a]
B .th	$L.C_{50}$	40	12.24	7.27	19.61	16.00	6.00	8.00	87.50	1 : 1.30	6.00	6.50
	$L.C_{25}$	40	11.97	6.22	18.19	29.00	9.00	15.00	82.76	1 : 1.67	6.82	6.88
Mean			12.18[a]	6.83[c]	18.90a	22.50[b]	7.06[b]	11.50[b]	85.04[b]		6.41[b]	6.60[ab]
Cont .		40	10.02[b]	4.92[d]	15.29[c]	37.00[a]	12.00[a]	22.00[a]	91.89[a]	1:1.83	9.30[a]	10.92[a]
Means of concentrations		50	12.08[a]	7.02[a]	19.22a	21.25[b]	6.75[b]	10.45[b]	79.31[a]		6.07[a]	6.73[a]
		25	11.36[b]	6.44[b]	17.92a	28.25[a]	8.50[a]	14.25[a]	78.67[a]		7.07[a]	7.61[a]
L.S.D ..... for pesticides =			0.561	0.401	2.310	2.447	2.247	2.436	2.488		2.547	2.425
L.S.D ..... for concentrations =			0.383	0.273	NS	1.730	1.530	1.630	NS		NS	NS
L.S.D ..... for (P XC.)-			0.767	NS	NS	3.494	NS	NS	3.55		NS	NS

- in ppm

Table 4. Effect of sublethal concentrations* of profenophos. lufenuron and *B .th.* on development of the endoparasitoid *M. rufiventris* after 48-72 hrs from parasitoidism on second instar larvae of *S. littoralis* .

Insecticides	Tested conc.	No of parasitized larvae	Egg- larval duration (in days)	Pupal duration (in days)	Total developmental period (in days)	No. of cocoon	No. of emerged adults . Female	Male	% emergence	Sex / ratio ♀ : ♂	Longevity(in days) Female	Male
Profenophos	$L.C_{50}$	40	11.03	7.48	18.51	19.00	6..00	9.00	78.95	1 : 1.5	6.20	7.00
	$L.C_{25}$	40	11.18	7.20	18.38	28.00	8.00	13.00	75.00	1 : 1.63	8.00	8.06
Mean			11.10a	7.33a	18.44a	23.50^{b}	7.00^{b}	11.00^{b}	76.97^{c}		7.10^{a}	7.53^{b}
Lufenuron	$L.C_{50}$	40	12.51	8.08	20.59	20.00	6.00	9.00	75.00	1 : 1.5	8.00	9.10
	$L.C_{25}$	40	11.39	7.09	18.48	29.00	8.00	13.00	72.41	1 : 1.63	9.00	9.50
Mean			11.94a	7.58a	19.53a	24.50^{b}	7.00^{b}	11.00^{b}	73.70^{ad}		8.50^{a}	8.88^{ab}
B .th	$L.C_{50}$	40	11.37	6.52	17.99	20.00	7.00	10.00	85.00	1 : 1.43	8.20	8.05
	$L.C_{25}$	40	10.99	6.05	17.04	30.00	9.00	16.00	83.33	1 : 1.78	8.89	9.22
Mean			10.15a	6.28b	17.51ab	25.00^{b}	8.00^{b}	13.00^{b}	84.16^{b}	84.16^{b}	9.09^{a}	8.95^{ab}
Cont .		40	10.02^{b}	4.92^{d}	15.29^{c}	37.00^{a}	12.00^{a}	22.00^{a}	91.89^{a}	1:1.83	9.3^{a}	10.92^{a}
Means of concentrations		50	11.28^{a}	6.75^{a}	18.09a	24.00^{b}	7.75^{a}	12.25^{b}	82.71^{a}		8.20^{a}	8.79^{a}
		25	10.33^{a}	6.31^{b}	17.29a	31.00^{a}	9.25^{a}	16.00^{a}	80.65^{b}		8.70^{a}	9.29^{a}
L.S.D ..... for pesticides =			NS	0.324	2.316	2.447	2.667	2.578	2.537		NS	NS
L.S.D ..... for concentrations =			NS	0.229	NS	1.730	NS	1.580	1.790		NS	NS
L.S.D ..... for (P XC.)=			NS	0.473	NS	3.494	NS	NS	NS		NS	NS

* in ppm

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سمية بروفينوفوس لوفينورون ، باسيللس ثورينجنسز للطفيل ميكروبليتس روفينفنترس

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يهدف هذا البحث إلى تقويم حساسية أحد الطفيليات الداخلية لدودة ورق القطن *Microplitis rufiventris* Kok. لثلاث من المبيدات الحشرية هي المركب الفوسفوري profenophos و المبيد المثبط لتخليق الكيتين lufenuron و المبيد الحيوي *Bacillus thuringiensis kurstaki* ومدي تأثير التطفل علي حساسية العائل للمبيدات المذكورة .

أوضحت النتائج أن التطفل يزيد من حساسية العائل للمبيدات سالفة الذكر . عند متابعة بيولوجية الطفيل بالأحياء من اليرقات الناتجة من التعرض لمستويين من الموت – هما 25 و 50 ٪ من اليرقات المعاملة – وجد أن المبيدات الثلاث أدت إلى إطالة فترات الأطوار الداخلية بالعائل (طوري البيضة و اليرقة) وكذلك طور العذراء . أدت المعاملة بالمبيدات كذلك إلى قلة عدد الشرانق المتكونة مما يشير إلى السمية الحادة للمبيدات علي طوري البيضة و اليرقة . وجد كذلك سمية متأخرة للمبيدات الثلاثة حيث قلت النسبة المئوية لخروج الحشرات الكاملة وقصر فترة حياتها . تفوق المبيد المثبط لتخليق الكيتيني علي المبيد التقليدي الفوسفوري في إحداث الأضرار سابقة الذكر وكان المبيد الحيوي أقلها تأثيرا . الفترة المحصورة بين بداية التطفل وتعريض العائل للمبيدات لم تلعب دورا ملموسا في هذا الشأن .