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Effect of chlorpyrifos/diflubenzuron (*) and gamma irradiation on the relative susceptibility of *Spodoptera littoralis* larvae to some insecticides

Abstract - Laboratory studies were carried out to evaluate whether irradiation of pupae resulting from larvae reared on untreated or Empire treated diet, could alter the susceptibility of F_1 fourth instar larvae of *S. littoralis* to some insecticides. Substerilizing doses (25, 50 and 100 Gy) of gamma radiation were used for pupal irradiation. The results revealed that: (1) pupal irradiation alone or preceded by chlorpyrifos/diflubenzuron (Empire) treatment had the same effect, both equally decreased the tolerance of F_1 larvae to deltamethrin (Decis) and cyanophos (Cyanox), but treatment with Empire alone didn't affect the response of these larvae; (2) no differences in the level of susceptibility of F_1 larvae to methomyl/diflubenzuron (Deenate) or chlorpyrifos/Dowco 439 (Delfos) was observed whether Empire and irradiation with lower doses were applied in a combination or alone; (3) the susceptibility of F_1 larvae to Empire wasn't affected as a result of separate treatments with Empire or irradiation, while insecticide-irradiation combination greatly enhanced it's effectiveness; and (4), the effect of irradiation alone was more pronounced in increasing the toxicity of chlorpyrifos (Dursban); cyfluthrin (Baythroid) and thiodicarb (Larvin), but when combined with Empire, different levels of tolerance to those insecticides were noticed. Thus, gamma irradiation when applied alone has increased the susceptibility of *S. littoralis* F_1 larvae to all tested insecticides, except Empire. Furthermore, F_1 larvae from two irradiated parents had some relative susceptibility to the tested insecticides, the degree was probably great enough to achieve more favorable integrated control by the application of any one of these insecticides in the field, being treated by the release of sterile moths of the cotton leaf worm, *S. littoralis*.

Riassunto - *Effetto di chlorpyrifos/diflubenzuron e radiazioni gamma sulla sensibilità delle larve di* Spodoptera littoralis *ad alcuni insetticidi.*
Esperimenti di laboratorio sono stati effettuati per verificare se l'irradiazio-

(*) Commercial name: Empire.

ne di pupe derivanti da larve allevate su una dieta artificiale trattata o meno con la miscela di chlorpyrifos/diflubenzuron (Empire) può modificare la sensibilità di larve di quarta età di F_1 a diversi insetticidi. I risultati ottenuti hanno evidenziato quanto segue: (1) l'irradiazione delle pupe ha il medesimo effetto, sia su individui non trattati che su quelli alimentati con Empire; in ogni caso diminuisce la tolleranza delle larve F_1 a deltametrina e cyanophos; (2) nessuna differenza di sensibilità delle larve F_1 nei riguardi di chlorpyrifos/Dwoco 439 e di methomyl/diflubenzuron è stata osservata se sono somministrate basse dosi di Empire e di raggi gamma, sia in combinazione tra loro che singolarmente; (3) la sensibilità delle larve F_1 ad Empire si manifesta come il risultato di un trattamento separato di Empire o di irradiazione, mentre la combinazione dei due trattamenti aumenta grandemente l'efficacia; (4) l'effetto dell'irradiazione aumenta la tossicità di chlorpyrifos, cyfluthrin e thiodicarb ma, se viene combinato con Empire, si hanno differenti livelli di tolleranza a questi insetticidi. Nel complesso, il trattamento con raggi gamma, se applicato da solo, incrementa la sensibilità delle larve F_1 di *S. littoralis* a tutti gli insetticidi utilizzati, tranne che Empire. Tuttavia, le larve F_1 che derivano da coppie irradiate presentano una suscettibilità maggiore agli insetticidi sperimentati; ciò risulta di particolare interesse nella difesa integrata del cotone, con uso in campo di questi insetticidi e con la liberazione di individui sterili di *S. littoralis*.

Key words: Gamma radiation, insecticides, combination of irradiation and insecticides, *Spodoptera littoralis*.

INTRODUCTION

Excessive use of pesticides has led to several long-term problems and deterioration of environmental quality. The studies on the combined effects of ionising radiation and insecticides indicate that the insecticidal toxicity can be altered if the insects are exposed to radiation preceding or following insecticidal treatment (Rush & Ware, 1969; Wolfenbarger & Graham, 1970; Moffitt & White, 1972; Bhatia & Sethi, 1980; Rizvi, 1980; El-Shal, 1983; Shanbaky et al., 1987; Selim et al., 1987 and Abdel-Salam, 1991). The use of sterilization techniques by gamma irradiation to control harmful insects to plant crops is one of the promising approaches which has been introduced to the integrated pest control programs. Before planning for a sterile-insect-release program against *S. littoralis*, it is very important to study the effects of insecticides on the irradiated released moths or their larval progeny in case that they mate with native moths.

The present study was therefore conducted in an attempt to determine the efficiency of combined treatments of chlorpyrifos/diflubenzuron (Empire) and gamma irradiation, compared with Empire or gamma irradiation, each applied alone, in altering susceptibility of *S. littoralis* F_1 fourth instar larvae to some insecticides. Such information is needed to predict the outcome of insecticide control programs when incorporated into sterile-insect release programs.

MATERIALS AND METHODS

A set of newly moulted 4th instar larvae of *S. littoralis* was obtained from the laboratory strain from the Central Agricultural Pesticides Laboratory Dokki, Egypt, for treatment with chlorpyrifos/diflubenzuron (Empire). Dipping technique was used, in which fresh castor bean leaves were dipped in a water dilution of EC 10 (a concentration that produces 10% inhibition of normal adult development of Empire) for 10 seconds. The treated leaves were left to air dry before being offered to the larvae. The larvae were fed on treated leaves for 24 hrs, and then allowed to feed on untreated castor bean leaves till pupation. Another set of P_1 larvae were fed on leaves dipped in water only. The resulting full grown pupae (2-3 days before emergence), from the two sets, treated and untreated larvae, were divided into two groups, the first one, from each set, was subjected to substerilizing doses of 25, 50 and 100 Gy of gamma irradiation emitted by a cobalt-60 source, which gave an average exposure of about 5.50 rad/sec. The second group was kept untread. The Empire treated pupae (T), irradiated pupae (I), Empire-irradiated pupae (TI) and untreated pupae (N) of both sexes were kept separately till emergence. To determine the effect of treatments on each sex, the following reciprocal matings between treated and untreated adults were made: (1) T ♂ × N ♀, (2) N ♂ × T ♀, (3) 1 ♂ × N ♀, (4) N ♂ × 1 ♀, (5) TI ♂ × N ♀, (6) N ♂ × TI ♀, (7) N ♂ × N ♀. Five replicates (5 pairs each), from each mating combination and each level of irradiation, were put in glass jars supplied with branches of Tafla and sugar solution. The same dipping technique was used to test the toxicity of different insecticides to fourth instar larvae resulting from selection for only one generation. Six concentrations of each insecticide were prepared for each treatment and 3 replicates with 10 larvae were used for each toxicant dilution. Insecticides used represent 3 broad classes of insecticides currently used in *S. littoralis* control (phosphates, carbamates and synthetic pyrethroid). 1- Organophosphorus compounds: chlorpyrifos/diflubenzuron 51% FL (Empire), chlorpyrifos 48% EC (Dursban), chlorpyrifos/Dowco 439 5% EC (Delfos) and cyanophos 50% EC (Cyanox). 2- Carbamate: methomyl/diflubenzuron 31% FL (Deenate) and thiodicarb 80% DF (Larvin). 3- Synthetic pyrethroids: deltamethrin 2.5% EC (Decis) and cyfluthrin 5% EC (Baythroid). The data for the mortality-regression lines of insecticides were subjected to probit analysis of Finney's method (1970). The tolerance ratio (TR) was estimated as follows:

$$TR = \frac{LC_{50} \text{ of the treated insects}}{LC_{50} \text{ of the untreated insects}}$$

RESULTS AND DISCUSSION

According to data summarized in Table 1, it is worthy to note that while most of the individuals of the F_1 larvae produced by female pupae irradiated with 50 and 100 Gy failed to reach to the 4th instar larvae, treatment of P_1 larvae with Empire prior to female pupal irradiation with 50 Gy resulted in enough number of F_1 larvae required for different treatments. These results were explained by Abu-Youssef et al., (1984) on *Drosophila melanogaster.* They suggested that chlorpyrifos can interfere with the recovery process from genetic damage induced by gamma-irradiation.

Organophosphorus insecticides:

1) *Empire and Dursban*

The values of LC_{50} revealed that neither treatment with Empire against the parent larval stage (P_1 larvae) nor gamma irradiation alone of the parent pupal stage (P_1 pupae), affected the response of F_1 larvae to Empire, while combination of both treatments increased its efficacy when both male and female parents were treated (Table 1). On the other hand, treatment with Empire didn't alter the response of F_1 larvae to Dursban also, but gamma irradiation with the doses of 25 and 50 Gy enhanced it's effectiveness. However, treatment with Empire followed by gamma irradiation with the same doses, increased the tolerance of F_1 larvae to Dursban, in treated males. In the females, whether treated with Empire alone or followed by gamma irradiation with 50 Gy, the response of the F_1 larvae increased to Dursban with the same level (TR = 0.67 fold). Treatment with a dose of 25 Gy alone induced an increase in the tolerance of F_1 larvae, but when it was preceded by insecticidal treatment the response of these larvae to Dursban was not affected.

2) *Delfos*

Results in Table 1 indicated that either insecticidal treatment, gamma irradiation alone or combination of both treatments, decreased tolerance of F_1 larvae to Delfos with similar level, where TR ranged from 0.12-0.75 fold in both treated males and females.

Table 1 - Effect of Empire and gamma irradiation on the relative susceptibility of the 4th instar F_1 larvae of S. littoralis *to some insecticides.*

Treatment*	Insecticides used**	Treated male × untreated female		Untreated male × treated female	
		LC_{50}	TR***	LC_{50}	TR***
		Organophosphorus insecticides			
Control		15.3	—	15.3	—
Empire		15.3	1.0	15.3	1.0
25 Gy		15.3	1.0	15.3	1.0
50 Gy	Empire	15.3	1.0	—	—
100 Gy		15.3	1.0	—	—
Empire + 25 Gy		10.2	0.67	10.2	0.67
Empire + 50 Gy		10.2	0.67	5.1	0.33
Control		7.2	—	7.2	—
Empire		7.2	1.0	4.8	0.67
25 Gy		4.8	0.67	9.6	1.33
50 Gy	Dursban	4.8	0.67	—	—
100 Gy		7.2	1.0	—	—
Empire + 25 Gy		9.6	1.33	7.2	1.0
Empire + 50 Gy		8.64	1.20	4.8	0.67
Control		20.4	—	20.4	—
Empire		10.2	0.50	5.1	0.25
25 Gy		5.1	0.25	10.2	0.50
50 Gy	Delfos	10.2	0.50	—	—
100 Gy		10.2	0.50	—	—
Empire + 25 Gy		15.3	0.75	7.65	0.38
Empire + 50 Gy		10.2	0.50	2.55	0.12
Control		450.0	—	450.0	—
Empire		500.0	1.11	400.0	0.89
25 Gy		200.0	0.44	250.0	0.56
50 Gy	Cyanox	100.0	0.22	—	—
100 Gy		50.0	0.11	—	—
Empire + 25 Gy		200.0	0.44	300.0	0.67
Empire + 50 Gy		150.0	0.33	200.0	0.44
		Carbamate insecticides			
Control		62.0	—	62.0	—
Empire		31.0	0.50	18.6	0.30
25 Gy		24.8	0.40	24.8	0.40
50 Gy	Deenate	24.8	0.40	—	—
100 Gy		93.0	1.50	—	—
Empire + 25 Gy		31.0	0.50	24.8	0.40
Empire + 50 Gy		46.5	0.75	15.5	0.25
Control		20.0	—	20.0	—
Empire		10.0	0.50	10.0	0.50

(continued Table 1)

Treatment*	Insecticides used**	Treated male × untreated female		Untreated male × treated female	
		LC_{50}	TR***	LC_{50}	TR***
25 Gy		24.0	1.20	24.0	1.20
50 Gy	Larvin	16.0	0.80	—	—
100 Gy		8.0	0.40	—	—
Empire + 25 Gy		24.0	1.20	40.0	2.00
Empire + 50 Gy		48.0	2.40	16.0	0.80
	Synthetic pyrethroid				
Control		1.0	—	1.0	—
Empire		1.0	1.00	1.0	1.00
25 Gy		0.25	0.25	0.25	0.25
50 Gy	Decis	0.50	0.50	—	—
100 Gy		0.80	0.80	—	—
Empire + 25 Gy		0.25	0.25	0.25	0.25
Empire + 50 Gy		0.50	0.50	0.20	0.25
Control		0.50	—	0.50	—
Empire		0.70	1.40	0.70	1.40
25 Gy		0.40	0.80	1.00	2.00
50 Gy	Baythroid	0.25	0.50	—	—
100 Gy		0.50	1.00	—	—
Empire + 25 Gy		0.90	1.80	0.50	1.00
Empire + 50 Gy		0.50	1.00	0.25	0.50

* Empire applied to P_1 fourth instar larvae and irradiation applied to P_1 pupae.
** Insecticides applied to F_1 fourth instar larvae.
*** TR = Tolerance Ratio.

3) *Cyanox*

Toxycity of Cyanox to F_1 larvae produced from treating the larval stage of the male parents with Empire was less than its toxicity to untreated larvae, the LC_{50} was 500, and 450 ppm, respectively. However, toxicity of Cyanox to F_1 larvae was increased gradually by increasing the doses of gamma radiation delivered to male pupae, the LC_{50} values were 200, 100 and 50 ppm for doses of 25, 50 and 100 Gy, respectively, as compared with 450 ppm for untreated control. Moreover, treatment with Empire in the larval stage preceding pupal irradiation showed the same effect. On the other hand, the three kinds of treatments against the female parents increased the response to Cyanox (Table 1).

The increased susceptibility of *S. littoralis* F_1 larvae to these organophosphate insecticides as a result of irradiation in their parental pupae coincides with Rush & Ware (1969) who found an increase in the susceptibility to azinphosmethyl in pink bollworm adults emerging from pupae irradiated with 100 Gy of gamma radiation. Also, irradiation of pupae increased susceptibility of *S. littoralis* F_1 4th instar larvae to chlorpyrifos (El-Shal, 1983) and *S. littoralis* adults to pyridaphenthion and fenitrothion (Shanbaky et al., 1987).

Carbamate insecticides:

1) *Deenate*

Toxicity of Deenate to F_1 larvae resulting from treated male and female parents was increased to the same level, whether Empire and gamma irradiation with 25 and 50 Gy were applied separately or in a combination, where TR for those types of treatments was less than 1.0 fold. However, increasing the dose of irradiation alone, to male pupae (100 Gy) increased tolerance of F_1 larvae to Deenate (Table 1).

2) *Larvin*

Although treatment of male or female parents larvae with Empire, decreased the tolerance of F_1 larvae to Larvin with the same level (TR=0.5 fold), pupal irradiation with 25 Gy applied alone or preceded with insecticidal treatment, reduced its effectiveness. (TR ranged from 1.2-2.0 fold). The tolerance of F_1 larvae to Larvin decreased gradually with increasing radiation doses, against the male parent pupae, from 25 to 100 Gy, where the LC_{50} decreased from 24 to 8 ppm.

Similarly, irradiation of pupae increased the susceptibility of *S. littoralis* to methomyl (Shanbaky et al., 1987) and *Tribolium confusum* to Duradin (Abdel-Salam, 1991). On the other hand, Rush & Ware (1969) found a slight increase in tolerance to carbaryl in pink bollworm adults emerging from pupae irradiated with 100 Gy, El-Shal (1983) found that susceptibility of F_1 fourth instar larvae of *S. littoralis* produced from unirradiated females mated to males irradiated as pupae with 50-300 Gy, to methomyl, gradually decreased with increasing radiation doses.

Synthetic pyrethroids

1) *Decis*

The results in Table 1 show that toxicity of Decis to F_1 larvae wasn't affected when male or female parents were treated in their larval stage with Empire. The susceptibility of F_1 larvae to Decis increased with the same level whether pupal irradiation (25 and 50 Gy) was applied alone or in a combination with Empire, TR ranged from 0.25-0.50 fold.

2) *Baythroid*

The same table illustrates that in both sexes, treatment with Empire suppressed the effectiveness of Baythroid against F_1 larvae (TR = 1.4 fold) while irradiation of male pupae with 25 or 50 Gy increased its toxicity where TR was 0.8 and 0.5 fold, respectively. Moreover, when the male pupae result from treated P_1 larvae with Empire was irradiated with 25 Gy, the tolerance of F_1 larvae to Baythroid was increased again (TR = 1.8 fold), but by increasing the irradiation dose to 50 Gy the response of these larvae was not altered (TR = 1.0 fold). The F_1 larvae resulting from female parents irradiated in their pupal stage with 25 Gy showed the highest level of tolerance to Baythroid (TR = 2.0 fold), but when this treatment was preceded by Empire the degree of susceptibility was not altered (Table 1).

These results are in agreement with Selim et al. (1987), who found that the susceptibility of F_1 larvae *S. littoralis* to fenvalerate was gradually increased by increasing the radiation doses for male parent pupae. El-Sayed et al. (1988), showed that gamma irradiation when applied at 80 Gy to the adults of *Callosobruchus maculatus* increased the toxicity of sumicidin but didn't affect the toxicity of ripcord. Rizvi 1980, suggested that the alteration in response may be due to an internal stimulus due to radiation which can not be solely attributed to the loss of detoxification mechanism.

From the obtained results it can be generally concluded that toxicity of all tested insecticides to *S. littoralis* was altered detectably in F_1 fourth instar larvae from parent moths treated in their larval stage with Empire and/or subjected to irradiation substerilizing doses, during pupal stage. There were some differences in response between the two sexes; F_1 larvae resulting from treated female parents were either more susceptible to the tested insecticides than treated males or equally susceptible (Table 1). However, irradiation applied alone increased the susceptibility of *S. littoralis* F_1 larvae to all tested insecticides, except Empire, as compared with treatment with Empire alone or followed by gamma irradiation. The lower dose of irradiation, 25 Gy, usually resulted in a greater degree of insecticide susceptibility than that of the larger doses (50 and 100 Gy). Furthermore, F_1 larvae from irradiated both parents had some relative susceptibility to the tested insecticides, the degree was probably great enough to achieve more favorable integrated control by the application of any one of these insecticides in the field, being treated by the release of sterile moths of the cotton leaf worm, *S. littoralis*.

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