

**TOXICITY OF SOME INSECTICIDES AND  
INSECTICIDE COMBINATIONS ON *PLUTELLA  
XYLOSTELLA* (LINN.)<sup>1</sup>**

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**ABSTRACT**

Four insecticides (cypermethrin, deltamethrin, chlorfluazuron and triazophos) and their joint actions were tested on *Plutella xylostella* (Linn) population from La Trinidad, Benguet. Tests show chlorfluazuron, a new chitin synthesis inhibitor, as the most toxic. The other compounds are effective against the insect; however, traces of resistance build-up were noted against deltamethrin and triazophos. Joint actions show the potentiation of cypermethrin + deltamethrin, cypermethrin + chlorfluazuron and deltamethrin + chlorfluazuron at LD<sub>25</sub> + LD<sub>25</sub> and at LD<sub>5</sub> + LD<sub>25</sub> combinations. Chlorfluazuron + triazophos exhibited strong antagonism.

**Key Words:** Joint action, potentiation, synergism, antagonism

**INTRODUCTION**

Diamondback moth (DBM), *Plutella xylostella* (Linn.), is a serious insect pest of crucifers and one of the most resistant insect species to insecticides. Reports show the insect resistant to organochlorines, organophosphates (OP), carbamates and lately, to synthetic pyrethroids (Magallona 1977-78, Barroga 1980, Sun et al. 1983). The development of resistance was attributed to the ability of the insect to detoxify the compounds.

Sun et al. (1983) investigated the biochemical basis of this mechanism and reported the involvement of insensitive acetylcholinesterase in OP and carbamate resistance, and that of mixed function oxidases and hydrolases in synthetic pyrethroid resistance. The wide-range development of resistance from OP to synthetic pyrethroids can be traced to what Beeman (1982) postulated as the genetic linkage between OP and pyrethroid resistance. This was supported by the report of Gammon and Holden (1979) and Gammon (1980) that an Egyptian cotton leafworm resistant to OP is also resistant to permethrin. In the Philippines, pyrethroid resistance in DBM remains undocumented.

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Such rapid resistance development of DBM against chemical compounds prompted the research and development of other compounds, thus, the birth of insect growth regulators (IGR), juvenile hormones (JH), and botanical compounds. Likewise, insecticide mixtures became common among farmers. The toxicity of a mixture containing insecticide A and B, for example, is not uniquely determined by the toxicity of A and B applied separately as what most farmers believe. Hence, the danger of antagonism (negative synergism) poses a problem. Using insecticide combinations among farmers could either be on a 'hit or a miss' basis. So far, several studies have been done to give a more scientific explanation of the toxicity of insecticide combinations. Morallo-Rejesus & Eroles (1976) paired several compounds and tested them on DBM while Barroga (1980) studied the mechanism of joint action of mixtures on malathion-resistant DBM.

This study was conducted at the Dept. of Entomology, UPLB to compare the topical activity of four insecticides and their combinations on DMB population of La Trinidad, Benguet, to determine joint action of the combinations, and to determine which of the test compounds and combinations could serve as a promising control agent of *Plutella*.

## MATERIALS AND METHODS

### Rearing of test insects

Larvae and pupae of *Plutella* were collected from La Trinidad, Benguet and reared in the laboratory. Adult emergence was allowed inside 16 x 16 x 16 in cages. Cotton wads soaked in sugar solution were placed inside the cages for the adults to feed on. Potted pechay plants were placed inside the cages for adult oviposition. These plants were changed every four hours to better monitor larval age. Test insects were reared continuously for the duration of the experiment. Host plants were likewise maintained throughout the rearing period through staggered planting.

### Testing and determination of the effects of insecticides and combinations

Graded concentrations of insecticides (Table 1) were dissolved in acetone and topically applied on the thorax of fourth instar larvae using an electrically driven microliter syringe at a rate of 0.04 ul per larva. Lethal dose values at 50% of test population ( $LD_{50}$ ) for each treatment were determined using probit analysis by Finney (1952). Each treatment consisted of 10 larvae for the initial bracketing test and 20 insects for the final tests. There were two replicates per treatment for three trials. Treated larvae were placed in Petri dishes with pechay leaves. Mortalities were recorded after 24 hours and continued until adult emergence for those larvae receiving sublethal dose. For chlorfluazuron-treated larvae,  $ED_{50}$  (effective dose causing 50% mortality-abnormality in test population) was calculated using data

Table 1. Insecticides used in the study.

Chemical name	Date of application a.i. per hectare	Chemical designation
cypermethrin	0.02 - 0.085 kg	(±) alpha-cyano-3-phenyxybenyl (±) cis, trans 3-(2,2-dichlorovinyl)- 2,2-dimethyl cyclopropane carboxylate
deltamethrin (decamethrin)	7.5 - 25 g	(S)-alpha-cyano-M-phenoxybenyl (1R, 3R)-3-(2,2-dibromovinyl)-2, 2-dimethyl cyclopropane-carboxylate
triazophos	0.02 - 1%	1-phenyl-3-(0,0 diethyl-thionosphos- phate)-1, 2, 4 - triazole
chlorfluzaron	0.0025% - 0.01%	N-[ [ [ 4- [ [ 3-chloro-5-(trifluoro- methyl)2-pyridinyl] oxy ] -3,5- dichlorophenyl ] amino ] carbonyl ] 2, 6-difluorobenzamide

obtained four days after treatment since it is a slow-acting compound. Number of eggs ( $F_2$ ) laid by surviving  $F_1$  adults were also counted to evaluate fecundity of treated insects.

To test the effect of insecticide combinations, the expected  $LD_{25}$  dosage (estimated from the plotted % mortality vs. dosage in the log-probit graph) of each of the insecticide and  $LD_5$  for the more potent +  $LD_{25}$  for the less toxic compound were used in the paired combinations. Following the same procedure as in single insecticide testing, only one dosage was tested on fourth instar larvae. The type of joint action was identified using Mansour et al. (1966) equation:

$$\text{Co-toxicity factor} = \frac{\text{observed \% mortality} - \text{expected \% mortality}}{\text{expected \% mortality}} \times 100$$

where +20 — potentiation  
 -20 — antagonism  
 +20 to -20 — additive effect

A 20% difference was allowed from the theoretical expected value for the experimental and biological variations because relatively few insects were used.

## RESULTS AND DISCUSSION

**Effect of single insecticide use.** Table 2 and Figure 1 show the toxicity values of the test compounds topically applied on the fourth instar DBM

Table 2. LD<sub>50</sub> (mg/g) of the test compounds on *Plutella xylostella* (Linn.)

Insecticide	LD <sub>50</sub> <sup>1</sup>	LD <sub>50</sub> Fiducial limits	Slope	SE slope
Cypermethrin	0.049	0.035 - 0.069	1.372	0.27
Deltamethrin	0.017	0.011 - 0.026	0.982	0.19
Chlorfluazuron	0.002	0.0027 - 0.0013	2.008	0.28
Triazophos	0.091	0.071 - 0.118	1.876	0.25

<sup>1</sup>Effective dose (ED<sub>50</sub>) for chlorfluazuron.

larvae. Since chlorfluazuron has a different mode of action from the other three standard insecticides, it will be discussed separately.

Based on the LD<sub>50</sub>, the order of toxicity is as follows:

deltamethrin > cypermethrin > triazophos.

Deltamethrin is twice more toxic than cypermethrin, and five times more than triazophos. Magallona (1977-78) observed similar trends in OP and synthetic pyrethroids. However, there has been a significant change from 1977-78 data, with the LD<sub>50</sub> of deltamethrin increasing 33-fold and triazophos seven-fold in the present study. This suggests the build-up of resistance against these insecticides in DBM.

In this study, synthetic pyrethroids are toxic to DBM and more effective than OP. However, at LD<sub>95</sub>, deltamethrin became the least toxic as predicted from the ld-p line with a slope (b) = 0.982 which is very low as compared with the others. This low slope value may indicate the inherent ability of the insect to tolerate deltamethrin more than the other two chemicals (Hoskuis and Gordon 1956). For cypermethrin and triazophos, ld-p line approached each other at higher dosage levels.

On the other hand, chlorfluazuron being an IGR, is a slow-acting compound and exerts its effects on the insect cuticle. Ingestion of the chemical makes it effective even at low concentrations; although it can act as a contact poison at higher concentrations. ED<sub>50</sub> is used in determining toxicity instead of LD<sub>50</sub> and this was based on data obtained 96 hours after treatment on the assumption that pupal development is completed by this time. Belonging to the benzoylphenyl urea group, chlorfluazuron has a similar mode of action as diflubenzuron and penfluron. Visible effects are mostly seen at the later part of the pupal stage. As shown in Table 3, maximum effects of this compound are seen between 72-96 hours after treatment. Treated larvae failed to molt normally and resulted to larval-pupal intermediates or deformed pupae during the first 48 hours. Later on these insects shrivel and then they dry up. Emerging adults were deformed and lived only for a few hours.

Earlier studies claim no direct ovicidal effects of chlorfluazuron, but treated adults lay non-viable eggs (Scheurer et al 1983, Colting 1985). Similar findings were observed in this study where eggs treated with chlorfluazu-

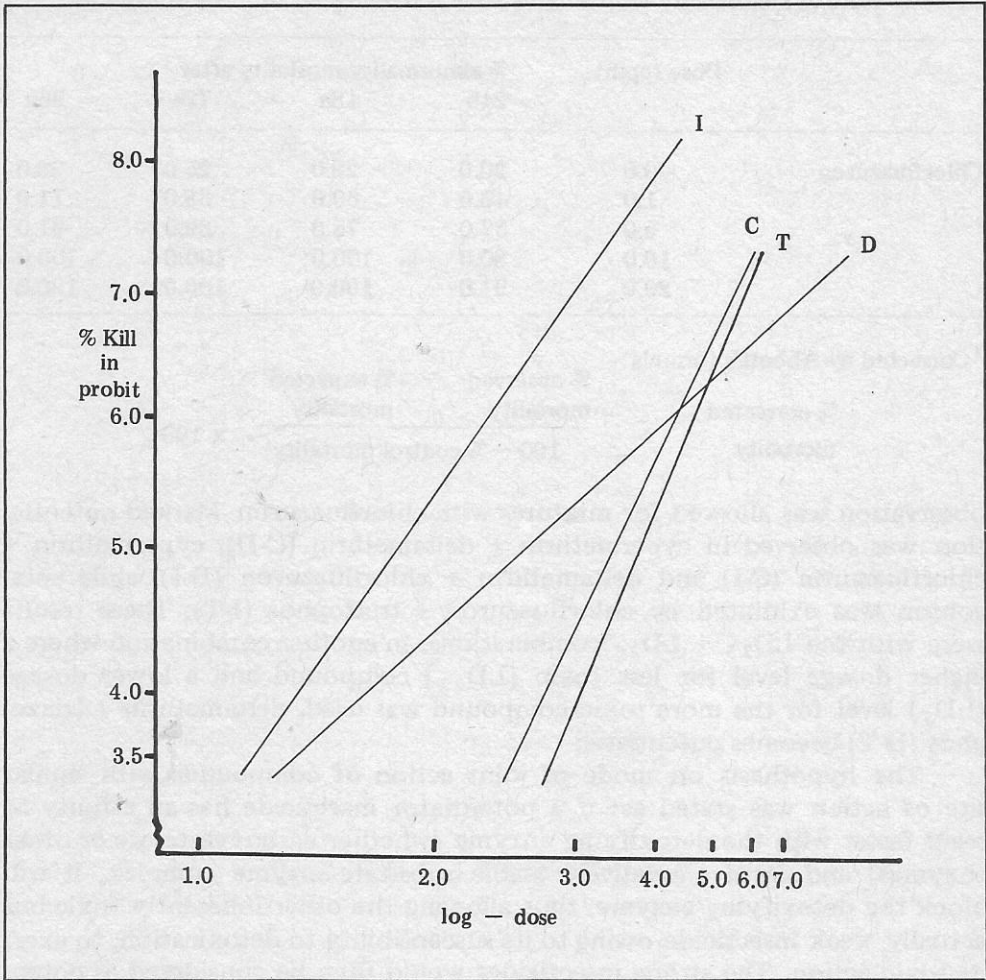


Figure 1. Ld-p lines of cypermethrin (C), deltamethrin (D), chlorfluazuron (I), and triazophos (T) tested on DBM.

ron at 0.5 ppm normally hatched. Probably at higher concentrations, chlorfluazuron acts as a contact poison. Non-viability of eggs may be correlated with the involvement of chitin in egg formation. Chang and Borkovec (1980) claimed that benzoylphenyl ureas are translocated into flies' eggs. However, sterility in houseflies is temporary since the compound decreased in concentrations from generation to generation. This is likely to take place in DBM, knowing its detoxifying mechanisms.

**Effect of insecticide combinations.** Synthetic pyrethroids, OP and insect growth regulators have different modes of action, and considering the tolerance of this insect species to a wide range of compounds, different slopes of ld-p lines were obtained (Figure 1 and Table 2). Table 3 shows DBM mortality after 24 hours of treatment with mixtures. Two to four days

Table 3. Rate of abnormality-normality of DBM larvae treated with chlorfluazuron.

	Dose (ppm)	% abnormality-mortality after <sup>1</sup>			
		24h	48h	72h	96h
Chlorfluazuron	0.5	20.0	22.0	25.0	28.0
	1.0	46.0	60.0	68.0	71.0
	5.0	57.0	75.0	89.0	91.0
	10.0	80.0	100.0	100.0	100.0
	20.0	91.0	100.0	100.0	100.0

<sup>1</sup>Corrected by Abbott's formula:

$$\% \text{ corrected mortality} = \frac{\% \text{ observed mortality} - \% \text{ expected mortality}}{100 - \% \text{ control mortality}} \times 100$$

observation was allowed for mixtures with chlorfluazuron. Marked potentiation was observed in cypermethrin + deltamethrin (C-D), cypermethrin + chlorfluazuron (C-I) and deltamethrin + chlorfluazuron (D-I) while antagonism was exhibited by chlorfluazuron + triazophos (I-T). These results were with the LD<sub>25</sub> + LD<sub>25</sub> combinations. In another combination where a higher dosage level for less toxic (LD<sub>25</sub>) compound and a lower dosage (LD<sub>5</sub>) level for the more toxic compound was used, deltamethrin + triazophos (D-T) becomes potentiated.

The hypothesis on mode of joint action of compounds with similar site of action was stated as: if a potentiator insecticide has an affinity to react faster with the detoxifying enzyme (whether carboxyesterase or other enzymes) and form a relatively stable substrate-enzyme complex, it will block the detoxifying enzyme, thus allowing the other inherently toxic but actually weak insecticide owing to its susceptibility to detoxication, to exert its toxic action. The strong insecticides would then be considered as potentiators and the weaker as potentiabile (Rosenberg & Coon 1958). This was likely demonstrated by C-D where marked potentiation was exhibited. In mixtures with different sites of action, different physiological systems of the insect species are acted upon. One component may augment or interfere with that of the other, thereby resulting to either potentiation, addition or antagonism. Generally, a strong insecticide potentiates a weaker one as exhibited by C-I and D-I. However, this may not hold true always as shown by I-T. Two possible mechanisms could have taken place in the mixture; either one component had interfered with the activity of the other or one has been detoxified already before reaching its site of action and the other had released only sublethal doses. Speeds of the acting compound and sites of action are among the contributory factors which influence the toxicity of a mixture.

Mode of joint action can not be generalized for any mixture of a specific insecticide for a specific group species. Le Pelley & Sullivan (1930) (as cited by Sakai 1960) concluded the absence of synergism on rotenone-py-

Table 4. Toxicity of insecticide mixtures on DBM<sup>a</sup>.

Insecticide combination	Expected % mortality	Observed % mortality <sup>b</sup>	Co-toxicity factor <sup>c</sup>	
at LD <sub>25</sub> + LD <sub>25</sub>				
cypermethrin +	deltamethrin	50	85.0	+70.0***
	chlorfluazuron	50	82.0	+64.0***
	triazophos	50	41.0	-18.0**
deltamethrin +	chlorfluazuron	50	54.0	+88.0***
	triazophos	50	41.0	-18.0**
chlorfluazuron +	triazophos	50	36.0	-28.0*
at LD <sub>5</sub> + LD <sub>25</sub>				
cypermethrin +	deltamethrin	30	44.0	+45.0***
	chlorfluazuron	30	54.0	+80.0***
	triazophos	30	34.0	+11.0**
deltamethrin +	chlorfluazuron	30	41.0	+37.0***
	triazophos	30	56.0	+88.0***
chlorfluazuron +	triazophos	30	15.0	-49.0*

<sup>1</sup> Based on 24 h data (96 h for mixtures with chlorfluazuron)

<sup>2</sup> Corrected by Abbott's formula

<sup>3</sup> Calculated using Mansour et al. (1966) equation: where: \*\*\* — potentiation  
 \*\* — additive effects  
 \* — antagonistic effect

rethroid sprays while H. Richardson (as cited by Sakai 1960) found a definite evidence of synergism in the same mixtures. Similar findings were also reported by Morallo-Rejesus & Eroles (1976) where methyl parathion + mevinphos at LD<sub>25</sub> each exhibited marked antagonism but became potentiated at LD<sub>10</sub> each.

Of the four compounds tested, deltamethrin, cypermethrin and chlorfluazuron are considered excellent against DBM with the latter giving the best results. Although chlorfluazuron may be least persistent, its use (IGRs in general) can be dangerous to aquatic life. On the other hand, the use of insecticide mixtures is not encouraged and may not agree with the concept of insect pest management. However, in some instances, mixtures make possible the suppression of resistant insect species. Wolfenbarger & Cantu (1975) stated that mixtures give the best control to several pests with varying susceptibilities to the different components of the mixture. Further, resistant insect species may become susceptible to a combination of toxicants. But proper dosage levels and compatible insecticides or any poison for that matter are very important to consider in using mixtures. Otherwise, such resources are wasted and a contributory factor in pollution.

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