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Acute Toxicity of New Molecular Insecticides to Diamond Back Moth, *Plutella xylostella* (L.)

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Studies were conducted to assess the acute toxicity of new molecular insecticides *viz.,* chlorfenapyr, indoxacarb and profenofos against Diamondback moth (DBM), *Plutella xylostella* (L.) in Tamil Nadu Agricultural University, Coimbatore. The LC_{50} and LC_{95} values of chlorfenapyr, indoxacarb (F_1 to F_{15} generation) and profenofos (F_1 to F_{13} generation) decreased from 2.104 to 0.432 and 16.942 to 7.490 ppm, 8.266 to 2.162 and 69.442 to 20.542 ppm and 20.307 to 9.430 and 149.473 to 70.373 ppm, respectively. Considering the susceptible population of DBM, the tentative discriminating doses (DD) by leaf disc method to third instar larvae arrived at were 7.50, 20.54 and 70.37 ppm for chlorfenapyr, indoxacarb and profenofos, respectively.

Key words: Acute toxicity, LC_{50}, Chlorfenapyr, Indoxacarb, Profenofos, diamond back moth

Diamondback moth (DBM), *Plutella xylostella* (L.) (Lepidoptera; Plutellidae) is a major pest of cruciferous vegetables throughout the world. The pest has become very serious in many regions because of its ability to establish in newer areas, coupled with high reproductive potential, shorter life cycle and the year-round availability of host plants. The crop loss due to DBM varied from 52 to 100 per cent (Calderon and Hare, 1986). The main method of control has been the use of insecticides.

In recent years, due to the availability of early varieties and the prospects of higher market value in the off season, cabbage and cauliflower crops are grown almost throughout the year and because of this, the use of insecticide has increased alarmingly in India. As a result of excessive selection pressure exerted by the intensive use of insecticides, the field population of DBM has become resistant to most of the insecticides. The resistance has been reported from Indonesia, India, Philippines, Australia, South America, Japan, China, Central America and North America. In India, the first incidence of DBM resistance was reported against DDT and parathion (Verma and Sandhu, 1968); but it has since developed resistance to various groups of insecticides (Chandrasekaran and Regupathy 1996; Raju, 1996; Sannaveerappanavar and Viraktamath, 1997; Mohan and Gujar, 2000; Singh, 2002). At present, several new groups of insecticides are intensively used against this pest. The status of resistance of the new insecticides in DBM is not yet known. Further the initial base line level of susceptibility of the new insecticides is essential, so that future comparisons can be made.

Chawla and Kalra (1976) had emphasized the need for establishing base line data for insecticide susceptibility using standard bioassay techniques for the correct appraisal of the problems of resistance in DBM. If base line data are not available and a strain known to be susceptible is not available, the alternative method is to make check tests at intervals before the suspected chemical is widely used or in an area where it is not used. The variability of these tests will give an idea of the normal limits of susceptibility of the pest (Dhingra *et al*., 1988). Susceptible strain is obtained by continuous unexposure of insects to insecticides over generations (Pradhan, 1983). Resistance monitoring programmes generally involve comparisons of LD_{50}'s, LD_{90}'s and slopes between field collected populations and laboratory strains or both (Twine and Reynolds, 1980). The slopes of the dose mortality line of the LD_{95} might be a better indicator of resistance (Roush and Miller, 1986). The base line susceptibility responses of DBM to several commonly used insecticides were established by earlier workers (Chandrasekaran and Regupathy, 1996; Lavanya, 2004; Sannaveerappanavar and Viraktamath, 2006). These base line values are used to quantify resistance in field population of DBM. Keeping the above in view, the present study was undertaken to assess the acute toxicity of new molecular insecticides *viz.,* chlorfenapyr, indoxacarb and profenofos to *P. xylostella.*

Materials and Methods

The test insects were collected from cabbage / cauliflower fields at Coimbatore district and maintained on mustard and cauliflower leaves at Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore at a photoperiod of 12:12 (light: dark) and at 30±4.0°C. The third instar larvae measuring 0.5±0.12cm in length with 1.83±0.28mg in weight were used for bioassay studies. The insecticide dilutions required for

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bioassay were prepared by dissolving the insecticide formulations in analytical grade acetone. The following insecticides were used in the present study.

a. Profenofos: Profenofos 50% a.i, Trade name – Curacron 50 EC. Manufactured by M/s. Syngenta India limited, Mumbai.
b. Indoxacarb: Indoxacarb 14.5% a.i, Trade name – Avaunt 14.5 SC. Manufactured by M/s. E. I. DuPont India private limited., Gujarat.
c. Chlorfenapyr: Chlorfenapyr 10% a.i, Trade name – Intrepid 10 SC. Manufactured by M/s. BASF India Ltd, Mumbai.

Median lethal concentration (LC_{50}) for the field collected population of insect to different insecticides was obtained by conducting bioassays. Then insects collected from field were cultured continuously without any selection pressure (without any insecticide exposure) throughout F_n generations. Preliminary range finding tests were done with laboratory cultured populations to fix the test dose range causing 20 to 80 per cent mortality approximately. Based on this, 4 to 6 doses were fixed in geometric progression for which dilutions were prepared with analytical grade acetone. The experimental insects were treated starting from lower to higher concentration.

The procedure originally described by Hirano (1979) and supplemented by Tabashnik and Cushing (1987) was followed. Leaf discs of 6-8cm diameter covering either side of the midrib were prepared. After cleaning the leaf disc thoroughly with cotton, leaf discs were dipped in required concentration of insecticides for about one minute and then shade dried. After complete evaporation, the leaves are transferred to clean bioassay containers over a moistened filter paper. Leaf discs were placed slantingly to rest on side of the container so that larvae can move on either side. Then 3rd instar larvae were transferred to each disc and container was closed. Mortality was recorded at 24 and 48 hours after treatment. The resistance percentage was calculated after correction using Abbott's formula and expressed with a standard error.

Statistical analysis

The corrected mortality (CM) was calculated as per Abbot (1925) formula:

$$CM = \frac{\text{Per cent test mortality - Per cent control mortality}}{\text{100 - Per cent control mortality}} \times 100$$

Pooled binomial standard error (SE) was calculated by using the formula (Regupathy and Dhamu, 2001),

$$SE = \sqrt{\frac{P(100 - P)}{n - 1}}$$

Where, P- per cent larvae surviving in discriminative dose, n- total number of larvae tested

The resistance percentage was worked out by the formula (Regupathy and Dhamu, 2001)

$$R = (100\text{-}CM) \pm SE$$

Where, CM - corrected mortality, SE - standard error

$$\text{Susceptibility index} = \frac{LC_{50}\ /_{99} \text{ of First generation}}{LC_{50}/_{99} \text{ of Last generation}}$$

$$\text{Slope function increase/ decrease \%} = \frac{\text{Slope of Last generation}}{\text{Slope of First generation}} - 1 \times 100$$

$$\text{Rate of resistance decline (R)} = \frac{\text{Log (final } LC_{50}) - \text{Log (initial } LC_{50})}{n}$$

Generations required for a 10-fold decrease in LC_{50} (G) = R^{-1}

Results and Discussion

The log-dose-probit -mortality (LDPM) curves were constructed for the populations collected from the cauliflower/ cabbage field (F_1) and up to 15 (F_{15}) generations without exposure to insecticides culturing under laboratory conditions. Information about the relative toxicity of the pesticide is helpful in developing effective pest management programmes (Flaherty and Huffaber, 1970). The LC_{50} and LC_{95} values of chlorfenapyr to *P. xylostella* were determined for F_1, F_3, F_6, F_{10}, F_{13}, F_{14} and F_{15} generations (Table 1). The LC_{50} and LC_{95} values of chlorfenapyr from F_1 to F_{15} generation decreased from 2.104 to 0.432 and 16.942 to 7.490 ppm, respectively. The LC_{50} and LC_{95} value for subsequent generations tested were found to be decreasing with succeeding generations, thus increasing the susceptibility of the pest. The susceptibility index (SI) of F_{15} generation over F_1 was 4.8703 and 2.2619 based on LC_{50} and LC_{95}, respectively (Table 4). The rate of resistance decline (R) was negative indicating that susceptibility increased with the subsequent generations (R value was -0.1114) Thus, the number of generations required for a 10-fold decrease in LC_{50} was calculated as 8.976. The LC_{50} value of chlorfenapyr has been worked out for other pests by several workers. The LC_{50} value was 5 ppm for Kanzawa spider mite, 4.80 ppm for beet armyworm, *Spodoptera exigua* (Hubner) (Mascarenhas *et al*., 1996), 70.10 ppm against tobacco bud worm *Heliothis virescens* (Fabricius) and 2.50 ppm against eggplant flea beetle, *Epitrix fuscula* Crotch (Leaf disc bioassay method) (Pimprale *et al.,* 1997). Considering the acute toxicity values obtained for F_{15} generation of DBM, tentative discriminating dose (DD) were arrived at 7.50 ppm by leaf disc method.

The LC_{50} of indoxacarb assessed for F_1 population of DBM was 8.266 ppm and LC_{95} value being 69.442 ppm (Table 2). The susceptibility of F_{15} generation was moderately increasing and were 2.162 and 20.542 ppm for LC_{50} and LC_{95}, respectively. The susceptibility gradually increased with the succeeding generations which are evident from the decline in LC_{50} and LC_{95} values tested. The susceptibility index (SI) of F_{15} generation over F_1 was 3.8233 and 3.3804 based on LC_{50} and LC_{95}, respectively (Table 4). The rate of resistance decline (R) was negative indicating

Table 1. Acute toxicity of chlorfenapyr to *Plutella xylostella larvae* by leaf disc method

Generation	Regression Equation	Chi square c_2	LC_{50} mg/larvae	Fiducial limits		LC_{95} mg/larvae	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
1.	Y = 4.411 + 1.826x	0.1951	2.104	1.586	2.791	16.942	8.215	34.939
3.	Y = 4.52 + 1.876x	0.734	1.798	1.357	2.383	14.182	7.144	28.155
6.	Y = 4.65 + 1.931x	0.8711	1.591	1.146	2.015	11.480	6.011	21.922
10.	Y = 5.04 + 1.801x	0.9212	0.966	0.722	1.291	8.050	3.713	17.488
13.	Y = 5.43 + 1.555x	1.8964	0.538	0.385	0.751	6.252	2.302	16.978
14.	Y = 5.55 + 1.559x	2.3424	0.462	0.332	0.642	5.475	2.069	14.487
15.	Y = 5.67+1.593x	0.9101	0.432	0.297	0.629	7.490	2.172	25.882

that susceptibility increased with the succeeding generations (R value was -0.4069) . Thus, the number of generations required for a 10 fold decrease in LC_{50} was calculated as 2.457. The tentative discriminating dose (DD) arrived based on LC_{95} of indoxacarb for F_{15} generation of laboratory population of *P. xylostella* was 20.54 ppm. The LC_{50} of profenofos assessed for F_1 population was 20.307 ppm and LC_{95} value was

Table 2. Acute toxicity of indoxacarb to *Plutella xylostella larvae* by leaf disc method

Generation	Regression Equation	Chi square c_2	LC_{50} mg/larvae	Fiducial limits		LC_{95} mg/larvae	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
1.	Y= 3.373+ 1.765x	0.440	8.266	6.190	11.037	69.442	34.59	139.397
3.	Y= 3.452+ 1.803x	0.229	7.173	5.373	9.577	57.155	30.028	108.789
6.	Y = 3.823+1.623x	0.219	5.358	3.896	7.367	54.882	21.970	137.094
10.	Y = 4.286+1.594x	0.578	2.848	2.057	3.944	30.441	11.772	78.716
13.	Y = 4.400+1.576x	0.901	2.449	1.773	3.383	27.329	10.665	70.025
14.	Y= 4.426 +1.700x	0.896	2.227	1.643	3.017	21.162	9.187	48.535
15.	Y= 4.4431+1.700x	0.707	2.162	1.597	2.928	20.542	9.008	46.844

149.473 ppm (Table 3). The LC_{50} and LC_{95}- values for subsequent generations tested were found to be slightly decreasing with succeeding generations, thus increasing the susceptibility of the pest. The susceptibility of F_{15} generation was moderately increasing and was of 9.430 and 70.373 ppm for LC_{50} and LC_{95}, respectively. The susceptibility index (SI) of F_{13} generation over F_1 was 2.1534 and 2.1240 based on LC_{50} and LC_{95},

Table 3. Acute toxicity of profenofos to *Plutella xylostella larvae* by leaf disc method

Generation	Regression Equation	Chi square c_2	LC_{50} mg/larvae	Fiducial limits		LC_{95} mg/larvae	Fiducial limits	
				Lower limit	Upper limit		Lower limit	Upper limit
1.	Y = 3.207 -1.910x	1.846	20.307	15.450	26.690	149.473	75.935	294.227
3.	Y=2.426 + 2.088x	0.7836	17.466	13.504	22.539	108.734	61.057	193.640
6.	Y = 2.898+1.887x	1.4165	13.424	10.105	17.834	101.959	48.439	214.616
10.	Y= 3.029 +1.880x	2.000	11.673	8.791	15.500	92.514	44.381	192.847
11.	Y= 2.985+ 2.022x	2.5496	10.432	7.946	13.694	75.013	38.188	147.348
12.	Y= 3.039+ 2.021x	2.025	9.680	7.365	12.723	69.270	35.398	135.554
13.	Y = 3.087 +1.991x	2.5183	9.430	7.147	12.443	70.373	35.457	139.673

respectively (Table 4). The rate of resistance decline (R) was negative indicating that susceptibility increased with the succeeding generations (R value was -0.7251) . Thus, the number of generations required for a 10-fold decrease in LC_{50} was calculated as 1.379. Sannaveerappanavar and Viraktamath (2006) reported an LC_{50} value of 29.26 ppm for profenofos to DBM by topical method. The tentative discriminating

Table 4. Susceptibility index of *P. xylostella*

Insecticide	Susceptibility index based on LC_{50}	Susceptibility index based on LC_{95}	R	G	I (%)	RP±SE
Chlorfenapyr at F_{15}	4.8703	2.2619	-0.1114	8.976	- 12.760	6.67±0.86
Indoxacarb at F_{15}	3.8233	3.3804	-0.4069	2.457	-3.682	10.00±1.03
Profenofos at F_{15}	2.1534	2.1240	-0.7251	1.379	-204.241	33.33±1.66

R - Rate of resistance decline, G - Generations required for a 10-fold decrease in LC_{50},
I - Increase / decrease in slope function (%), RP - Resistance Percentage

dose (DD) arrived at based on LC_{95} of profenofos for F_{13} generation of laboratory population of *P. xylostella* was 70.37 ppm.

The tentative discriminating dose arrived for chlorfenapyr, profenofos and indoxacarb in the present study was used to assess the current resistance level in DBM. Chlorfenapyr and indoxacarb resistance level was very low (6.67 and 10.00 %, respectively), whereas the resistance level of profenofos was 33.33 per cent (Table 4). The present finding is in agreement with the findings of Sannaveerappanavar and Viraktamath (2006) who reported 39.09 per cent

resistance for profenofos by topical application method in Karnataka.

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