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Full Research Article

Cross-Resistance Variation among Different Populations of Diamondback Moth, *Plutella xylostella* (Linn.) to New Insecticides

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Abstract

Field populations of DBM sampled from Andhra Pradesh, Karnataka and Delhi were used in bioassays with insecticides acephate, cypermethrin, spinosad, cartap hydrochloride and Cry2Ab toxin over a period of three generations (F_1-F_3) to obtain respective insecticide resistant strains. The individual insecticide resistant strains were used to estimate the cross resistance against emamectin benzoate, chlorantraniliprole and flubendiamide. The cross-resistance ratios (CRR) obtained based on median lethal concentrations for resistant and unselected strains revealed that CRR of all the three DBM populations in acephate resistant strain (AR) ranged from 0.52-0.82, 0.31-0.64 and 0.37-0.75; cypermethrin resistant strain (CyR) ranged from 0.41-0.76, 0.29-0.80 and 0.37-1.12; spinosad resistant strain (SR) ranged from 0.35-0.64, 0.29-0.65 and 0.37-0.62 cartap hydrochloride resistant strain (ChR) ranged from 0.58-0.88, 0.45-0.50 and 0.50-0.87. Cry2Ab resistant strain (CryR) ranged from 0.29-0.82, 0.33-0.69 and 0.62-0.75 respectively. Except for cypermethrin resistant strain of DBM from Delhi that showed cross resistance to flubendiamide no other DBM insecticide resistant strains exhibited cross resistance to emmamectin benzoate, chlorantraniliprole and flubendiamide.

1. Introduction

Diamondback moth (DBM), *Plutella xylostella* (L) (Lepidoptera: Yponomeutidae), is an most important destructive insect pest of cruciferous crops and ubiquitous in distribution (CIE, 1967). In India, DBM was reported in 1914 on cruciferous vegetables and is now the most devastating pest of cole crops in the states of Punjab, Haryana, Himachal Pradesh, Delhi, Uttar Pradesh, Bihar, Andhra Pradesh, Tamil Nadu, Maharashtra and Karnataka (Uthamasamy et al., 2011). The infestation increases gradually from first fortnight of August and leads to total loss of the crop (Dhaliwal et al., 2010). The insect is resistant to many classes of insecticides and causes 50 to 80% loss in marketable yield with an estimate of US$ 168 million per year. Control measures residing on insecticides alone itself constitute about 38% of the cost of production in cole crops in India.

DBM ranks second in the Arthropode Pesticide Resistance Database (APRD) for the highest number of insecticides with reported resistance in at least one population globally (APRD, 2012). DBM has the credential of becoming resistant to 82 compounds which have being used against it. DBM was the first species to develop field resistance to *Bacillus thuringiensis (Bt)* Cry toxins (Talekar and Shelton, 1993), and is one of only three insect species to have developed field resistance to Bt based spray products and this resistance is also wide spread (APRD, 2012).

Major outbreaks of *P.xylostella* are more likely in the fields that are sprayed frequently and heavily with insecticides. The absence of effective natural enemies and fast development of insecticide resistance are believed to be the major causes of increasing pest status of DBM in most parts of the country.

Many of the newer insecticides introduced to control DBM over the past 25 to 30 years have been more selective and thus are more compatible with natural enemies. Relics of DBM evidently prove that it may only take two to three years for problematic levels of resistance to develop following the introduction of a new insecticide. Recent examples of field resistance developed to relatively selective compounds, such as indoxacarb, avermectins, spinosad, Bt- based products, benzyl ureas and chlorantraniliprole. Rotation of insecticides is a IRM strategy employed for effective management of

DBM. But ample scope for development of cross resistance may render the new insecticide chemistry to be ineffective. Previous studies already demonstrated globally and in Indian conditions that DBM has the ability of developing cross resistance to insecticides owing similarities in target sites. Different group of insecticides such as organo chlorines, organo phoshates, carbamates and synthetic pyrethroids fall under this category. In recent past, commercial insecticides used either categorized to target acetylcholine esterase activity, perturb voltage gated Na^+ channel activity, chitin synthesis. Currently insecticides targeting unconventional target sites are gaining promise viz. mitochondrial respiration, GABA sites, ryanodine receptors and post synaptic nicotinic acetyl choline receptors (agonists/antagonists). Studies pertaining to the development of cross resistance by DBM towards these new insecticides are still wanting or scanty. Emmamectin benzoate is a avermectin compound that activate the GABA gated chloride channel, causing an inhibitory effect, when excessive, results in the insect's death. This channel normally blocks reactions in some nerves, preventing excess stimulation of CNS. Chlorantraniliprole and flubendiamide are novel anthranilicdiamides insecticide, exhibits larvicidal activity by targeting and disrupting the Ca^{2+} balance. Both insecticides activates ryanodine receptors via stimulation of the release of calcium stores from the sarcoplasmic reticulum of muscle cells causing impaired regulation, paralysis and ultimately death of sensitive species.Hence, a study/ attempt were made to check the cross resistance pattern of different DBM populations to recent commercially available insecticides viz. emamectin benzoate, chlorantraniliprole and flubendiamide at laboratory level.

2. Materials and Methods

Investigations were carried out during 2011-2012 in the Bt Lab, Department of Entomology, College of Agriculture, Rajendranagar, Hyderabad. For determining the cross - resistance pattern in DBM, *P. xylostella* three different populations (Andhra Pradesh, Karnataka and New Delhi) were used against following insecticides viz., emamectin benzoate, chlorantraniliprole and flubendiamide which are commonly used by farmers in cabbage agro-ecosystem. Initially, these DBM populations collected from the respective fields were reared separately on unsprayed cabbage leaves that served as natural feed and were subjected to bioassays during the third instar stage with four test insecticides four viz., acephate (Organophosphate), cypermethrin (Synthetic pyrethroid), spinosad (Spinosyn), cartap hydrochloride (Neriestoxin) and Cry2Ab for inducing selection pressure and resistance development. The DBM populations were subjected to bioassay at narrow ranges and then LC_{50} was deduced.

100 ml of one per cent stock solution of four test insecticides was used for the preparation of serial dilutions. The technical formulation of Cry2Ab (3.93 mg g^{-1}) was supplied by CICR, Nagpur.100 mg of the toxin was dissolved in 5 ml distilled water to obtain a stock solution of 60 µg ml^{-1} concentration. The stock solution was subjected to serial dilutions to obtain different concentrations and 0.2% Tween-80 was added. Similarly 0.2%, Tween-80 was added to control treatment also.

2.1. Bioassay

Bioassays were done by leaf dip method and each treatment had 10 third instar larvae and was replicated thrice along with a control. Mortality of the treated larvae was recorded at 24, 48 and 72 HAT by counting the larvae as dead or moribund when they did not resume activity after repeated proddings. LC_{50} values for the test insecticides were carried out by probit analysis (Finney, 1971). Bioassays were repeated for treatments wherein control mortality exceeded 20%.

Initially broad range concentrations were used in bioassay with the third instar larva. Based on 20-80% larval mortality again narrow range concentrations were used to determine the LC_{80} value of insecticides and Cry2Ab against DBM larvae,

2.2. Insecticidal resistant strains of DBM

Using this concentration (LC_{80}) from F_0 generation onwards bioassays were done to deduce a concentration that caused 80% larval mortality and sparing 20% larval survival for next succeeding generation. This bioassay procedural for inducing selection pressure was continued till F_3 generation using the LC_{80} concentration of preceding generation. Each bioassay was done by using 100 third instar larvae and was replicated thrice.The survivals of all the three DBM populations from third generation (F_3) were reared to fourth generation (F_4), where no selection pressure was given and the line thus selected was designated as particular insecticide resistant strain viz., acephate- resistant strain (AR), cypermethrin-resistant strain (CyR), spinosad-resistant strain (SR), cartap hydrochloride-resistant strain (ChR), Cry2Ab-resistant strain (CryR), which were used for further cross - resistance studies.

2.3. Test insect population

To assess the magnitude of cross- resistance levels among the DBM populations of three regions Andhra Pradesh, Karnataka and New Delhi, a separate DBM population, referred as unselected population was used by collecting the DBM larvae from farmers' fields and reared in the laboratory on unsprayed fresh cabbage leaves and after reaching third instar stage larvae were subjected to bioassay using three insecticides viz., emamectin benzoate, chlorantraniliprole and flubendiamide. 100 ml of one per cent stock solution of above test insecticides was initially prepared from which serial dilutions were prepared. Initially broad range concentrations were used in bioassay with the third instar larvae. Based on 20-80% larval mortality again narrow range concentrations were used to determine the LC_{50} value of individual insecticide. Each treatment had 10 third instar larvae and replicated thrice along with a control. Bioassay was repeated for treatments were control mortality exceeded 20%.These deduced LC_{50} values were considered as LC_{50} values of unselected population.

2.4. Assessment of the cross-resistance pattern in P. xylostella

Cross - resistance spectrum of the Andhra Pradesh (AP), Karnataka(K) and Delhi (D) DBM populations of the AR-strain (Acephate Resistant), CyR-strain(Cypermethrin Resistant), SR-strain (Spinosad Resistant), ChR-strain (Cartap hydrochloride Resistant), CryR-strain(Cry2Ab Resistant) was documented by testing the toxicity of emamectin benzoate, chlorontraniliprole and flubendiamide against resistant strains by using leaf dip method bioassay. LC_{50} values of the three insecticides (emamectin benzoate, chlorontraniliproleand flubendiamide) were estimated by using third instar larvae of the AR -strain, CyR-strain, SR-strain, ChR-strain, CryR-strain of all the three geographical populations. The degree of cross - resistance acquired by AR-strain, CyR-strain, SR- strain, ChR-strain, CryR- strain of *P.xylostella* from Andhra Pradesh, Karnataka and Delhi, respectively was calculated by dividing LC_{50} value of resistant strain with the LC_{50} value of unselected population for test insecticides and thus the relative degree of cross-resistance was assessed by using the formulae suggested by Ramasubramanian and Regupathy (2004).

$$\text{Cross-Resistance Ratio (CRR)} = \frac{LC_{50} \text{ of Fn (selected)}}{LC_{50} \text{ of unselected}}$$

If the CRR ratio is less than one indicates no cross resistance and more than one indicates positive cross resistance.

CRR=>1 (Positive); CRR=<1 (Negative)

3. Results and Discussion

3.1. Evaluation of cross-resistance pattern in P. xylostella to new insecticides

The LC_{50} values documented for DBM population collected from farmers field for studying the cross resistance patterns against emamectin benzoate, chlorontraniliprole and flubendiamide were 0.0017%, 0.1265% and 0.0008% to, respectively. (Table 1a).

3.2. Cross-resistance pattern of acephate resistant (AR) strain in three populations

The LC_{80} concentration for acephate that caused 80.00% mortality commencing from F_0 till F_3 generation for the three geographic populations of DBM (Andhra Pradesh, Karnataka and Delhi) was 0.085%, 0.015% and 0.112%, respectively. LC_{50} deduced from the bioassay with 3rd instar larvae from F_4 generation of respective populations against test insecticides (emamectin benzoate, chlorontraniliprole and flubendiamide) were 0.0009%, 0.0400% and 0.0003% for AP-AR strain; 0.0009%, 0.0423% and 0.0003% for K.AR strain while the D.AR recorded LC_{50} of 0.0014%, 0.0810% and 0.0006% respectively. The magnitude of cross - resistance pattern was estimated based on cross - resistance ratios. The Cross-resistance ratios of acephate resistant strain (AR) obtained for three populations against emamectin benzoate, chlorantraniliprole and flubendimide ranged from 0.52-0.82, 0.31-0.64 and 0.37-0.75 respectively. AP.AR,K.AR and D.AR strains did not exhibited cross - resistance against emamectin benzoate, chlorantraniliprole and flubendiamide, respectively, as cross - resistance ratios were less than one. The chi-square test revealed that the population used in the study was homogenous (p<0.05%) (Table 1b). Basanth et al., (2013) reported that acephate showed resistance ratio of 5.32 -1.34 among 5 populations of BPH sampled from Karnataka. Basavannagoud and Lingappa (2001) by using a *H.armigera* strain that had six continuous generations exposure to cypermethrin reported cross resistance to fenvelrate, monocrotophos, endosulphan by a magnitude of 2.57, 2.23 and 1.18. Field studies from elsewhere in India showed very high level of resistance to cypermethrin (2880 folds) and also cross resistance to cartap hydrochloride, diafenthiouron and flufenoxuron (Joia and Chawla 1996 and Joia et al., 1996).

3.3. Cross-resistance Pattern of cypermethrin selected (CyR) strain in three populations

Bioassay using F_4 generation Cypermethrin selected (CyR) Strain 3rd instar larvae of respective populations against test insecticides (emamectin benzoate, chlorontraniliprole and flubendiamide) revealed the LC_{50} values 0.0007%, 0.0374% and 0.0003% for AP-CyR strain; 0.0007%, 0.0784% and 0.0003% for K.CyR strain while for D.CyR the LC_{50} values were 0.0013%, 0.1023% and 0.0009% respectively. The

Table 1a: LC_{50} values of test insectcides for unselected population

A	B	C	D	E	F
Emamectin benzoate	1.0697	Y=9.7043+ 1.6982x	0.0017	0.0010-0.0020	1.6982+ 0.3807
Chloron-tranilipro l	0.7608	Y=7.2807+ 2.5404x	0.1265	0.0838-0.1619	2.5404+ 0.4381
Flubendi-amide	0.2063	Y=7.1158+ 0.6796	0.0008	0.0001-0.0021	0.6796+ 0.1448

A: Insecticide; B: Heterogenity (χ^2); C: Regression equation; D: LC_{50} (%); E: Fiducial limits; F: Slope ±S.E magnitude of cross - resistance pattern was estimated based on cross- resistance ratios. The Cross - resistance ratios of cypermethrin resistant strain (CyR) obtained for three populations (Andhra Pradesh-AP. CyR, Karnataka-K.CyR and Delhi-D.CyR) against emamectin benzoate, chlorantraniliprole and flubendiamide ranged from 0.41-0.76, 0.29-0.80 and 0.37-1.12 respectively. AP.CyR, K.CyR strains did not exhibited cross - resistance against emamectin benzoate, chlorantraniliprole and flubendiamide as cross - resistance ratios were less than one while D.CyR strain showed cross resistance to flubendiamide which recorded CRR of more than one and no cross-resistance developed against emamectin benzoate and chlorontraniliprole. The chi-square test revealed that the population used in the study was homogenous (P<0.05%) (Table 1c.) Cypermethrin usage in combating DBM is a regular management tactic in Indian scenario but did not proved to be cross resistant to either of the three novel insecticides. But studies elsewhere showed a 145 fold cross resistance to deltamethrin (Kim et al., 1990). The cross resistance mechanism attained here is attributed to similar mode of action as mentioned by authors and negative cross resistance was observed to acephate, fenitrothion, phenthoate and carbofuran.

An apprehension made in the present study was cypermethrin resistant strain of Delhi DBM population was ought to develop a cross resistance to flubendiamide, though the target systems for these two insecticides are different affect of biochemical and enzymes involved can be nullified by the use of synergists like PBO, DEF and TPP as a customary practice in diminishing the resistance menace as far as usage of synthetic pyrethroids are considered. Moreover, lack of cross-resistance of the insecticides acephate, cypermethrin, spinosad, Cry2Ab and cartap hydrochloride to emmamectin benzoate, flubendiamide and chlorantraniliprole suggest that rotation insecticides could be an effective resistance management strategy.

3.4. Cross-resistance pattern of spinosad selected (sr) strain in three populations

The survivals of spinosad selected strain of AP.SR, K.SR and D.SR were reared to F_4 generation and third instar larvae of F_4 generation were subjected to bioassay against test insecticides (emamectin benzoate, chlorontraniliprole and flubendiamide).

Spinosad selected strain of DBM treated with three insecticides showed LC_{50} values of emamectin benzoate, chlorontraniliprole and flubendiamide obtained were 0.001%, 0.0615% and 0.0003% in AP.SR strain,0.0006%, 0.0369% and 0.0004% for K.SR strain while , for D.SR strain the LC_{50} values were 0.0011%, 0.0830% and 0.0005% respectively.(Table 1d). The Cross - resistance ratios of spinosad resistant strain (SR) obtained for three populations against emamectin benzoate, chlorantraniliprole and flubendimide ranged from 0.35-0.64, 0.29-0.65 and 0.37-0.62 respectively.

Spinosad selected strains (AP.SR, K.SR and D.SR) of DBM larvae showed no cross - resistance to either of the three test insecticides emamectin benzoate, chlorontraniliprole and flubendiamide as cross - resistance ratios were obtained less than one in all the three populations. Arora (2003) reported a spinosad selected strain of DBM was not cross resistant to cypermethrin owing to the differential mode of action. Shino and scott (2002) reported the same findings similar to the present findings in a strain of house fly *(Musca domestica)* that showed >150 fold resistance to spinosad over a period of 10 generations and showed slight cross resistance in a range of 1.6 – chlorfenapyr, 42- fipronil, 43- cyfluthrin, 3.4 abamectin folds but the authors conclude this might be due to pre-exposure of the strains to other insecticides in the original population or due to selection pressure as such. In the present study though a meager resistance development was observed in the DBM

Table 1b: Cross-resistance pattern of acephate selected strain in three population

Insecticides	Heterogenity (χ2)	Regression equation	LC_{50} (%)	Fiducial limits	Cross-resisance ratio	Slope±S.E
Andhra Pradesh						
Emamectin benzoate	0.3226	Y=10.1766+1.7109x	0.0009	0.0006-0.0013	0.52	1.71+0.2878
Chlorontraniliprole	0.1722	Y=07.6919+1.9265x	0.0400	0.0241-0.0551	0.31	1.92+0.3533
Flubendiamide	0.5311	Y=10.5622+1.6072x	0.0003	0.0002-0.0005	0.37	1.60+0.2985
Karnataka						
Emamectin benzoate	0.2472	Y=11.0595+1.9900x	0.0009	0.0006-0.0012	0.52	1.99+0.3119
Chlorontraniliprole	0.1029	Y= 07.5862+1.8800x	0 .0423	0.0257-0.0582	0.33	1.88+0.3417
Flubendiamide	0.1192	Y=11.7520+1.9500x	0.0003	0.0002-0.0015	0.37	1.95+0.3308
Delhi						
Emamectin benzoate	0.1880	Y=9.2761+1.4900x	0.0014	0.0004-0.0019	0.82	1.49+0.2675
Chlorontraniliprole	0.1974	Y= 6.5381+1.4000x	0.0810	0.0515-0.1152	0.64	1.40+0.2719
Flubendiamide	0.3073	Y=10.2875+1.6200x	0.0006	0.0004-0.0008	0.75	1.62+0.2826

populations collected from various geographical locations over a period of three generations (F_1-F_3) no cross resistance to the test insecticides emamectin benzoate, flubendiamide and chlorantraniliprole was observed. The same pattern was observed in a field population of *Spodoptera litura* against spinosad for which a continues exposure of 11 generations resulted in a hike of 3921-fold resistance over the susceptible lab strain and was not cross resistant to emamectin benzoate, methoxyfenozide, fipronil, indoxacarb, profenofos, lufenuron or deltamethrin (Rehan and Freed, 2014).

Likewise, a field strain of house fly *(Musca domestica)* (Spin-SEL) also attained a 155 fold resistance ratio to spinosad but did not show cross-resistance to abamectin, indoxacarb or deltamethrin (Khan et al., 2013). Being relatively new, coupled with varied mode of action of the above insecticides cross resistance might have not been observed in the present study. Since Brassica crops receive heavy and frequent sprays of insecticides studies pertaining to the efficient use of synergists like PBO, DEF would be of great hand for maintaining the persistence of the insecticides in effective management of DBM.

3.5. Cross-resistance pattern of cartap hydrochloride selected (ChR) strain in three populations

Cartap hydrochloride selected strains (AP.ChR, K.ChR and D.ChR) of DBM larvae in the F_4 generation when treated with test insecticides emamectin benzoate, chlorantraniliprole and flubendiamide recorded LC_{50} values of 0.0011%, 0.0570% and 0.0007% in Andhra Pradesh population(AP.ChR). 0.0010%, 0.0835% and 0.0004% for K. ChR strain and 0.0015%, 0.0637% and 0.0005%, for D.ChR strain respectively (Table 1e).The Cross - resistance ratios of cartap hydrochloride resistant strain (ChR) obtained for three populations against emamectin benzoate, chlorantraniliprole and flubendimide ranged from 0.58-0.88, 0.45-0.50 and 0.50-0.87 respectively.

Cross-resistance pattern based on cross-resistance ratios of cartap hydrochloride selected strain were less than one to emamectin benzoate, chlorantraniliprole and flubendiamide which indicates that no cross **-** resistance was developed in all the three populations. Renuka and Regupathy 1996 reported resistance to cartap hydrochloride varying in the range of 17-52.4 folds among various populations of DBM in Tamil Nadu. Cho and Lee 1994 reported that cartap hydrochloride showed 9.1 folds resistance at 8^{th} generation and was 19.9 fold cross resistance to lambda cyhalothrin and was not cross resistant to *Bacillus thuringensis* commercial formulation, triflumuron and prothiophos**.**

3.6. Cross-resistance pattern of cry2Ab selected strains (CryR) in three populations

The LC_{50} values obtained in F_4 generation for Cry2Ab selected strain of *P. xylostella* larvae (AP.CryR, K.CryR and D.CryR) against test insecticides emamectin benzoate, chlorantraniliprole and flubendiamidewere 0.0014%, 0.1339%, 0.0006% in AP.CryR strain, 0.0005%, 0.0423% and 0.0005% for K.CryR strain whereas, LC_{50} values of D.CryR were 0.0011%, 0.0880% and 0.0005%, respectively.(Table 1f). Cross - resistance ratios of Cry2Ab resistant strain (CryR) obtained for three populations against emamectin benzoate, chlorantraniliprole and flubendimide ranged from 0.29-0.82, 0.33-0.69 and 0.62-0.75 respectively. Since CRR was less than one no cross - resistance has been observed in Cry2Ab selected strain of DBM when treated with emamectin benzoate, chlorontraniliprole and flubendiamide in Andhra Pradesh, Karnataka and Delhi populations. Cross resistance to Cry2Ab had been reported elsewhere for instance Tabashnik et al 2009 by conducting bioassays with a laboratory colony of pink bollworm against Cry2Ab resulted in 420- fold cross-

Table 1c: Cross-resistance pattern of cypermethrin selected strain in three population

Insecticides	Heterogenity (χ2)	Regression equation	LC_{50} (%)	Fiducial limits	Cross - resistance ratio	Slope±S.E
Andhra Pradesh						
Emamectin benzoate	0.4694	Y=10.5921+1.7805x	0.0007	0.0005-0.0010	0.41	1.78+0.3065
Chlorontraniliprole	0.1614	Y=07.4607+1.7245x	0.0374	0.0202-0.0536	0.29	1.72+0.3330
Flubendiamide	0.2487	Y=11.9211+1.9476x	0.0003	0.0002-0.0004	0.37	1.94+0.3520
Karnataka						
Emamectin benzoate	0.1879	Y=9.5121+1.4300x	0.0007	0.0004-0.0011	0.41	1.43+0.2786
Chlorontraniliprole	0.3212	Y=6.3900+1.2570x	0.0784	0.0461-0.1158	0.61	1.25+0.2655
Flubendiamide	0.0762	Y=8.5238+1.0053x	0.0003	0.0001-0.0005	0.37	1.00+0.2029
Delhi						
Emamectin benzoate	0.1507	Y= 8.3908+1.1700x	0.0013	0.0008-0.0030	0.76	1.17+0.2545
Chlorontraniliprole	0.1273	Y= 6.0504+1.0609x	0.1023	0.0585-0.1654	0.80	1.06+0.2563
Flubendiamide	0.1883	Y=8.4350+1.1270x	0.0009	0.0006-0.0015	1.12	1.12+0.2583

Table 1d: Cross-resistance pattern of spinosad selected strain in three populations

Insecticides	Heterogenity ($\chi 2$)	Regression equation	LC_{50} (%)	Fiducial limits	Cross - resistance ratio	Slope±S.E
Andhra Pradesh						
Emamectin benzoate	0.4179	Y=09.3230+1.4440x	0.0010	0.0006-0.0014	0.58	1.44+0.2694
Chlorontraniliprole	0.8425	Y=06.9982+1.6497x	0.0615	0.0395-0.0845	0.48	1.64+0.2918
Flubendiamide	0.1330	Y=11.2947+1.7734x	0.0003	0.0002-0.0004	0.37	1.77+0.3283
Karnataka						
Emamectin benzoate	0.1139	Y=07.6363+0.8100x	0.0006	0.0001-0.0011	0.35	0.81+0.2501
Chlorontraniliprole	0.1335	Y=07.4390+1.6800x	0.0369	0.0195-0.0533	0.29	1.68+0.3295
Flubendiamide	0.2772	Y=09.3507+1.2976x	0.0004	0.0003-0.0006	0.50	1.29+0.2702
Delhi						
Emamectin benzoate	0.0205	Y= 09.0501+1.3700x	0.0011	0.0007-0.002	0.64	1.37+0.2642
Chlorontraniliprole	0.0655	Y=06.6480+1.5248x	0.0830	0.0553-0.1154	0.65	1.52+0.2773
Flubendiamide	0.1578	Y= 10.2759+1.5880x	0.0005	0.0003-0.0007	0.62	1.58+0.2844

Table 1e: Cross-resistance pattern of cartap hydrochloride selected strain in three populations

Insecticides	Heterogenity ($\chi 2$)	Regression equation	LC_{50} (%)	Fiducial limits	Cross-resistance ratio	Slope±S.E
Andhra Pradesh						
Emamectin benzoate	0.4179	Y=09.3230+1.4440x	0.0010	0.0006-0.0014	0.58	1.44+0.2694
Chlorontraniliprole	0.8425	Y=06.9982+1.6497x	0.0615	0.0395-0.0845	0.48	1.64+0.2918
Flubendiamide	0.1330	Y=11.2947+1.7734x	0.0003	0.0002-0.0004	0.37	1.77+0.3283
Karnataka						
Emamectin benzoate	0.1139	Y=07.6363+0.8100x	0.0006	0.0001-0.0011	0.35	0.81+0.2501
Chlorontraniliprole	0.1335	Y=07.4390+1.6800x	0.0369	0.0195-0.0533	0.29	1.68+0.3295
Flubendiamide	0.2772	Y=09.3507+1.2976x	0.0004	0.0003-0.0006	0.50	1.29+0.2702
Delhi						
Emamectin benzoate	0.0205	Y= 09.0501+1.3700x	0.0011	0.0007-0.002	0.64	1.37+0.2642
Chlorontraniliprole	0.0655	Y=06.6480+1.5248x	0.0830	0.0553-0.1154	0.65	1.52+0.2773
Flubendiamide	0.1578	Y= 10.2759+1.5880x	0.0005	0.0003-0.0007	0.62	1.58+0.2844

resistance to Cry1Ac as well as 240-fold resistance to Cry2Ab. Inheritance of resistance to high concentrations of Cry2Ab was recessive. Kao and Cheng (1994) reported a field strain of DBM was 118.3 resistance ratio to thuricide a commercial formulation of *Bacillus thuringiensis kurstaki* having mixture of toxins (Cry1Aa Cry1Ab, Cry1Ac, Cry2A and Cry2B) and 55.4-118.3 fold resistance ratio to other commercial formulations Dipel 2X, Javelin, MVP, Xentari and Turex. Thuricide resistance proved to be cross resistance to Xentari a commercial formulation of *Bacillus thuringiensis aizwai* having mixture of toxins (Cry1Aa Cry1Ab, Cry1C, Cry1D and Cry2B).

Present findings reveal that selected strain of *P. xylostella* of all the insecticides does not show any cross - resistance to three test insecticides in all the three populations. Except D.CyR strain showed cross-resistance against flubendiamide. All the insecticides used in the present study are different insecticide groups with different modes of action. However, chlorontraniliprole, flubendiamide belonging to same mode of action group and the possibility of development of cross - resistance between these two insecticides should be considered.

The susceptibility of all the resistant strains to three test insecticides (emamectin benzoate, chlorontriliprole and flubendiamide) could be due to the fact that three insecticides are new and showed excellent efficacy against *P. xylostella* and has not been found to exhibit cross - resistance with existing insecticides (Lahm et al., 2009). These favorable characteristics provide an additional management tool to control *P. xylostella* and to make it a good tool for Integrated Pest Management (IPM) and Integrated Resistant Management (IRM) strategies.

Table 1f: Cross-resistance pattern of Cry2Ab selected strain in three populations

Insecticides	Heterogenity (χ2)	Regression equation	LC_{50} (%)	Fiducial limits	Cross-resistance ratio	Slope±S.E
Andhra Pradesh						
Emamectin benzoate	0.1082	Y=8.5253+1.234x	0.0014	0.0009-0.0021	0.82	1.234+0.22563
Chlorontraniliprole	0.0764	Y= 6.0721+1.2277x	0.1339	0.0881-0.2112	0.60	1.2277+0.2618
Flubendiamide	0.0962	Y=9.9937+1.5325x	0.0006	0.0004-0.0008	0.75	1.5325+0.2778
Karnataka						
Emamectin benzoate	0.4188	Y= 9.2307+1.2800x	0.0005	0.0002-0.0008	0.29	1.2858+0.2810
Chlorontraniliprole	0.1678	Y=7.0678+1.5067x	0.0423	0.022-0.0619	0.33	1.5000+0.3008
Flubendiamide	0.7511	Y=10.0466+1.5281x	0.0005	0.0003-0.0007	0.62	1.5281+0.2797
Delhi						
Emamectin benzoate	0.4974	Y= 9.2808+1.4509x	0.0011	0.0007-0.0016	0.64	1.4509+0.2679
Chlorontraniliprole	0.1678	Y=6. 3949+1.3215x	0.0880	0.0553-0.128	0.69	1.3200+0.2668
Flubendiamide	0.0599	Y=9.7257+1.4479x	0.0005	0.0004-0.0008	0.62	1.4479+0.2737

4. Conclusion

Toxins might be a reason for cross resistance to be inevitable. In the present study though selection pressure over three successive generations did not cause a much significant increase in the median lethal concentration of acephate, cypermethrin cartap, spinosad and Cry2Ab toxin the resistance ratios almost remained to be constant for all the geogarphicaly sampled DBM populations.

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