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Assessment of insecticides and *Cry2AB* toxin resistance development in Karnataka population of *Plutella xylostella* (Linn.)

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Abstract: Insecticidal resistance studies against third instar larvae of DBM (*Plutella xylostella* L.) were carried out to know the rate of development of resistance from F_1 to F_3 generations in Karnataka population. The third instar larvae obtained from field were subjected to bioassay studies with tested against acephate, cypermethrin, spinosad, cartap hydrochloride and Cry2Ab toxin using leaf dip method to calculate LC_{50} values. The LC_{50} values of the insecticides were further used to quantify the resistance in *P. xylostella* of parental generation (F_0) from Karnataka field population. The survivals from F_0 generation were reared to next generation (F_1). Resistance development studies was assessed from F1 to F3 generation with third instar larvae in every generation with a concentration that caused 80.00 % mortality for all the test insecticides and Cry2Ab toxin. Results revealed that ((0.17 folds) no resistance was developed against acephate in F_3 generation. In case of cypermethrin 2.33 folds. Resistance studies further revealed that 1.50 folds resistance was developed against spinosad, 2.28 folds against cartap hydrochloride and Cry2Ab toxin 1.49 folds resistance was recorded in F_3 generation. The rate of development of resistance from F_1 to F_3 generations increased in all the test insecticides and Cry toxin, except against acephate in Karnataka population. This data will be useful in the development of insecticide resistance management approach for DBM.

Keywords: DBM, Insecticide Resistance, Karnataka, Toxin

INTRODUCTION

India is the world's largest cauliflower (*Brassica oleracea* var. *botrytis* L) grower and second largest cabbage (*B. oleracea* var. *capitata* L.) grower next to China occupying an area of 2,32,800 and 2,78,800 hectares, respectively (Uthamasamy *et al.,* 2011). Diamondback moth (DBM), *Plutella xylostella* (L) (Lepidoptera: Yponomeutidae), is important pest of cruciferous crops and ubiquitous in nature (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 of the pest increases gradually from first fortnight of August and leads to total loss of the crop (Dhaliwal *et al*., 2010). In India it causes significant economic losses up to 50% with an estimate of US$ 168 million per year. Absence of effective natural enemies and rapid development of insecticide resistance to many classes of insecticides, which account for 30-50% of the total cost of production are considered to be the major causes of increasing pest status of DBM in most parts of the country. DBM occupies second position in being resistant to 82 compounds of insecticides (APRD, 2012) and to be the first species to develop field resistance to *Bacillus thuringiensis (Bt)* Cry toxins, and is one amongst three insect species to have developed field resistance to Bt based spray products (Talekar and Shelton, 1993).

It is documented that resistance is inevitable within a span of two to three years for following the introduction of a new insecticide. Recent examples of field resistance developed to relatively selective new compounds, include indoxacarb, avermectins, spinosad, Bt-based products, benzyl ureas and chlorantraniliprole (Furlong *et al.* 2013). Hence, the present study was undertaken for quantifying the resistance levels in DBM from Karnataka against four commonly used insecticide groups with diverse modes of action and one entomocidal toxin.

MATERIALS AND METHODS

Laboratory investigations were carried out during 2011 -2012 in the Bt Lab, Department of Entomology, Col-

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lege of Agriculture, Rajendranagar, Hyderabad.

LC_{50} Calculation

Test Insect Population: Cabbage cultivar "Charmant" nursery was raised in the greenhouse and one month old seedlings were transplanted in the main field and raised without any insecticide application. DBM, larvae were collected from farmers' cabbage fields in and around Hyderabad, to establish culture. Leaves were harvested daily washed with tap water and provided as feed to the larvae.

Larvae were allowed to pupate in the jars and the pupae were kept in petri plates and placed in a cage for moth emergence. The emerged adult moths were allowed to lay eggs on mustard seedlings. Adults were provided with 10% honey solution fortified with multivitamins and proteinex on a cotton swab for better egg laying. Mustard seedlings with eggs of DBM were collected from the cage and kept in glass jars for hatching. The neonates were reared on insecticide free cabbage leaves. At every successive instar, the larvae were shifted to clean jars and fresh cabbage leaves were provided. Larvae in the third instar stage were used in bioassay studies.

Test insecticides and cry toxin: To determine the LC_{50} values of insecticides and Cry toxin against DBM larvae, four insecticides *viz.*, acephate (Organophosphate), cypermethrin (Synthetic pyrethroid), spinosad (Spinosyn), cartap hydrochloride (Neriestoxin) and Cry2Ab toxin were used. 100 ml of one per cent stock solution of all the above test insecticides were used for the preparation of serial dilutions. Initially broad range concentrations were tested for each test insecticide and toxin depending on the 20 to 80% mortality observed, narrow range concentrations were tested. A control was also maintained at each time of experimentation and the mortality data was corrected by using modified Abbotts formula (Flemings and Ratnakaran, 1985). Bioassay was repeated for treatments wherein control mortality exceeded 20%.

Stock solution preparation for Cry toxin: The technical formulation of Cry2Ab (3.93 mg/g) was supplied by Central Institute of Cotton Research, Nagpur. 100 mg of the toxin was dissolved in 5 ml distilled water to obtain a stock solution of 60 µg/ml concentration. The stock solution was subjected to serial dilutions to obtain different concentrations and a drop of Tween-80 was added. Similarly a drop of Tween-80 was added to control also.

Bioassay : Bioassays were conducted with third instar larvae of *P. xylostella* by using a standard leaf dip method (Sayyed *et al.,* 2000). A bioassay was conducted to deduce the LC_{50} of all the four test insecticides and Cry2Ab toxin, this LC_{50} concentration was used in assessment of resistance in Karnataka populations of DBM from parental generation F_0 to F_3 generation.

The leaf discs (5 cm) were used for bioassay studies. The leaf discs were dipped in 10ml of aqueous solution of various concentrations of test insecticides and Cry toxin, whereas, control leaf discs were immersed in distilled water having a drop of Tween-80 for about fifteen seconds and shade dried before transferring onto a moistened filter paper in a petri plate. Ten third instar larvae were released on each treated leaf disc in each concentration. Each treatment was replicated thrice. Larval mortality was recorded at 24, 48, 72 hours after treatment (HAT) by counting the larvae as dead or moribund when they did not resume activity after repeated proddings. The mortality at 72 HAT was considered as end point for the assessment of toxicity of test insecticides and Cry toxin (Fisk and Wright, 1992). LC_{50} values of all test insecticides and Cry2Ab toxin were determined by probit analysis (Finney, 1971). The calculated LC_{50} was used in quantifying the resistance in different populations by inducing selection pressure.

Quantification of insecticidal resistance in Karnataka population: To assess the resistant levels in Karnataka DBM population, larvae were collected from Kolar District of Karnataka and reared on insecticide free cabbage leaves in the laboratory. Larvae in the third instar were used for bioassay studies.

Bioassay and lab selection: The larvae obtained from the field collected population were designated as F_0 population and the subsequent generations (obtained from previous generations) were designated as F_1 (First generation), F_2 (Second generation), F_3 (Third generation). The process of selection pressure for insecticides and Cry toxin was initiated in the parental generation (F_0) and continued up to F_3 generation.

The calculated LC_{50} values of each insecticide and Cry2Ab toxin was subjected to preliminary bioassay. Individual DBM population was subjected to five concentrations ($LC_{50,}$ two concentrations higher than LC_{50} and two concentrations lower the value of LC_{50}) of each individual insecticide and Cry2Ab toxin and a control with ten third instar larvae per treatment and replicated thrice. Larval mortality was recorded at 24, 48 and 72HAT.The concentration (LC_{80}) that gave 80% mortality was selected from the preliminary bioassay and the survivals at other concentrations were rejected. Using this LC_{80} concentration of all the test insecticides and Cry2Ab subsequent bioassays were conducted with Karnataka DBM populations using 100 third instar larvae per treatment (individual insecticide and Cry2Ab) and replicating the same thrice for inducing selection pressure from the parental generation (F_0) onwards along with a control. The survivals in the bioassay were raised to first generation (F_1) again during third instar F_1 larvae were subjected to bioassay in the above mentioned manner till F_3 generation.

The concentrations were adjusted in subsequent generations depending on the per cent larval survivals obtained in the previous generation.

Assessment of insecticidal resistance in *P. xylostella*: The degree of development of resistance through different generations was determined by working out LC_{50} values in each generation and thus comput-

ing the resistance ratio (RR) by dividing the LC_{50} value for F_n generation with LC_{50} value of the F_1generation (Arora, 2003).

$$\text{Resistance ratio (RR)} = \frac{LC_{50} \text{ value of } F_n \text{ generation}}{LC_{50} \text{ value of } F_1 \text{ generation}}$$

RESULTS AND DISCUSSION

The initial LC_{50} calculated for the DBM population collected around Hyderabad cabbage agro-ecosystem for all the four test insecticides and Cry2Ab was acephate (0.1%), cypermethrin (0.008%), spinosad (0.003%), cartap hydrochloride (0.01%) and Cry2Ab (0.3µg/ml). these LC_{50} values were used for assessing the resistance development for Karnataka DBM population.

Resistance development in DBM against acephate : The concentrations of acephate which was used in bioassay varied from 0.01 % to 0.20 % in F_1, F_2 and F_3 generations. The LC_{80} which was used for inducing selection pressure varied in all three F_1, F_2 and F_3 generations. The documented LC_{80} against third instar larvae of DBM collected from Karnataka was 0.20%, 0.15%, 0.10% .

The calculated LC_{50} values obtained in F_1, F_2 and F_3 generations were 0.027%, 0.022% and 0.015%. Resistance ratios obtained in F_2 and F_3 generations in comparison to F_1 generation were 0.81 and 0.55 folds. The results obtained in the present study indicated there is no development of resistance to acephate because the resistance ratios being less than one (Table 1). The LC_{50} values reduced from F_1 to F_3 generation whereas lowest LC_{50} was recorded in F_3 generation (0.015 %). The results obtained in the present study indicate that the resistance ratios of F_2 and F_3 generations were less than one which indicated there is no development of resistance to acephate in Karnataka population. In the present study DBM did not developed resistance against acephate. DBM population sampled from Karnataka if got exposed to more selection pressures with acephate for several generations, than resistance might have been developed similar to that of Sannaveerappanavar (1995) who documented 100 fold resistance to acephate in DBM population collected from Bangalore. Calibration of resistance ratios would been precise with that of laboratory strain maintained for several generations without insecticidal exposure as recorded elsewhere.

Resistance development in DBM against cypermethrin : LC_{50} concentration of 0.008% was obtained in bioassay using DBM population sampled from cabbage agro-ecosystem in and around Hyderabad the same was used to obtain the survivals of DBM in Karnataka population with cypermethrin. Concentrations in the range of 0.002% to 0.064% were used bioassays in all three different generations (F_1, F_2 and F_3) to get $LC_{80}$and LC_{50} against DBM third instar larvae of Karnataka population. The calculated $LC_{80}$for applying selection pressure were 0.016%, 0.064%, 0.032% in F_1, F_2 and F_3 generations, respectively. The LC_{50} recorded from the bioassays were 0.003 %, 0.011 % and 0.007% in F_1, F_2 and F_3 generations respectively. Resistance ratios in F_2 and F_3 generations over the F_1 generation were 3.66 and 2.33 (Table 1). The highest median lethal concentration for cypermethrin was documented in Karnataka population LC_{50}-0.011% in F_2 generation. In general the median lethal concentration followed increasing trend up to F_2 generation for Karnataka population and then decreased in F_3 generation. Enzymatic role of mixed function oxygenases coupled with target site nerve insensitivity (kdr) (Holden, 1979; Gammon, 1980) are regarded as the most common mechanisms of resistance by DBM to synthetic pyrethroids, reduced insecticide penetration through the cuticle is also cited to occur. The low magnitude of resistance development in the present study might be due to the less usage of synthetic pyrethroids (cypermethrin) in cabbage agro-ecosystem. The resistance folds developed by DBM against cypermethrin are in accordance with other reports -144 fold against cypermethrin in DBM at Panipat (Haryana) and -115 fold resistance to pyrethroids at Delhi and Karnataka (Saxena *et al.,* 1989),-25 fold resistance against pyrethroids (Raju and Singh, 1995),-26507 folds resistance against cypermethrin by DBM population sampled from Bangalore (Sannaveerappanavar, 1995). In the present study there was a decrease in susceptibility pattern upto F_2 generation for Karnataka populations followed by increase in the susceptibility in the F_3 generation (as depicted by increase in LC_{50} values) showing a moderate level of resistance development. Further studies are required with regards to calibration of variation in enzyme titers *viz.,* mixed function oxidases, glutathione S transferases using specific substrates that play vital role in resistance development against synthetic pyrethroids as reported in other insects as such. The present results are in corroboration with Liu *et al.* (1982) who reported resistance ratio of 21 fold for Peng Hu strain that is very similar to many populations of three ecological zones in Pakistan which fell in moderate resistance and 899 fold for Ban-Chu strain in China that is very high resistance zone. Khaliq *et al.* (2007) reported the LC_{50} (mg ml^{-1}at 48 h) values of cypermethrin for 18 field populations of *P. xylostella* from three different zones in Pakistan ranged from 0.19-1.88. The reason for low toxicity of pyrethroids was due the evolution of multiple resistance mechanisms. Resistance to cypermethrin was also documented in all populations of third instar by Liu *et al.* (1981) in Taiwan , Chawla and Joia (1991) in Punjab (India), and Yu and Nguyen (1992) in USA and Perez *et al.* (2000) in Nicaragua.

Resistance development in DBM against spinosad (Spinosyns) : The median lethal concentration of 0.003% for spinosad was recorded in the bioassay con-

ducted with DBM larval population from Hyderabad. Using this LC_{50} bioassays were conducted with third instar larvae of DBM collected from Karnataka population to get 80% larval mortality and for inducing selection pressure for resistance development. Concentrations ranging from 0.0015% to 0.004% were used in bioassays studied in F_1, F_2, F_3generations, respectively.LC_{80} obtained was 0.0035% in F_1, F_2 generations and 0.004%in F_3 generation of Karnataka population. The LC_{50} values calculated were 0.002%, 0.002% and 0.003% in F_1, F_2 and F_3 generations (Table 1).

Resistance ratios in DBM population of F_2 and F_3 generations in relation to F_1 generation were 1.00 and 1.50 fold. Studies indicates that resistance was developed against spinosad in Karnataka population.

Resistance development studies for the Karnataka population of DBM against spinosad showed neutral results. Susceptibility pattern for Karnataka population showed miscellaneous results (as depicted by LC_{50} values) ultimately showing negligible resistance development. The reason for the *P. xylostella* populations either developing moderate resistance or no resistance may be due to the fact that spinosad being a novel insecticide and the usage pattern and selection pressure by this insecticide is relatively new in cabbage agro ecosystem, alternatively the pest was never predisposed to spinosad sprays in this areas as such. The findings of the present studies are in corroboration with the findings of Peter *et al.* (2000), Dey and Somchoudhary (2001) and Vadodaria *et al*. (2000), who earlier reported the higher field efficacy of spinosad against *P. xylostella*.

The present data confirms the findings of Zhao *et al.* (2002) who determined the toxicity ratio of 1.3 to 1.2 from seven zones and 0.8 to 316 from six zones in Geneva. Kao and Cheng (2001) reported LC_{50} of 24.06 ppm and 26.77 ppm from Lu Chu and His-hu strain, respectively, in China during 2001. Shelton *et al.* (1996) showed tolerance ratio of more than 100 to spinosad in DBM population of California (USA), which indicated high levels of resistance than the present study. Walker *et al.* (2002) showed no significant resistance in field population of diamondback moth (New Zealand) which is in conformity with the findings of the present study.

The present study indicated that population of *P. xylostella* in Karnataka remain susceptible to spinosad. However, resistance to spinosad occurred in Hawaii (2000), Georgia (2001) and California (2002) as a consequence of multiple years of extensive application. A major reason for the rapid resistance development to spinosad in Hawaii was the lack of suitable alternatives and the unsynchronized use of insecticide classes that led to continuous population exposure to spinosad as it happened in South East Asia (Sayyed *et al.,* 2003, 2004) and North America (Zhao *et al*., 2002).

The propensity for the selection of spinosad resistance may have arisen from pre - existence of resistance al-

Table 1: Toxicity of insecticide and toxin to third instar larvae of *P. xylostella* in different generations of Karnataka population.

Generation	Heterogenity (χ^2)	Regression equation	LC_{50} (%)	Fiducial limits	Resistance ratio	Slope ± S.E
Acephate						
F_1	0.904	Y=6.4154+0.9019x	0.027	0.007 - 0.050	1.00	0.9019 ± 0.2429
F_2	6.058	Y=6.6575+0.9977x	0.022	0.006 - 0.039	0.81	0.9976 ± 0.2460
F_3	4.940	Y=7.2742+1.2379x	0.015	0.015 - 0.026	0.55	1.2379 ± 0.2597
Cypermethrin						
F_1	0.706	Y=8.3308+1.3049x	0.003	0.001- 0.004	1.00	1.3049±0.2933
F_2	0.633	Y=7.5252+1.2999x	0.011	0.006-0.017	3.66	1.2999±0.2797
F_3	1.380	Y=8.2334+1.4813x	0.007	0.003- 0.010	2.33	1.4813±0.3017
Spinosad						
F_1	1.839	Y=21.5207+6.2115x	0.002	0.001-0.002	1.00	6.2115±1.2354
F_2	0.375	Y=15.4870+3.9491x	0.002	0.002-0.003	1.00	3.9491±1.0790
F_3	0.517	Y=14.9325+3.8552x	0.003	0.002-0.003	1.50	3.8552±1.0609
Cartap Hydrochloride						
F_1	1.307	Y=7.9558+1.3867x	0.007	0.002-0.011	1.00	1.3867±0.4478
F_2	0.966	Y=9.8129+2.6246x	0.015	0.010-0.018	2.14	2.6246±0.6762
F_3	2.249	Y=10.2740+2.9525x	0.016	0.009-0.020	2.28	2.9525±0.8741
Cry2Ab						
F_1	6.597	Y=7.3013+3.3705x	0.208	0.160-0.249	1.00	3.3705 ±0.5906
F_2	1.758	Y=6.6862+3.2750x	0.306	0.237-0.361	1.47	3.2750±0.7060
F_3	0.563	Y=6.2574+2.4814x	0.311	0.217-0.385	1.49	2.4814±0.6713

leles from the past use of organochlorine insecticides as the mode of action of organochlorine and spinosad as GABA/nicotinic acetyl choline receptor as a target (Ortells and Lunt 1995, Massol *et al.,* 2000). However, the possibility of *P.xylostella* carrying spinosad resistance allele may have been introduced from other areas via transportation of cabbage also cannot be ruled out (Khaliq *et al.,* 2007). Our results indicated that *P. xylostella* from Karnataka were susceptible to spinosad. From this it can be construed that spinosad can be used commercially as an alternative to particularly those insecticides against which *P. xylostella* has developed resistance.

Globally, results pertaining to development of resistance manifested by DBM against spinosad showed an 22.4- and 60.7-folds increase in resistance ratios over the parental DBM strain and laboratory susceptible strain in conjunction to the results the genetics of resistance in this strain revealed to be autosomal, incomplete recessive and probably polygenic in association with fitness costs, showed no cross resistance towards abamectin, fipronil, chlorfenpyr and indoxacarb and target site sensitivity was attributed for spinosad resistance (Xiang and Dong, 2006).

Sayyed *et al.* (2008) also reported field collected DBM population from Pakistan showed a rise in resistance ratio to the tune of 283-, 13- and 67- folds against spinosad, indoxacarb and acetamprid over the parental population, moreover genetics of resistance to spinosad in this population was inherited as autosomal, incompletely recessive trait coupled with fitness cost attributes.

Log dose regression analysis of F1 reciprocal crosses between laboratory susceptible strain and field population of DBM collected from Cameroon Highlands revealed that spinosad resistance was inherited as codominant. At the highest dose of spinosad tested, resistance was verge of being completely recessive and at the lowest dose it was incompletely dominant. (Sayyed *et al.,* 2004)

Resistance development in DBM against cartap hydrochloride (Nereistoxin) : The median lethal concentration of 0.01% for cartap was recorded in the bioassay conducted with DBM larval population from Hyderabad. Using this LC_{50} bioassays were conducted with third instar larvae of DBM collected from Karnataka populations to get 80% larval mortality and for inducing selection pressure for resistance development. Concentrations ranging from 0.005% to 0.045% were used in bioassays studied in F_1, F_2, F_3generations, respectively. LC_{80} obtained were 0 0.025%, 0.030% and 0.03%, in F_1, F_2 and F_3 generations, respectively. The LC_{50} calculated for Karnataka population were 0.007 %, 0.015 %, 0.016 % in F_1, F_2 and F_3 generations (Table 1).

Resistance ratios in DBM population of F_2 and F_3 generations in relation to F_1generation were 2.14 to 2.28 fold for Karnataka population. The results clearly showed that the resistance ratios were more than one in F_3 generation, which indicates that resistance developed against cartap hydrochloride in Karnataka population.

Studies pertaining to resistance development for the *P. xylostella* populations from Karnataka showed considerable decrease in susceptibility pattern over the three generations and developed moderate resistance over a magnitude of 2 folds.

Branco and Gatehouse (2001) calculated the resistance ratio for a cartap hydrochloride resistant field strain of DBM that ranged from 2.8 to 7.1. On contrary, Vastrad *et al.* (2004) documented low to moderate level of resistance to cartap hydrochloride against all the DBM populations sampled from three locations that were monitored continuously and for DBM populations sampled from 12 locations that were monitored randomly in Karnataka.

The present study is in accordance with that of Chandrasekharan and Reghupathy (1996) who found resistance levels (expressed as % survival) varied from 17.9 to 52.4 against cartap hydrochloride and Vastrad *et al.* (2002) who recorded the moderate survival percentage (1.11) of DBM treated with cartap hydrochloride. Sannaveerappanavar and Viraktamath (1997) reported the development of resistance to cartap hydrochloride at recommended field concentrations.

The results of the present studies, by and large, fall in line with those obtained by Joia and Udeaan (1997) who obtained LC_{50} values 0.015% to 0.020 % with cartap against a multi-resistant *P. xylostella* population from Punjab. In the present study in LC_{50} of cartap in comparison with doses of other test insecticides and Cry2Ab was in the range of 0.007-0.029 which proves the effectiveness of cartap in the management of DBM.

In another study conducted elsewhere using a strain of DBM showing 14.2- resistance folds to cartap and no cross resistance to abamectin implicating the rotation of both these insecticides cartap and abamectin as a successful pest management strategy (Ninsin, 2015).

Resistance development in DBM against Cry2Ab: The median lethal concentration of 0.3µg/ml for Cry2Ab was recorded in the bioassay conducted with DBM larval population from Hyderabad. Using this LC_{50} bioassays were conducted with third instar larvae of DBM collected from Karnataka population to get 80% larval mortality and for inducing selection pressure for resistance development. Concentrations ranging from 0.1µg/ml to 1.0µg/ml were used in bioassays studied in F_1, F_2, F_3 generations, respectively. LC_{80} obtained was 0.4µg/ml, 0.5µg/ml, 0.4µg/ml in F_1, $F_2$and F_3 generations for Karnataka population. The LC_{50} calculated for Karnataka population were 0.208µg/ml, 0.306 µg/ml and 0.311µg/ml in F_1, F_2 and F_3generations, respectively (Table 1).

Resistance ratios in DBM population of F_2 and F_3 generations in relation to F_1 generation were 1.47, 1.49 fold .The results clearly showed that the resistance ratios were more than one in F_3 generation, which indicates that resistance developed against Cry2Ab in Karnataka population. The resistance development in *P.*

xylostella populations sampled from Karnataka gradually increased from F_1 to F_3 generations against Cry2Ab. Although geographical differences in susceptibility of *P. xylostella* to *Btk* products in India has also been reported by Chandrasekaran and Regupathy (1996), Perez *et al.* (1997), Mohan and Gujar (2000).

In general the susceptibility of *P. xylostella* to *Bt* strains and their toxins was found to be significantly lower in populations that originated from Southern India followed by those from Western and Northern India (Mohan and Gujar, 2002). This suggests the possibility of diamondback moth adaptation in the populations where *Btk* formulations are regularly used (Sawant, 1998). However, in our studies the susceptibility patterns indicate decreasing in susceptibility. This may be due to the fact that baseline susceptibility of local insect populations depends not only on the extent of selection pressure (amount of insecticide used) but the other factors like relative dominance of resistant alleles, level of immigration of susceptible individuals (gene flow), population structure, and exposure to the pesticide time of year are also responsible. In addition, insect behaviour also plays a major role (Roush and Daly, 1990). Further studies are required in understanding the mechanism underlying the resistance though reduced binding of Cry toxin to BBMV's and reduced activation of proteases coupled with faster degradation of proteases are already documented to be resistance mechanism(Forcada *et al*., 1996), studies with regards to type of mid-gut proteases involved in activation of Cry toxin for these populations are required to understand cross resistance mechanisms and for successful management of DBM using IRM strategies.

Sayyed and Wright (2006) by using a field population of DBM collected from Cameroon Highlands revealed 1285- and 171- folds resistance ratio in second generation against Cry1Ac toxin and spinosad respectively in comparison with a laboratory susceptible population.

Median lethal concentration of biopesticide *Bacillus thuringiensis* var.*kurstaki* (Btk), spinosad and beta-cypermethrin ranged from 0.99 mg/L - 9.18mg/L,0.54 mg/L - 4.13 mg/L and 89.02 mg/L - 4338.21 mg/Lfor 14 different DBM populations in China depicting a narrow to moderate increase in resistance against the test insecticides.(Jiang *et al.,* 2015).

The DBM popopulation used in the present study might have never been exposed to sprays of any commercial formulation of Bt containing Cry2Ab toxin in it is also evident that commercial cultivation of transgenic brassicas in India are still at infancy hence, higher susceptibility of this population cannot be ruled out. Esterases and glutathione-S-transferases among the test population is to studied as prior studies elsewhere viz. esterases imparting resistance to organophosphates and indoxacarb (Sayyed and Wright (2006). CarE to spinosad which ultimately result in cross resistance and proving insecticidal rotational strategy to be vane (Gong *et al.,* 2013). Though insecticides with diversity in their mode of action are evaluated for resistance over generations the core understanding of resistance mechanisms that are incurred due enzyme detoxification, target site insensitivity and cross resistance among the insecticidal groups are to be investigated further, owing to suitable substrates.

Conclusion

The results indicated that Karnataka population of DBM developed resistance against all insecticides and Cry toxins except against acephate. These results can be used as base data for further comparision to monitoring changes in susceptibility of DBM to these insecticides.

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