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Toxicological studies on *Helicoverpa armigera* in pigeonpea growing in Vidarbha region of Maharashtra, India

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Abstract: Insecticide resistance level in pigeonpea pod borer, *Helicoverpa armigera* (Hubner) to technical grade insecticides collected from major pigeonpea growing districts of Vidarbha viz., Akola, Amravati, Buldhana, Yavatmal and Washim was worked out. LDP indicated LD_{50} of Cypermethrin in the range of 1.402 to 9.209 ppm with maximum in Yavatmal (9.209 ppm); LD_{90} within range of 6.021 to 18.427 ppm. LD_{50} of Quinalphos in the range of 1.303 to 4.789 ppm with maximum in Yavatmal (4.789 ppm); LD_{90} within range of 3.150 to 14.194 ppm. LD_{50} of Methomyl in the range of 1.297 to 3.792 ppm with maximum in Yavatmal (3.792 ppm); LD_{90} within range of 4.993 to 16.737 ppm. LD_{50} of Indoxacarb in the range of 0.521 to 2.709 ppm with maximum in Yavatmal (2.709 ppm); LD_{90} within range of 2.819 to 20.947 ppm. LD_{50} of Spinosad in the range of 0.713 to 2.408 ppm with maximum in Buldhana (2.408 ppm); LD_{90} within range of 6.413 to 18.349 ppm. The resistance level is visibly high in cypermethrin, moderate to indoxacarb, quinalphos, spinosad and low to methomyl; Yavatmal and Washim strains expressed higher resistance level to cypermethrin, quinalphos and methomyl, whereas Yavatmal and Buldhana strains expressed higher resistance level to indoxacarb and spinosad. The investigation will help to track resistence level in *Helicoverpa armigera* to different groups of insecticides.

Keyword: Cypermethrin, Indoxacarb, Methomyl, Quinalphos, Spinosad

INTRODUCTION

Helicoverpa armigera (Hubner) (Lepidoptera: Noctuidae) is the most dreaded species commonly known as cotton bollworm, pigeonpea pod borer, American bollworm. It is a polyphagous pest of worldwide occurrence inflicting annual crop damage worth US $1 billion in India (Upendhar *et al.,* 2011). In India this insect has been recorded on more than 200 hosts (Pawar, 1998). About 250 species of insects belonging to 8 orders and 61 families have been found to attack on pigeonpea, among these gram pod borer, *H. armigera* (Hubner), tur plume moth, *Exelastis atomosa* (Walsh) and tur pod fly, *Melanogramyza obtusa* (Mall) are important pod feeders of pigeonpea (Durairaj, 1999) causing considerable losses in grain yield ranging from 3-100 per cent (Sharma *et al.,* 2001). The losses due to *H. armigera* alone contribute upto 50 per cent (Thakre, 2001).

The management of *Helicoverpa* has become increasingly difficult due to indiscriminate and extensive use of chemical insecticides which led to development of resistance to insecticides (Armes *et al.,* 1996; Kranthi et al., 1997; Ramasubramaniam and Regupathy 2004). Insecticide resistance in *H. armigera* in India was first recorded in 1987 when widespread field control failure were reported by farmers growing cotton and pulse crop in Andra Pradesh (McCaffery *et al.,* 1989). This trend still exists though, not documented in the literature reviewed. Resistance monitoring is an indispensable prerequisite in designing any IPM programme. Thus, an assessment of resistance profile and mechanism of resistance would be key for the management of *H. armigera* and to decide the management strategy for management of insecticide resistant strains which could help in high production and productivity in pigeonpea. The present investigation was carried out to evaluate the level of resistance to commonly used insecticides in *H. armigera* collected from different locations of Vidarbha that will help to track the level of resistance to different groups of insecticides.

MATERIALS AND METHODS

Test insect: *H. armigera* (Hub.) larvae were collected from different fields of pigeonpea during 2013-14 from five major pigeonpea growing districts of Vidarbha (Amravati, Yavatmal, Buldhana, Akola and Washim). Field collected populations were reared on semi synthetic diet using standard rearing technique (Armes *et al.,* 1992) in laboratory. Eggs collected from mating chamber were allowed to hatch and third instar larvae of F_1 generation were used to evaluate the level of resistance of *H. armigera* to commonly used insecticides.

Method of preperation of artificial semi-synthetic diet (Armes *et al.*, 1992): All ingredients were kept together, one fraction was prepared by boiling agar upto boiling point in half quantity of water, then yeast was added and the mixture was again boiled. The second fraction containing other ingredients except sorbic acid, vitamin suppliment and streptomycin were weighed as per recipe and were homogenously mixed in mixer. These two fractions were thoroughly mixed together after adding remaining ingredients. The semi-liquid diet was poured in petri-plates. The diet was allowed to cool and then pour into multicellular tray for easy feeding. For the preservation of diets for few days, they were stored in refrigerator. The recipe of ingredients of different diets is given in Table 2.

Insecticide: Five technical grade insecticides from different groups were used, cypermethrin 10 EC (synthetic pyrethroid), quinalphos 25 EC (organophosphate), methomyl 40 SP (carbamate), indoxacarb 15.8 EC (oxidiazin) and spinosad 45 SC (microcyclic lactone).

Bioassay: The bioassays were conducted by topical application using Hamilton micro applicator to evaluate the toxicity of test insecticides (Kranthi *et al.*, 2002).

Topical application method: Graded stock solutions through serial dilution technique of the test insecticide were prepared from the technical grade products by dissolving the required quantities after accurate weighing in acetone. One microlitre of the test insecticide solution was applied on the dorsum of thoracic segment by micro applicator. Three replications were maintained for each concentration with 30 larvae for each concentration and minimum 5-7 insecticide concentration. Larvae were held individually in 12-well tissue culture plates containing artiÞcial diet, at 25 ± 2 ^{0}C for 6 d when mortality was recorded. All rearing and bioassay operations were carried out at 25 ± 2°C under a photoperiod of 12:12 (L:D) hours (Kranthi *et al.*, 2002).

Maintenance of susceptible strain: *H. armigera* were collected from pigeonpea field (unsprayed) from different locations and maintained in laboratory for seven generation without exposure to insecticides.

Table 1. Ingredients for the preparation of semisynthetic diet used for rearing of *H. armigera* larvae.

S. N.	Ingredients	Quantity
Part – A		
1	Chickpea flour	160 g
2	Wheat germ	60 g
3	Ascorbic acid	5.3 g
4	Methyl-4-hydroxybenzoate	3.3 g
5	Sorbic acid	1.7 g
6	Aureomycin	2.5 g
7	Formaldehyde (10 % v/v)	13.5 ml
8	Distilled water	550 ml
Part – B		
1	Yeast (Dried)	53 g
2	Agar-agar	16 g
3	Distilled water	550 ml

RESULTS AND DISCUSSION

Log dose probit assays were carried to determine the median lethal concentration and level of resistance of different insecticides in *H. armigera* (Hubner) strains collected from the different locations of Vidarbha, M.S., India, during 2013-14.

Toxicity and level of resistance of insecticides to *H. armigera* (Hubner)

Cypermethrin: Cypermethrin indicated significant levels of resistance in field collected populations from Yavatmal and Washim. LD_{50} values of Cypermethrin ranged from 1.402 to 9.209 ppm (Table 2). High level of resistance was observed in Yavatmal population. The LD_{50} of Yavatmal strain was 9.209 ppm, while its LD_{90} was 18.427 ppm. It was followed by Washim with LD_{50} value 8.176, Amaravati with LD_{50} value 7.551, Akola with LD_{50} value 7.354 and Buldhana with LD_{50} value 6.803 ppm, respectively. The laboratory strain was found most susceptible amongst the strain tested. LD_{50} for laboratory strain was 1.402 ppm whereas, LD_{90} was 6.021 ppm. The fiducial limits at 95 per cent of LD_{50} were between 0.941 and 2.890 ppm. The DrPDKV strain was followed by laboratory strain having 5.841 ppm LD_{50} value and LD_{90} was 10.887 ppm.

Yavatmal strain showed resistance ratio of 6.56 and 1.57 as compare to laboratory and DrPDKV strain, followed by Washim with 5.83 and 1.40, Amaravati with 5.38 and 1.29, Akola with 5.24 and 1.25, Buldhana with 4.85 and 1.16, respectively. The order of toxicity of Cypermethrin to *Helicoverpa armigera* (Hubner) was Yavatmal > Washim > Amravati > Akola> Buldhana> DrPDKV.

Quinalphos: Quinalphos indicated significant levels of resistance in field collected populations from Yavatmal and Amaravati. LD_{50} values of Quinalphos ranged from 1.303 to 4.789 ppm (Table 2). High level of resistance was observed in Yavatmal population. The LD_{50} of Yavatmal strain was 4.789 ppm, while its LD_{90} was 14.194 ppm. It was followed by Washim with LD_{50} value 4.250, Amaravati with LD_{50} value 4.089, Akola with LD_{50} value 3.968 and Buldhana with LD_{50} value 3.466 ppm, respectively. The laboratory strain was found most susceptible amongst the strain tested. LD_{50} for laboratory strain was 1.303 ppm whereas, LD_{90} was 3.150 ppm. The fiducial limits at 95 per cent of LD_{50} were between 0.591 and 1.759 ppm. The DrPDKV strain was followed by laboratory strain having LD_{50} value 3.466 ppm.

Yavatmal strain showed resistance ratio of 3.67 and 1.53 as compare to laboratory and DrPDKV strain, followed by Washim with 3.26 and 1.36, Amaravati with 3.13 and 1.31, Akola with 3.04 and 1.27, Buldhana with 2.66 and 1.11, respectively. The order of

Table 2. Toxicity and level of resistance of different insecticides to *H. armigera* (Hub) (150 larvae/concentration).

Insecticides	Strain	LD_{50} (95% FL)	LD_{90} (95% FL)	Slope (±SE)	Chi Square	RR over FS	RR over LS
Cypermethrin	Akola	7.354 (6.444 - 8.450	12.765 (10.3 -24.123)	5.351 ± 1.42	0.022	1.25	5.24
	Amaravati	7.551 (6.637 - 8.76)	13.014 (10.45- 25.33)	5.420 ± 1.46	0.108	1.29	5.38
	Buldhana	6.803 (5.816 - 7.70)	12.171 (9.885 - 22.45)	5.073 ± 1.35	0.184	1.16	4.85
	Washim	8.176 (7.03 - 9.886)	13.127 (10.52 - 33.16)	6.233 ± 2.04	0.149	1.40	5.83
	Yavatmal	9.209 (7.786 -16.75)	18.427 (12.4- 192.52)	4.254 ± 1.56	0.108	1.57	6.56
	DrPDKV (Field)	5.841 (4.337 - 6.82)	10.887 (9.12 - 22.45)	4.623 ± 1.36	0.230		
	Lab Sus-ceptible	1.402 (0.941 - 2.89)	6.021 (3.138 - 7.954)	4.05 ± 1.85	0.526		
Quinalphos	Akola	3.968 (3.10 - 5.025)	10.521 (7.308 - 28.94)	3.026 ± 0.77	0.320	1.27	3.04
	Amaravati	4.089 (3.27 - 5.354)	12.209 (7.99 - 40.00)	2.697 ± 0.68	0.456	1.31	3.13
	Buldhana	3.466 (2.66 - 4.118)	7.308 (5.795 - 12.44)	3.955 ± 0.91	0.747	1.11	2.66
	Washim	4.250 (3.25 - 5.817)	12.799 (8.114 - 56.37)	2.676 ± 0.75	0.646	1.36	3.26
	Yavatmal	4.789 (3.56 - 7.371)	14.194 (8.53 -121.57)	2.716 ± 0.88	0.043	1.53	3.67
	DrPDKV (Field)	3.121 (2.337 - 4.82)	6.887 (5.12 - 22.453)	4.023 ± 1.06	0.267		
	Lab Sus-ceptible	1.303 (0.591 - 1.76)	3.150 (2.545 - 4.352)	3.34 ± 0.85	0.840		
Methomyl	Akola	3.099 (2.03 - 4.379)	12.058 (7.126 - 67.54)	2.172 ± 0.62	0.514	1.45	2.38
	Amaravati	3.640 (2.446 - 5.51)	13.698 (7.736 -125.3)	2.226 ± 0.70	0.567	1.61	2.66
	Buldhana	2.738 (2.03 - 3.556)	10.151 (6.56 - 29.46)	2.252 ± 0.51	1.589	1.28	2.11
	Washim	3.452 (2.383-5.329)	15.809 (8.367-143.15)	1.939 ± 0.56	0.890	1.70	2.80
	Yavatmal	3.792 (2.496 - 6.44)	16.737 (8.53 -298.99)	1.987 ± 0.65	0.437	1.77	2.92
	DrPDKV (Field)	2.138 (1.73 - 3.156)	8.151 (6.56 - 19.46)	2.282 ± 0.91	1.589		
	Lab Sus-ceptible	1.297 (0.691 - 1.76)	4.993 (3.617 - 9.922)	2.188 ± 0.50	2.886		
Indoxacarb	Akola	1.574 (0.974 -2.263)	9.150 (5.17 -36.887)	1.676 ± 0.39	0.909	1.47	3.02
	Amaravati	1.802 (1.026 - 2.77)	11.285 (5.839 - 73.62)	1.608 ± 0.42	0.435	1.68	3.45
	Buldhana	2.311 (1.31 - 3.547)	10.852 (5.91 - 81.94)	1.907 ± 0.56	0.710	2.15	4.43
	Washim	2.148 (1.29 - 3.288)	11.449 (6.096 - 72.53)	1.763 ± 0.47	0.784	2.0	4.12
	Yavatmal	2.709 (1.548 -5.538)	20.947 (8.36 -799.64)	1.442 ± 0.45	0.504	2.52	5.19
	DrPDKV (Field)	1.074 (0.574 -1.963)	7.150 (4.17 -26.887)	1.963 ± 0.39	1.209		
	Lab Sus-ceptible	0.521 (0.38 - 0.948)	2.819 (2.02 - 5.06)	2.07 ± 0.40	1.049		
Spinosad	Akola	1.694 (0.85 - 2.722)	16.299 (7.48 -154.41)	1.303 ± 0.34	2.040	1.41	2.37
	Amaravati	2.025 (1.12 - 3.135)	14.909 (7.34 -121.43)	1.478 ± 0.39	2.227	1.68	2.84
	Buldhana	2.408 (1.20 - 3.711)	13.523 (6.83 -233.89)	1.709 ± 0.56	0.507	2.01	3.37
	Washim	1.607 (0.94 - 2.414)	13.634 (6.90 -72.681)	1.380 ± 0.32	2.967	1.33	2.25
	Yavatmal	2.334 (1.001- 3.416)	10.340 (5.75- 189.48)	1.982 ± 0.70	1.084	1.94	3.27
	DrPDKV (Field)	1.207 (0.689 - 2.31)	18.349 (9.84 -183.41)	1.113 ± 0.38	1.349		
	Lab Sus-ceptible	0.713 (0.36 - 1.266)	6.413 (3.79 - 19.38)	1.429 ± 0.31	6.677		

toxicity of Quinalphos to *Helicoverpa armigera* (Hubner) was Yavatmal > Washim > Amravati > Akola > Buldhana > DrPDKV.

Methomyl: Methomyl indicated significant levels of resistance in field collected populations from Yavatmal and Amaravati. LD_{50} values of Methomyl ranged from 1.297 to 3.792 ppm (Table 2). High level of resistance was observed in Yavatmal population. The LD_{50} of Yavatmal strain was 3.792 ppm, while its LD_{90} was 16.737 ppm. It was followed by Washim with LD_{50} value 3.640, Amaravati with LD_{50} value 3.452, Akola with LD_{50} value 3.099 and Buldhana with LD_{50} value 2.738 ppm, respectively. The laboratory strain was found most susceptible amongst the strain tested. LD_{50} for laboratory strain was 1.297 ppm whereas, LD_{90} was 4.993 ppm. The fiducial limits at 95 per cent of LD_{50} were between 0.691 and 1.762 ppm. The LD_{50} of DrPDKV strain was followed by laboratory strain having values 2.138 ppm.

Yavatmal strain showed resistance ratio of 2.92 and 1.77 as compared to laboratory and DrPDKV strain, followed by Washim with 2.80 and 1.70, Amravati with 2.66 and 1.61, Akola with 2.38 and 1.45, Buldhana with 2.11 and 1.28, respectively. The order of toxicity of Methomyl to *Helicoverpa armigera* (Hubner) was Yavatmal > Amravati > Washim > Akola > Buldhana > DrPDKV.

Indoxacarb: Indoxacarb indicated significant levels of resistance in field collected populations from Yavatmal and Buldhana. LD_{50} values of Indoxacarb ranged from 0.521 to 2.709 ppm (Table 2). High level of resistance was observed in Yavatmal population. The LD_{50} of Yavatmal strain was 2.709 ppm, while its LD_{90} was 20.947 ppm. It was followed by Buldhana with LD_{50} value 2.311, Washim with LD_{50} value 2.148 and Amaravati with LD_{50} value 1.802, Akola with LD_{50} value 1.574 ppm, respectively. The laboratory strain was found most susceptible amongst the strain tested. LD_{50} for laboratory strain was 0.521 ppm whereas, LD_{90} was 2.819 ppm. The fiducial limits at 95 per cent of LD_{50} were between 0.382 and 0.948 ppm. The DrPDKV strain was followed by laboratory strain having 1.074 ppm LD_{50} value and LD_{90} was 16.299 ppm.

Yavatmal strain showed resistance ratio of 5.19 and 2.52 as compared to laboratory and DrPDKV strain, followed by Buldhana with 4.43 and 2.15, Washim with 4.12 and 2.0, Amaravati with 3.45 and 1.68, Akola with 3.02 and 1.47, respectively. The order of toxicity of Indoxacarb to *Helicoverpa armigera* (Hubner) was Yavatmal > Buldhana > Washim > Amravati > Akola > DrPDKV.

Spinosad: Spinosad indicated significant levels of resistance in field collected populations from Buldhana and Yavatmal. LD_{50} values of Spinosad ranged from 0.713 to 2.408 ppm (Table 2). High level of resistance was observed in Buldhana population. The LD_{50} of Buldhana strain was 2.408 ppm, while its LD_{90} was 13.523 ppm. It was followed by Yavatmal with LD_{50} value 2.334, Amaravati with LD_{50} value 2.025, Akola with LD_{50} value 1.694 and Washim with LD_{50} value 1.607 ppm, respectively. The laboratory strain was found most susceptible amongst the strains tested. LD_{50} for laboratory strain was 0.713 ppm whereas, LD_{90} was 6.413 ppm. The fiducial limits at 95 per cent of LD_{50} were between 0.365 and 1.266 ppm. The LD_{50} of DrPDKV strain was followed by laboratory strain having values 1.207 ppm.

Buldhana strain showed resistance ratio of 3.37 and 2.01 as compared to laboratory and DrPDKV strain, followed by Yavatmal with 3.27 and 1.94, Amaravati with 2.84 and 1.68, Akola with 2.37 and 1.41, Washim with 2.25 and 1.33, respectively. The order of toxicity of Spinosad to *Helicoverpa armigera* (Hubner) was Buldhana > Yavatmal > Amravati > Akola > Washim > DrPDKV.

LDP during 2013-14 indicated LD_{50} value of Cypermethrin in the range of 1.402 to 9.209 ppm with maximum in Yavatmal (9.209 ppm) and LD_{90} value within range of 6.021 to 18.427 ppm. LD_{50} value of Quinalphos in the range of 1.303 to 4.789 ppm with maximum in Yavatmal (4.789 ppm) and LD_{90} value within range of 3.150 to 14.194 ppm. LD_{50} value of Methomyl in the range of 1.297 to 3.792 ppm with maximum in Yavatmal (3.792 ppm) and LD_{90} value within range of 4.993 to 16.737 ppm. LD_{50} value of Indoxacarb in the range of 0.521 to 2.709 ppm with maximum in Yavatmal (2.709 ppm) and LD_{90} value within range of 2.819 to 20.947 ppm. LD_{50} value of Spinosad in the range of 0.713 to 2.408 ppm with maximum in Buldhana (2.408 ppm) and LD_{90} value within range of 6.413 to 18.349 ppm.

The pesticides continue to play a pivotal role in pest control because of which the situation is accentuated by rapid rise in resistance toward insecticides. Several new insecticide molecules are being introduced in the market for controlling pests. In the present investigation, efforts were made to monitor levels of insecticide resistance in *H armigera* (Hubner) collected from various locations of Vidarbha, M.S., India.

These findings are in the same line asthe results reported by previous workers. Rao *et al.* (2005) carried out Insecticide Resistance studies on *Helicoverpa armigera* (Hubner) against cypermethrin 0.1μg/μl, fenvalrate 0.2 μg/μl, endosulfan 10μg/μl, quinalphos 0.75μg/μl, and methomyl 0.1μg/μl. Synthetic pyrethroids showed high resistance, low to moderate against methomyl and quinalphos showed moderate to above moderate level. The *Helicoverpa armigera* has developed 946 folds resistance against cypermethrin whereas, only 13 folds resistance was observed against quinalphos. Sampath kumar *et al.* (2007, 2008) carried out toxicity of indoxacarb and spinosad against spotted boll worm, *Earias vittella* revealed an LD_{50} value of 0.09270 μg/ larva and 0.00188 μg/larva respectively. Ghodaki (2009) reported the resistance development due to constant selection pressure with indoxacarb in *Helicoverpa armigera* was 1238.86 fold as compared to the laboratory susceptible strain. Rao and Grace (2008) carried out the experiment on *H. armigera.* The LC_{50} (μg/ larva) values of indoxacarb, spinosad, methomyl, quinalphos and cypermethrin were 0.22, 0.11, 0.45, 0.53 and 8.70 respectively. Of all the insecticides tested spinosad was the most toxic and cypermethrin was the least toxic.

Nimbalkar *et al.* (2009) studied the Resistance Management in *Helicoverpa armigera*, recorded a maximum LD_{50} value of 0.070μg/larva for quinalphos in October with a minimum of 0.025 mg/larva in August in Jalna district. Resistance to quinalphos was greatest in Jalna (2.50-7.00 fold) followed by Aurangabad (3.0-5.0 fold) and Parbhani (3.7-4.9 fold). The LD_{50} value for cypermethrin was highest, during October (1.459 mg/larvae in Aurangabad) and lowest during August in Parbhani (0.157 μg/larva).

Upendhar *et al.* (2011) conducted bioassay on *H. armigera* larvae of the Mahaboobnagar district and recorded a LD_{50} of 29.125 μg/larva and 59.609 μg/larva at LD_{90} for cypermethrin. The LD_{50} and LD_{90} values of cypermethrin for Raichur population were 32.481 and 38.172 μg/larva, respectively. Toxicity of cypermethrin to Nagpur population showed that the LD_{50} and LD_{90} values were 20.069 and 54.708 μg/larva, respectively. The H. armigera larvae of the Mahaboobnagar district recorded a LD_{50} of 3.651 μg/larva and 10.287 μg/larva at LD_{90} for methomyl. The LD_{50} and LD_{90} values of methomyl for Raichur population was 3.630 and 10.417 μg/larva, respectively, while toxicity of methomyl to Nagpur population showed that the LD_{50} and LD_{90} values were 2.652 and 7.214 μg/larva. Sen *et al.,* (2012) reported in *Helicoverpa assulta*, after 13 generations of selection with indoxacarb in the laboratory, the resistance ratio of the third instar larvae increased by 4.19-fold.

Conclusion

The present investigation on the status of insecticide resistance in *Helicoverpa armigera* to commonly used insecticides of different groups viz., Synthetic pyrethroid, organophosphorous, carbamate, oxydiazine and macrocyclic lactones and with different mode of action in major pigeonpea growing districts of Vidarbha, Maharashtra, indicated that the resistance level is visible as high in cypermethrin, moderate to indoxacarb, quinalphos, spinosad and low to methomyl. In the present investigation, Yavatmal and Washim strains showed higher resistance level to cypermethrin, quinalphos and methomyl; Yavatmal and Buldhana strains showed higher resistance level to indoxacarb and spinosad. On the basis of present findings, it could be concluded that *H. armigera* population of Yavatmal was found most resistant whereas, Dr.Panjabrao Deshmukh Krishi Vidyapeeth population was found susceptible to technical grade insecticides from all the populations collected during 2013-14. Evaluation of insecticide resistance in *H. armigera* revealed highest resistance in technical grade of cypermethrin followed by indoxacarb, quinalphos, spinosad and lowest in methomyl.

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