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Bade AS
Department of Agricultural Entomology, College of Agriculture, Latur, Maharashtra, India

Bhamare VK
Department of Agricultural Entomology, College of Agriculture, Latur, Maharashtra, India

Corresponding Author:
Bade AS
Department of Agricultural Entomology, College of Agriculture, Latur, Maharashtra, India

Detection of insecticidal resistance in *Helicoverpa armigera* (Hubner) infesting pigeonpea and chickpea

Bade AS and Bhamare VK

Abstract
A laboratory experiment was conducted at Post Graduate Laboratory, Department of Agricultural Entomology, College of Agriculture, Latur, during 2017-18. Resistance to five insecticides (*viz*., chlorantraniliprole, cyantraniliprole, emamectin benzoate, indoxacarb and spinosad) was investigated against *H. armigera* infesting pigeonpea and chickpea from different locations of Western Maharashtra (Ahmednagar, Kolhapur, Nasik, Pune, Sangali, Satara and Solapur). Results revealed that, all the insecticides tested were showed susceptibility to test insecticides except indoxacarb 15.8% EC showed decreased susceptibility to low level resistance against all field populations of *H. armigera* infesting pigeonpea and chickpea.

Keywords: Detection, resistance, *H. armigera*, pigeonpea, chickpea

Introduction
Grain legumes are chief source of proteins in human diet hence globally recognized as poor man's meat. Pigeonpea (*Cajanus cajan* (L) Millspaugh) and Chickpea (*Cicer arietinum* L.) are the important grain legume crops of tropical and subtropical countries in Asia, Africa and Latin America which revealed high potential in addressing human nutrition, soil health and crop productivity. Pigeonpea and chickpea contributes to 16% and 47% of total pulse production respectively followed by black gram (10%) and remaining 27% by other pulses in India (Varshney *et al*., 2016) [26]. Various biotic and abiotic stresses are reported for lower production of pulses. Biotic stresses like insect-pests, diseases and nematodes damage reported yield loss up to 30-50% in pulses (Singh, 2017) [20]. More than 200 species of insects live and feed on pigeonpea and chickpea crop. *Helicoverpa armigera* (Hubner) is a prominent polyphagous pest damaging pigeonpea and chickpea in many global agricultural systems. Due to its high reproductive rate, high voracity, high dispersal rate and development of resistance against several insecticides (Yang *et al*., 2013) [28], *H. armigera* caused economic and environmental problems that had been estimated to result in a loss of more than US $ 2 billion annually worldwide (Tay *et al*., 2013) [24]. Almost 30% of all pesticides used worldwide are directed against *H. armigera* (Ahmad, 2007) [4]. *H. armigera* is the noctuid species reported enormous cases of insecticide resistance worldwide with evolved resistance against organochlorines, organophosphates, carbamates, pyrethroids (Kranthi *et al*., 2002) [14] and www.pesticideresistance.org), spinosad (Aheer *et al*., 2009) [3], emamectin benzoate, indoxacarb (Qayyum *et al*., 2015) [18] and *Bacillus thuringiensis*-derived toxins (Zhang *et al*., 2011) [29]. The field populations of *H. armigera* also indicated development of resistance to multiple insecticides (Faheem *et al*., 2013) [9]. Insecticide resistance in *H. armigera* is reported due to the combined effects of insensitivity of acetylcholine esterase to insecticides, expression of higher levels of esterases, phosphatases and a specific protein called p-glycoprotein ATPase (Srinivas *et al*., 2004) [21]. Hence the change in susceptibility in insect-pests to different insecticides is need to be detected from time to time which alert growers about changes in resistant populations, development of novel resistance and helping them in taking correct pest control decisions and improve the sustainable use of insecticides. Keeping this in view, the present investigation was planned to detect the levels of insecticidal resistance in the field populations of *H. armigera* infesting pigeonpea and chickpea grown in different districts of Western Maharashtra region during 2017-18.

Material and Methods

Large sized larvae of *H. armigera* were collected from pigeonpea and chickpea fields grown in different districts of Western Maharashtra region of Maharashtra separately in clean plastic vials along with sufficient green pods to avoid starvation. Immediately these larvae were carried to Post Graduate Laboratory, Dept. of Agril. Entomology, College of Agriculture, Latur for further culturing. The collected L1 larvae were individually reared on natural diet (green pods) till pupation in round plastic vials (measuring 4 cm diameter and 5 cm height). The pupae were transferred to round clean plastic containers (measuring 16 cm diameter and 16 cm height) covering top with muslin cloth secured firmly with rubber band. The sexes were determined in pupal stages on the basis of distance between genital and anal apertures. It is less in the case of male and more in the case of female (Srivastava and Pande, 1966 and Dani *et al*., 1980) [22, 7]. The freshly emerged adults were released into standard oviposition cage (measuring 50 cm x 30 cm) covered with black muslin cloth. The oviposition cage was placed over the water trough in order to create humidity. The proportion of female and male in the cage was 1:1 in order to get fertilized eggs. Cotton swab dipped into 10% honey solution was provided to serve as food for the adults. A strip of cotton cloth toweling (6 x 17 cm) and/or pigeonpea and/or chickpea pods were hung vertically inside each oviposition cage as oviposition substrate. The eggs laid on the toweling and/or pigeonpea and/or chickpea pods were kept in a transparent plastic box (measuring 26 cm L x 17 cm W x 6 cm H). The eggs from each pair were kept separately. After hatching, neonate larvae were transferred separately into plastic vials to avoid cannibalism. Daily the larvae were fed on natural diet. The 2nd instar larvae of 'F-1' generation were used for conducting the bioassay studies. The rearing of *H. armigera* population collected from different districts of Western Maharashtra region was carried out separately at ambient room temperature of 28 ± 3^0 C. The susceptible population of *H. armigera* was developed by rearing five generations of *H. armigera* without selection pressure of any insecticide under laboratory conditions (Tripathy and Singh, 2000) [25].

The insecticides which are commonly used by farmers (*viz*., chlorantraniliprole, cyantraniliprole, emamectin benzoate, indoxacarb and spinosad) were selected for studying the levels of insecticide resistance in *H. armigera* infesting pigeonea and chickpea. All the insecticides were procured from market and dilutions required were prepared from the formulated product only with distilled water. In *H. armigera* bioassay, each insecticide was used in five concentrations (two lower than recommended, one recommended and two higher than recommended) rendering 20 to 80% mortality in pilot tests. However, care was taken to retain the recommended dosage of each insecticide as one of the concentrations. Newly moulted 2nd instar larvae of *H. armigera* from F_1 laboratory culture were exposed to different insecticides using pod dip method (IRAC Method No. 7) recommended by Insecticide Resistance Action Committee with slight modification. Formulated insecticides were diluted using distilled water as a solvent. Sufficient number of non-infested, untreated and fresh pods was collected from unsprayed pigeonpea and chickpea plots. Then these pods were dipped into the test solution for 60 seconds, dried on paper towel and transferred to lebelled clean plastic rearing vials. Two treated pods per treatment were maintained in each vial to avoid starvation stress during the test. One newly moulted 2nd instar F1 larva was placed on these dried pods and then the vial was covered with a plastic lid. Ten larvae per treatment per replication were exposed to treated pods. Three replicates each of five concentrations and one control (distilled water) were used for each test insecticide at ambient room temperature. Observation on larval mortality was recorded at 48 hrs. after exposure period. Larvae regarded as dead when they were not able to move on probing with a blunt probe or brush. The setup of bioassays was maintained separately for every location. The mortality data of each treatment was corrected with respect to control mortality as per Abbott (1925) [1] for *H. armigera* bioassays.

The value of median lethal concentration (LC_{50}) for each insecticide was worked out using profit analysis by Finney (1971) [10] and by computer software Polo Plus 1.0 (LeOra software, 2003) [15]. Similarly LC_{50} values of these insecticides against the susceptible population of *H. armigera* infesting pigeonpea and chickpea were calculated. LC_{50} values of field collected population were compared with the LC_{50} values of susceptible strain to know the level of resistance. The resistance intensity of insect population to particular insecticide is quoted as Resistance Ratio (RR). Sometimes it is also called Resistance Factor (RF) which was calculated with formula given by Pate and Bhamare, 2016 [17]. The RR values were used to indicate resistance levels or categories as per given by Reddy and Bhamare, 2016 [19].

Results and Discussion

Detection of Insecticidal Resistance in *Helicoverpa armigera* (Hubner) infesting Pigeonpea

The resistance ratios of test insecticides for all 7 locations of Western Maharashtra was found to be in the range of 1.97 to 2.44 fold for chlorantraniliprole 18.5%SC, 2.47 to 2.93 fold for cyantraniliprole 10.26%OD, 1.14 to 1.66 fold for emamectin benzoate 5%SG, 4.09 to 5.74 fold for indoxacarb 15.8%EC and 1.11 to 1.50 fold for spinosad 45%SC. The toxicity of test insecticides was noticed in the order of spinosad 45% SC > emamectin benzoate 5%SG > chlorantraniliprole 18.5% SC > cyantraniliprole 10.26%OD > indoxacarb 15.8%EC.

Spinosad 45% SC, emamectin benzoate 5% SG, chlorantraniliprole 18.5% SC and cyantraniliprole 10.26% OD were more toxic and *H. armigera* populations were found to be susceptible to these insecticides whereas decreased susceptibility to low level of resistance developed for indoxacarb 15.8% EC against *H. armigera*. These results are in conformity with the findings of Karjule *et al*. (2017) [12] who monitored development of insecticide resistance in *H. armigera* infesting pigeonpea from Marathwada region and exhibited that all the populations indicated susceptibility to chlorantraniliprole 18.5%SC (1.13 to 1.96 fold), cyantraniliprole 10.26% OD (1.74 to 2.10 fold) and emamectin benzoate 5%SG (2.09 to 2.54 fold). From Telangana, Deepa (2015) [8] indicated that *H. armigera* larvae collected from Mahaboobnagar population recorded the resistance factor of 1.3, 2.0 and 2.6 fold to chlorantraniliprole at 24, 48 and 72 hours, respectively and 1.1, 1.7 and 2.5 fold resistance ratio to emamectin benzoate at 24, 48 and 72 hours, respectively. Similarly, Bird (2015) [6] revealed that the resistance ratio for chlorantraniliprole was 2.9 fold. Bird *et al*. (2017) [5] could not detected resistance in *H. armigera* populations to emamectin benzoate and also reported low but

detectable levels of survival of *H. armigera* at discriminating concentrations of indoxacarb. Gill and Dhawan (2006) [11], Stanley *et al*. (2009) [23], Khan *et al*. (2010) [13] and Pan *et al*. (2017) [16] revealed that *H. armigera* was highly susceptible to spinosad. Wang *et al*. (2017) [27] showed that the indoxacarb-selected population, Yishui population, Shandong and Handan populations exhibited decreased sensitivity, low-level resistance and moderate-level resistance to indoxacarb 15.8% EC, with the resistance ratios of 4.36, 8.06 and 15.34 fold, respectively.

More or less similar trend of results were obtained by Agboyi *et al*. (2016) [2] revealed that spinosad was more toxic to *P. xylostella* populations than the other insecticides with LC_{50} and LC_{90} values less than 1 and 15 µg per ml, respectively. Reddy and Bhamare (2016) [19] exhibited that *Earias vittella* population from different locations of Marathwada region registered variations in susceptibility to chlorantraniliprole 18.5% SC, cyantraniliprole 10.26% OD and emamectin benzoate 5% SG.

Table 1: Insecticidal resistance in field population of *H. armigera* infesting pigeonpea

Sr. No.	Strain	Chlorantraniliprole 18.5% SC		Cyantraniliprole 10.26% OD		Emamectin benzoate 5% SG	Indoxacarb 15.8% EC			Spinosad 45% SC	
		LC_{50} ml/g/l	RR	LC_{50} ml/g/l	RR	LC_{50} ml/g/l	RR	LC_{50} ml/g/l	RR	LC_{50} ml/g/l	RR
1	Ahmednagar	0.0340	2.26	0.0582	2.93	0.0160	1.26	0.0836	5.42	0.0099	1.45
2	Kolhapur	0.0361	2.40	0.0554	2.79	0.0176	1.39	0.0630	4.09	0.0091	1.33
3	Nashik	0.0367	2.44	0.0567	2.86	0.0193	1.53	0.0802	5.20	0.0076	1.11
4	Pune	0.0337	2.24	0.0528	2.66	0.0210	1.66	0.0854	5.54	0.0102	1.50
5	Sangali	0.0296	1.97	0.0490	2.47	0.0154	1.22	0.0676	4.38	0.0101	1.48
6	Satara	0.0307	2.04	0.0530	2.67	0.0144	1.14	0.0720	4.67	0.0089	1.30
7	Solapur	0.0389	2.59	0.0576	2.90	0.0147	1.16	0.0885	5.74	0.0082	1.20
8	Susceptible	0.0150	-	0.0198	-	0.0126	-	0.0154	-	0.0068	-

Detection of Insecticidal Resistance in *Helicoverpa armigera* (Hubner) infesting Chickpea

Resistance ratios of test insecticides for all 7 locations of Western Maharashtra was found to be in the range of 2.47 to 2.93 fold for chlorantraniliprole 18.5% SC, 2.31 to 2.93 fold for cyantraniliprole 10.26% OD, 1.55 to 1.94 fold for emamectin benzoate 5% SG, 5.23 to 7.00 fold for indoxacarb 15.8%EC and 1.52 to 1.98 fold for spinosad 45% SC. The toxicity of test insecticides was noticed in the order of emamectin benzoate 5% SG > spinosad 45% SC > chlorantraniliprole 18.5% SC > cyantraniliprole 10.26% OD > indoxacarb 15.8% EC.

Emamectin benzoate 5%SG, Spinosad 45%SC, chlorantraniliprole 18.5%SC and cyantraniliprole 10.26%OD were more toxic and *H. armigera* populations were found to be susceptible to these insecticides whereas decreased susceptibility to low level of resistance developed for indoxacarb 15.8%EC against *H. armigera* infesting chickpea. These results are in agreement with the findings of Karjule *et al*. (2017) [12] who monitored development of insecticide resistance in *H. armigera* infesting pigeonpea from Marathwada region and exhibited that all the populations of pod borer indicated susceptibility with varied level of resistance to chlorantraniliprole 18.5%SC (1.13 to 1.96 fold), cyantraniliprole 10.26% OD (1.74 to 2.10 fold), emamectin benzoate 5% SG (2.09 to 2.54 fold) and indoxacarb 15.8%EC (6.94 to 10.89 fold). Similarly Bird (2015) [6] revealed that the LC_{50} value of chlorantraniliprole to *H. armigera* was 0.03 mg per ml diet and variation in susceptibility to chlorantraniliprole was 2.9-fold. From Telangana, Deepa (2015) [8] indicated that *H. armigera* larvae collected from Mahaboobnagar population recorded the LC_{50} values of 0.04, 0.06 and 0.04 mg per *l* to chlorantraniliprole and 0.011, 0.017 and 0.025 mg per *l* to emamectin benzoate at 24, 48 and 72 hours, respectively with resistance factor of 1.3-, 2.0- and 2.6-fold and 1.1-, 1.7- and 2.5-fold, respectively on comparing with baseline data. Bird *et al*. (2017) [5] could not detected resistance to emamectin benzoate and low but detectable levels of survival of *H. armigera* at discriminating concentrations to indoxacarb. These results are in conformity with the findings of Pan *et al*. (2017) [16] revealed that Qiuxian population of *H. armigera* expressed susceptibility to spinosad. Khan *et al*. (2010) [13] showed that spinosad 240 SC was very effective against *H. armigera*. Stanley *et al*. (2009) [23] revealed that *H. armigera* was highly susceptible to spinosad with LC_{50} of 2.94 ppm. Resistance was not observed in field-collected populations of *H. armigera* to spinosad collected from Coimbatore and Madurai. Gill and Dhawan (2006) [11] revealed that population of *H. armigera* was susceptible to spinosad with LD_{50} value of 0.09 µg per larva and resistance ratios of 1.5-folds.

More or less similar trend of results were obtained by Reddy and Bhamare (2016) [19] exhibited that *Earias vittella* population from different locations of Marathwada region registered variations in susceptibility to chlorantraniliprole 18.5%SC (1.30 to 2.1 fold), cyantraniliprole 10.26%OD (1.94 to 1.40 fold) and emamectin benzoate 5%SG (2.37 to 1.56 fold). Agboyi *et al*. (2016) [2] revealed that spinosad was more toxic to *P. xylostella* populations than the other insecticides with LC_{50} and LC_{90} values less than 1 and 15 µg per ml, respectively.

Table 2: Insecticidal resistance in field population of *H. armigera* infesting chickpea

Sr. No.	Strain	Chlorantraniliprole 18.5% SC		Cyantraniliprole 10.26% OD		Emamectin benzoate 5% SG		Indoxacarb 15.8% EC		Spinosad 45% SC	
		LC_{50} ml/g/l	RR	LC_{50} ml/g/l	RR	LC_{50} ml/g/l	RR	LC_{50} ml/g/l	RR	LC_{50} ml/g/l	RR
1.	Ahmednagar	0.0343	2.28	0.0509	2.57	0.0202	1.60	0.1035	6.72	0.0124	1.82
2.	Kolhapur	0.0388	2.58	0.0517	2.61	0.0224	1.77	0.0806	5.23	0.0104	1.52
3.	Nashik	0.0354	2.36	0.0582	2.93	0.0217	1.72	0.0959	6.22	0.0119	1.75
4.	Pune	0.0375	2.50	0.0570	2.87	0.0245	1.94	0.1078	7.00	0.0122	1.79
5.	Sangali	0.0328	2.18	0.0521	2.63	0.0196	1.55	0.0815	5.29	0.0135	1.98
6.	Satara	0.0363	2.42	0.0528	2.66	0.0209	1.65	0.0961	6.24	0.0125	1.83
7.	Solapur	0.0372	2.48	0.0459	2.31	0.0232	1.84	0.1037	6.73	0.0120	1.76
8.	Susceptible	0.0150	-	0.0198	-	0.0126	-	0.0154	-	0.0068	-

Conclusions

Among the insecticides tested, spinosad 45% SC, emamectin benzoate 5% SG, chlorantraniliprole 18.5% SC and cyantraniliprole 10.26% OD were found to be highly toxic to all the field populations of *H. armigera* infesting pigeonpea and chickpea evidenced susceptibility. However, indoxacarb 15.8% EC registered increasing trends towards resistance but still at decreased level or low level of resistance to most of populations, hence it should be used wisely. Spinosad 45% SC, emamectin benzoate 5% SG, chlorantraniliprole 18.5% SC and cyantraniliprole 10.26% OD can be used in rotation with the other insecticides to suppress the resistant population of *H. armigera* in pigeonpea and chickpea ecosystem.

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