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Negative cross resistance of *Helicoverpa armigera* Hubner on okra to green insecticide molecule spinetoram 12 SC W/V (11.7 W/W)

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Abstract
Repeated application of insecticides on okra causes in many of the lepidopteran pests may have developed resistance. In general, newly developed compounds are preferred not to show cross resistance with existing insecticides. With this background, field population of *Helicoverpa armigera* from okra which was known to be resistant to most of the conventional insecticides were collected and subjected to the *in vivo* toxicity of spinetoram 12 SC to assess whether cross resistance exists or not. After 48 hours, on spinetoram 12 SC treated fruits, LC_{50}s of field larvae were 0.23, 0.84, 3.47 and 6.85 ppm for 2nd, 3rd, 4th and 5th instars of *H. armigera* respectively. However, in the laboratory strain, these values were 1.00, 3.54, 31.54 and 85.27 ppm for 2nd, 3rd, 4th and 5th instars of *H. armigera* respectively. Laboratory strain of 2nd instar larvae showed higher LC_{50} than 2nd and 3rd instars of field strain. Resistance ratio was 0.23, 0.24, 0.11 and 0.08 for 2nd instar up to 5th instar of *H. armigera*.

Keywords: Spinetoram 12 SC, okra, negative cross resistance, *Helicoverpa armigera*, probit analysis

Introduction
Okra, (*Abelmoschus esculentus* (L.) Moench) commonly known as lady's finger is cultivated in rainy and summer seasons in an area of 0.36 million hectares with a total annual production of 3.42 million tonnes in India. Although there are larger areas under cultivation, productivity remains low. There are many factors for the stagnant or low productivity, and insect pests are one of the major direct causes for yield reduction. Nearly 72 insect pests attack okra [1]. Among these shoot and fruit borer, *E. vittella; Aphis gossypii* Glover; *Amrasca devastans* (Dist.) and *Bemisia tabaci* (Gennadius) are quite serious [2]. The damage due to okra fruit borer alone accounts for 48.9 per cent in Tamil Nadu [3], 45 per cent in Karnataka [4], 22.5 per cent in Uttar Pradesh [5], from 25.9 to 40.9 per cent in Madhya Pradesh [6] and 54.0 per cent in Rajasthan [7]. Generally in vegetable ecosystem due to poor natural enemy complex and the concealed nature of the pests, need based insecticide application along with other IPM strategies were developed and used to mitigate pests [8] especially on okra. Insecticides have been used extensively for the control of these insect-pests for quicker remedy. But these chemicals with varied mode of action due to indiscriminate use carry the danger of resistance development, pest resurgence, outbreaks of secondary pests, reduction in biodiversity of natural enemies, and bio-concentrations of residues in consumable produce at harvest [9, 10]. Available reports reveal that repeated applications of synthetic chemical insecticides dominate the other means for the control of the pest and their indiscriminate use has led to the resurgence of whitefly, aphid and mite [11].

The insecticides used mostly are organophosphates, carbamates and synthetic pyrethroids. However, insecticides such as quinalphos, monocrotophos, carbaryl, carbofuran and fenvalerate have been reported to fail in controlling the fruit borer on okra effectively which consequently increased cost of production [12]. The Scientist [13] used the term "resistant" (1) to describe a resistant population which contains a single individual capable of surviving a laboratory dose of insecticide which would kill almost all (at least 99.9%) of a laboratory strain and (2) to describe only a field population which could not be controlled by field dose properly applied [13]. For resistance management, it is important that resistance is detected at the earliest stage. For effective implementation of resistance management practices, it is essential to have some estimate of changes of resistance level throughout the area when a particular chemical is applied [14].

These estimates rely on initial baseline levels of susceptibility and variability in population so that future comparisons can be made [15]. Spinetoram is effective against insect pest, *P. xylostella* which is resistant to the existing insecticides (organophosphates, chitin biosynthesis inhibitor and synthetic pyrethroids) in crop protection [16]. However, there are no reports on negative cross resistance of okra fruit borer, *Helicoverpa armigera*. Therefore, this study was undertaken with the objective to investigate the negative cross resistance of the *Helicoverpa armigera* population on okra to spinetoram 12 SC in the laboratory.

Materials and Methods

Mass culturing of fruit borer, *Helicoverpa armigera* Hubner

Culturing of fruit borer commenced with collection of large number of late instar larvae from infested fields during the flush season on okra. The larvae were reared on fresh okra fruits in 32 well multi cavity trays (TNAU model) individually till pre-pupal stage (Figure 1). Once the larvae reached the pre-pupal stage they were placed in bread box having sand and saw dust mix bedding material for pupation. During rearing, strict quarantine was made to eliminate parasitized, diseased and unhealthy larvae/pre pupae. Only healthy pupae were sexed and sterilized by dipping in 0.2 per cent sodium hypochlorite solution. Air dried pupae were placed on fine saw dust bed and kept in moth emergence cage for eclosion. The pupae were observed periodically for adult emergence 26±1ºC, RH 75±5% and photoperiod 16:8 h scoto/photo regimes.

Adult diet of 10 per cent honey solution enriched with multivitamin was prepared and provided in a glass vial plugged with a sterile absorbent cotton swab. Five pairs of adults were released for oviposition in the oviposition cage enclosed in cloth cover. The cages were checked daily and cloth containing eggs were dipped in a small bucket containing pure water with 0.1 per cent sodium hypochlorite solution. Then water containing eggs was poured to another bucket through a sterile muslin cloth. The muslin cloth containing eggs was spread inverted over a wide mouth plastic box with the help of rubber bands for egg hatching. Once larval emergence started, the neonates were shifted to multicavity tray containing fresh small pieces of okra at the rate of two neonates/ well [17]. After rearing several generations in the laboratory, the culture was used for experiment.

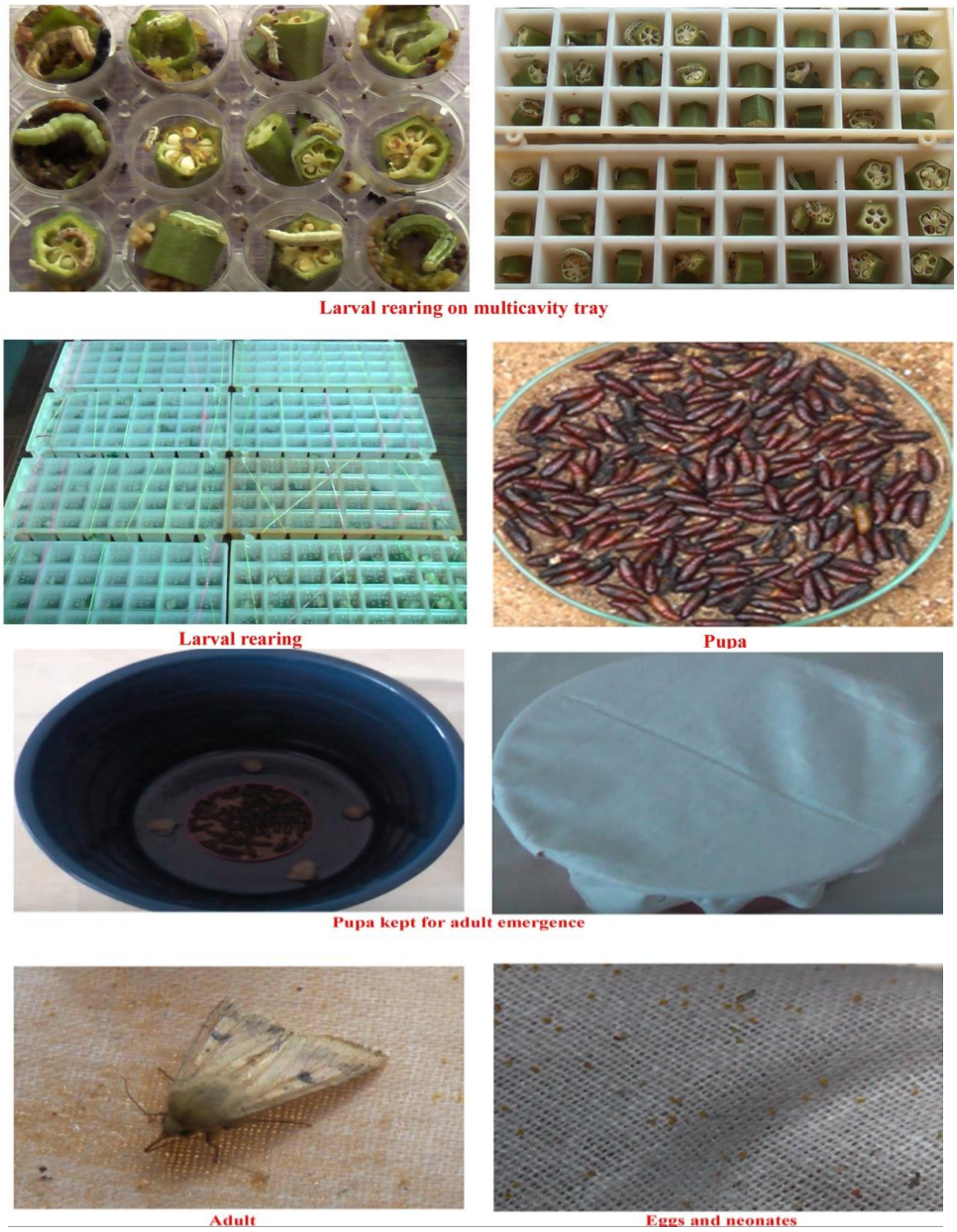

Fig 1: Mass culturing of okra fruit borer, *Helicoverpa armigera*

Negative cross resistance of *H. armigera* population on okra to spinetoram 12 SC

Okra farmers often use several insecticides of varied chemical nature and mode of action. This situation leads to the development of resistance to the borer pest *H. armigera*. There is also a hypothesis that new chemistry molecules are desired not to show any cross resistance to the existing insecticides used against crop pests. In order to test verify the hypothesis, conventional insecticides resistant field populations of *H. armigera* from okra were collected and assessed for their susceptibility to various concentrations of spinetoram 12 SC.

H. armigera larvae reared in the laboratory for five generations without any exposure to conventional insecticides were used as standard reference. Field population of *H. armigera* larvae were collected from farmer's holding (Mr. K. Alagar) at Pudhusukkampatty, Melur Block, Madurai District, which might had been exposed to different insecticides (profenophos, quinalphos, cypermethrin and carbaryl) every year. This field collected *H. armigera* populations were reared in the Insectary of Agricultural College and Research Institute, Madurai separately.

Acute toxicity experiment of spinetoram 12 SC against *H. armigera* was done by fruit dip technique [18]. Spinetoram 12 SC was diluted with water to obtain nine different concentrations (1.2 ppm, 4.8 ppm, 8.4 ppm, 12.0 ppm, 15.6 ppm, 19.2 ppm, 22.8 ppm, 26.4 ppm and 30.0 ppm). Tender small sized okra fruits obtained from potted plants were dipped for 30 seconds in different concentrations of spinetoram 12 SC and left dry under laboratory condition for one hour. Fruits of untreated control were dipped in water. All the treated fruits were placed in large plastic containers separately and covered with muslin cloth. Laboratory reared *H. armigear* larvae (20) were released on treated fruits. Similarly, 20 numbers of field collected *H. armigera* larvae were also placed on another set of treated fruits. All the two experiments were replicated three times. The larvae were considered dead if they became desiccated with shortened body and dark cuticle, and/or unable to move in a coordinated manner when disturbed with a needle. In this acute toxicity experiment, observations on larval mortality were fixed till 72 hours of exposure as spinetoram 12 SC tested was lepidoptericide characterized by stomach action showing slower mortality [18]. The cumulative mortality data were observed till 72 h at 24 h intervals and corrected by Abbott's formula.

Statistical Analysis

The statistical analysis of the data on mortality was subjected to the Abbott formula [19] for correction wherever required. Probit analysis was used to calculate LC_{50} and LC_{95} values [20] through software computer programme.

Results and Discussion

As a result of recurrent application of insecticides on vegetables like okra most of the lepidopteran pests may have developed resistance. Generally, newly developed compounds are preferred not to show cross resistance with existing insecticides. With this background, field population of *H. armigera* from okra which was known to be tolerant to most of the conventional insecticides were collected and subjected to the *in vivo* toxicity of spinetoram 12 SC to assess whether cross resistance exists. Probit analysis criteria of field and laboratory strain of *H. armigera* larvae are presented in Table 1. Untreated larvae of both field and laboratory strains showed no mortality upto 48 hours of feeding. After 48 hours, on spinetoram 12 SC treated fruits, LC_{50}s of field larvae were 0.23, 0.84, 3.47 and 6.85 ppm for 2nd, 3rd, 4th and 5th instars of *H. armigera* respectively. However, in the laboratory strain, these values were 1.00, 3.54, 31.54 and 85.27 ppm for 2nd, 3rd, 4th and 5th instars of *H. armigera* respectively. Results confirmed greater LC_{50} of the laboratory strain than of field strain. The laboratory strain of the 2nd instar showed higher LC_{50} than 2nd and 3rd instars of field strain. Resistance ratio was 0.23, 0.24, 0.11 and 0.08 for the 2nd instar upto the 5th instar of *H. armigera* (Table 2).

Spinosad and spinetoram 12 SC has both contact and stomach toxicity, appears to be unique with primary site of attack being the nicotinic acetyl choline receptor and a secondary site of attack being GABA receptors[21, 22]. This mechanism of action suggests that resistance due to changes in the target sites of many other insecticides [23] might not result in cross resistance to spinetoram 12 SC as well. These results substantiate with the findings of scientist who concluded that spinosad selected strain of *Plutella xylostella* (L.) did not show any cross resistance to certain conventional insecticides [24]. Since toxicity of many insecticides decline as larvae age [25, 26], insecticides should be applied against early larval stages to maximize control efficacy. Variable degrees of cross – resistance in a variety of pest species between spinosad and other few insecticides have been reported [27, 28, 29, 30, 31]. A study of cross resistance with spinosad in tobacco bud worm has been recently reported [32]. Spinetoram is effective against insect pests (*P. xylostella* and *Adoxophyes honmai* Yasuda) which are resistant to the existing insecticides (organophosphates, chitin biosynthesis inhibitor and synthetic pyrethroids) in crop protection (Anonymous, 2012).

Table 1: Probit analysis of larval instars of field *H. armigera* versus laboratory strain

Larval instars	LC_{50} (ppm)	Lower limit	Upper limit	Folds	LC_{95} (ppm)
Field 2nd instar	0.23	0.12	0.40	1.00	1.90
Field 3rd instar	0.84	0.61	1.03	3.65	3.99
Field 4th instar	3.47	2.34	4.78	15.09	42.81
Field 5th instar	6.85	5.79	7.92	29.80	80.04
Laboratory 2nd instar	1.00	0.74	1.87	4.35	7.98
Laboratory 3rd instar	3.54	2.36	5.30	15.40	54.10
Laboratory 4th instar	31.54	19.77	45.99	137.20	155.23
Laboratory 5th instar	85.27	68.34	102.37	370.92	297.75

Table 2: Resistance ratio between field strain of *H. armigera* and laboratory strain

Larval instars	LC_{50} of field strain	LC_{50} of laboratory strain	Resistance ratio
2nd instar	0.23	1.00	0.23
3rd instar	0.84	3.54	0.24
4th instar	3.47	31.54	0.11
5th instar	6.85	85.27	0.08

Conclusion

Chemicals with varied mode of action due to indiscriminate use carry the danger of resistance development, pest resurgence, outbreaks of secondary pests, reduction in biodiversity of natural enemies and bio-concentrations of residues in consumable produces at harvest have opened the new era of eco friendly insecticides having a novel mode of action with higher activity against target insects. Traditional resistance management plans have often used a "use and discard" approach, changing the chemical to target a different mode of action in the pest species once resistance becomes a problem in the field. An alternative strategy is to identify compounds that confer negative cross-resistance (NCR), where the NCR compound is more toxic to pesticide resistant insects as compared to their pesticide susceptible counterparts. Such an insecticide spinetoram 12 SC shows results for laboratory strains of 2nd to 5th instar of *H. armigera* registered higher LC_{50}s from 1.00 to 85.27 ppm. Thus field collected larvae of *H. armigera* from okra showed higher sensitivity towards spinetoram 12 SC.

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