

Original Research Article

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Negative Cross Resistance of *Leucinodes orbonalis* Population of Brinjal to Newer Molecule Spinetoram 12 SC W/V (11.7 W/W)

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ABSTRACT

Keywords

Spinetoram 12 SC, Brinjal, Negative cross resistance, *Leucinodes orbonalis*, Probit analysis.

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As a result of repeated application of insecticides on brinjal most of the lepidopteron pests may have developed resistance. In general, newly developed compounds are desired not to show cross resistance with existing insecticides. With this background, field population of *L. orbonalis* from brinjal 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. Untreated larvae of both field and laboratory strains showed no mortality during the 48 hours of feeding. After 48 hours of feeding on spinetoram 12 SC-treated fruits, LC₅₀s of field larvae were 0.097, 0.45, 3.13 and 6.58 ppm for the 2nd, 3rd, 4th and 5th in stars of *L. orbonalis* respectively. However, in the laboratory strain these values were 0.76, 1.92, 17.37 and 49.55 ppm for 2nd, 3rd, 4th and 5th in stars of *L. orbonalis* respectively. The results confirmed the greater LC₅₀ of the laboratory strain than of the field strain.

Introduction

Eggplant or Brinjal, *Solanum melongena* L. is native to India and cultivated almost throughout the year in all parts of the world. It contributes to 9% of the total vegetable production of the country (Sidhu and Dutta, 2007). People relish brinjal as a table vegetable throughout India due to its nutritive value. It is grown in almost all states of India in an area of 5.10 lakh hectares with the production of 88.0 lakh tonnes of fruits, making country the second largest producer after China. Although India ranks second after China in global eggplant production, crop productivity is relatively low. This commercial crop is infested by more than 23 pests (Muthukrishnan *et al.*, 2005) from the

time of planting to harvest. According to Asian Vegetable Research and Development Centre (Anon., 2009) the key pests responsible for deterioration of quality and quantity in brinjal is shoot and fruit borer, *Leucinodes orbonalis* (Guenee), aphid (*Aphis gossypii* Glover), jassid (*Amrasca biguttula biguttula* Ishida), epilachna beetle, *Henosepilachna vigintioctopunctata* Fabricius and white fly (*Bemisia tabaci* Gennadius).

Shoot and fruit borer, *L. orbonalis* Guenee (Lepidoptera: Pyralidae) is the key pest among these pests throughout Asia (Ghosh *et al.*, 2003). In India, this pest has a countrywide distribution and has been

categorized as the most destructive and serious pest causing huge losses in brinjal (Patil, 1990). Larvae bore into the young shoots and feed on internal tissues resulting in wilting of the shoot, which reduces plant growth and number and size of fruits (Atwal and Dhaliwal, 2007). Fruit infestation ranged from 31 to 90% in Bangladesh (Rahman, 1997), 37-63% in different states in India (Dhankar, 1988) and 50-70% in Pakistan (Saeed and Khan, 1997). The pest has been reported to inflict losses to the tune of 20.7 – 60.0% in Tamil Nadu (Raja *et al.*, 1999), 70% in Andhra Pradesh (Sasikala *et al.*, 1999), 80% in Gujarat (Jhala *et al.*, 2007) and 41% in Himachal Pradesh (Lal *et al.*, 1976).

Insecticides have been used extensively for the control of these insect-pests for quicker remedy. 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 (Rahman, 1997). 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 shoot and fruit borer effectively which consequently increased cost of production (Radhika *et al.*, 1997).

Muggleton (1984) used the term “resistant” (i) 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 (ii) to describe only a field population which could not be controlled by field dose properly applied. For resistance management, it is important that resistance is detected at the earlier stage. For effective implementation of resistant management practices, it is essential to have some estimate of changes of resistant level throughout the area when a particular chemical

is applied (Taylor, 1983). These estimates rely on initial baseline levels of susceptibility and variability in population so that future comparisons can be made (Georghiou and Mellon, 1983). 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 (Anonymous, 2012). However, there are no reports on negative cross resistance of shoot and fruit borer, *Leucinodes orbonalis*. Therefore, this study was undertaken with the objective to investigate the negative cross resistance *Leucinodes orbonalis* population of brinjal to spinetoram 12 SC in the laboratory.

Materials and Methods

Mass culturing of brinjal shoot and fruit borer, *Leucinodes orbonalis*

Mass culturing of brinjal shoot and fruit borer (BSFB) was done as per the procedures described by Selvi (2010). BSFB damaged fruits harbouring larvae were collected from the farmers’ field. The fruits were placed in plastic trays containing dry river sand in a culture room at $26\pm 1^{\circ}\text{C}$, RH $75\pm 5\%$ and photoperiod 16:8 h scoto/photo regime. The fully grown larvae came out of the fruits were allowed to pupate in dry river sand.

The pupae were collected and placed in a plastic jar covered with muslin cloth for adult emergence. Subsequently, adults were sexed and confined at 20 pairs per oviposition cage with tender brinjal twigs/muslin cloth as oviposition substrate. Cotton swabs dipped in honey solution (10% sugar and 0.1% multivitamin drops) were provided as adult feed. Flat creamy, yellow, scaly eggs were laid on the leaf surface/muslin cloth. Eggs laid on twigs/muslin cloths were placed in plastic containers lined with muslin cloth for larval emergence.

After 3 – 4 days the newly hatched neonate larvae were transferred to potato. Medium sized young potatoes were washed well in water and a suspension containing 500 mg / l of carbendazim and 40 mg / l streptomycin sulphate. The tubers were slightly peeled in three to four places and neonate larvae at the rate of 30-40 larvae / tuber were placed over the peeled region. The tubers were placed over dry river sand individually, inside a plastic container covered with muslin cloth. The matured larvae came out of potato and pupated in dry sand medium. Subsequent to pupation, adult emergence and oviposition were followed as described earlier for field collected brinjal fruits. Newly hatched first instar larvae were transferred to peeled regions of potato tubers and reared for many generations in the laboratory conditions for further laboratory experiments.

Negative cross resistance of *L. orbonalis* population of brinjal to spinetoram 12 SC

Brinjal 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 *L. orbonalis*. There is also a hypothesis that new chemistry molecules are desired not to show any cross resistance with the existing insecticides used against crop pests. In order to test verify the hypothesis, conventional insecticides resistant field populations of *L. orbonalis* from brinjal were collected and assessed for their susceptibility to various concentrations of spinetoram 12 SC.

L. orbonalis larvae reared in the laboratory for five generations without any exposure to conventional insecticides were used as standard reference. Field population of *L. orbonalis* larvae were collected from farmer's holding (Mr. Balamurugan) at Thaniyamangalam, Melur Block, Madurai District, which might had been in selection

pressure due to the continuous application of insecticides like insect growth regulators, organophosphates (profenophos and quinalphos), pyrethroids (cypermethrin) and carbamates (carbaryl). These field collected *L. orbonalis* populations were reared in the Insectary of Agricultural College and Research Institute, Madurai separately.

Experiment on acute toxicity of spinetoram 12 SC against *L. orbonalis* was done by fruit dip technique (Ahmad *et al.*, 2003). Spinetoram 12 SC was diluted with water to obtain six different concentrations (1.2 ppm, 2.4 ppm, 3.6 ppm, 4.8 ppm, 6.0 ppm and 7.2 ppm). Tender small sized brinjal fruits obtained from potted plants were dipped for 60 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 *L. orbonalis* larvae (20) were released on treated fruits. Similarly, 20 numbers of field collected *L. orbonalis* 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 (Ahmad *et al.*, 2003). The cumulative mortality data were observed till 72 h at 24 h interval and corrected by Abbott's formula.

Statistical analysis

The statistical analysis of the data on mortality was subjected to the Abbott formula

(Abbott, 1925) for correction wherever required. Probit analysis was used to calculate LC₅₀ and LC₉₅ values (Finney, 1971) through software computer programme.

Results and Discussion

Due to frequent application of insecticides on vegetables like brinjal most of the lepidopteron pests may have developed resistance. In general, newly developed compounds are desired not to show cross resistance with existing insecticides. With this background, field population of *L. orbonalis* from brinjal 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 all *L. orbonalis* instars are presented in Table 1. Untreated larvae of both field and laboratory strains showed no mortality during the 48 hours of feeding. After 48 hours of feeding on spinetoram 12 SC-treated fruits, LC₅₀s of field larvae were 0.097, 0.45, 3.13 and 6.58 ppm for the 2nd, 3rd, 4th and 5th instars of *L. orbonalis* respectively. However, in the laboratory strain these values were 0.76, 1.92, 17.37 and 49.55 ppm for 2nd, 3rd, 4th and 5th instars of *L. orbonalis* respectively. The results confirmed the greater LC₅₀ of the

laboratory strain than of the field strain. The laboratory strain of the 2nd instar showed higher LC₅₀ than 2nd and 3rd instars of field strain. Resistance ratio was 0.13, 0.23, 0.18 and 0.13 for the 2nd instar up to the 5th instar of *L. orbonalis* (Table 2). These results corroborate with the findings of Arora (2002) who concluded that spinosad selected strain of *Plutella xylostella* (L.) did not show any cross resistance to certain conventional insecticides. Since toxicity of many insecticides decline as larvae age (Mayuravalli *et al.*, 1985; Rao and Dhingra, 1996), insecticides should be applied against early larva 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 (Dunley *et al.*, 2006; Magna *et al.*, 2007; Lambkin and Rice, 2007; Bielza, 2007; Sayyed *et al.*, 2008b).

A study of cross resistance with spinosad in tobacco bud worm has been recently reported by Roe (2010). The estimated decrease in resistance was 11.36, 11.11, 16.67 and 9.61 for imidacloprid, spinosad, emamectin benzoate and profenofos, respectively and suggested that spinosad could be included in the control program of *S. litura*, due to its lower stability and higher reversion rate with insecticides bearing novel modes of action (Adeel *et al.*, 2011).

Table.1 Probit analysis of larval instars of field *L. orbonalis* versus laboratory strain

Larval instars	LC ₅₀ (ppm)	Lower limit	Upper limit	Folds	LC ₉₅ (ppm)
Field 2 nd instar	0.097	0.085	0.11	1.00	1.26
Field 3 rd instar	0.45	0.32	0.67	4.64	2.14
Field 4 th instar	3.13	2.11	4.09	32.24	44.82
Field 5 th instar	6.58	5.42	7.59	67.77	89.75
Laboratory 2 nd instar	0.76	0.51	1.02	7.82	3.36
Laboratory 3 rd instar	1.92	1.53	2.40	19.78	7.21
Laboratory 4 th instar	17.37	12.31	23.75	178.91	89.65
Laboratory 5 th instar	49.55	40.26	62.71	510.37	167.99

Table.2 Resistance ratio between field strain of *L. orbonalis* and laboratory strain

Larval instars	LC ₅₀ of field strain	LC ₅₀ of laboratory strain	Resistance ratio
2 nd instar	0.097	0.76	0.13
3 rd instar	0.45	1.92	0.23
4 th instar	3.13	17.37	0.18
5 th instar	6.58	49.55	0.13

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).

During the 20th century, pesticide use has become integral for current agricultural practices, as has the challenge linked with pesticide resistance. 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 *L. orbonalis* registered higher LC₅₀s from 0.76 to 49.55 ppm. Thus field collected larvae of *L. orbonalis* from brinjal showed higher sensitivity towards spinetoram 12 SC.

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