Uttar Pradesh Journal of Zoology

Volume 45, Issue 11, Page 78-92, 2024; Article no.UPJOZ.3435
ISSN: 0256-971X (P)

Assessment of Insecticide Resistance in *Spodoptera litura* (Fabricius) in Vegetable Crop Ecosystem

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2024/v45i114072

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/3435

Original Research Article

Received: 24/02/2024
Accepted: 28/04/2024
Published: 07/05/2024

ABSTRACT

One of the most prevalent insect pests is the tobacco caterpillar or *Spodoptera litura* (Fabricius), which results in significant crop losses developed resistance against different insecticides over time. This laboratory experiment was carried out in the Department of Entomology, College of Agriculture, Rajendranagar, Professor Jayashankar Telangana State Agricultural University (PJTSAU), Hyderabad during *Karif*, 2022. The susceptible population was reared in laboratory continuously for ten generation without exposing it to insecticides. The susceptibility of field populations of *S. litura* that were collected from many vegetable crops in the intense vegetable crop cultivating mandals *i.e.,* Chevella and Maheshwaram of Rangareddy district to commonly used insecticides *viz.,*

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chlorantraniliprole, emamectin benzoate and flubendamide was then compared with a laboratory reared susceptible population The bioassay studies revealed that the LC_{50} values of these insecticides were significantly higher in the field population collected from Chevella and Maheshwaram compared to the susceptible laboratory population. Based on the LC_{50} values the Resistant ratio (RR) was calculated for the field populations. It was found that Chevella population developed 35.34 folds resistance and Maheshwaram population also developed nearly 34.90-fold resistance against emamectin benzoate. Both Chevella and Maheshwaram populations were found to develop moderate resistance of 28.23 and 27.35-fold against flubendamide and comparatively lower resistance of 21.05 and 23.44-fold resistance against chlorantraniliprole when compared to susceptible population. These findings further helps in developing better IRM strategies.

Keywords: Susceptible population; toxicity; dose mortality responses; resistance and resistance ratios.

1. INTRODUCTION

India's agriculture relies heavily on vegetable crops since a sizable section of the populace follows a vegetarian diet. India is second only to China in terms of global vegetable output, with an overall area of 11.37 million hectares and a 2021–2022 yield of 209.14 million metric tonnes (Indiastat.com). Vegetable gardening is not without its challenges, though; insect infestations are the main one. It is estimated that 40% of vegetable production is lost to insect pests. One of the most prevalent insect pests is the tobacco caterpillar or *Spodoptera litura* (Fabricius), which results in significant crop losses [1].

According to Shankaramurthy [2], the tobacco caterpillar, *S. litura* is a persistent polyphagous pest of both field and horticultural crops. Asia, spanning the eastern region of the globe from North Africa to Japan, Australia and New Zealand, is home to it [3]. A considerable amount of economically significant crops, such as tobacco, cotton, groundnut, castor, chilli, potato, soybean, cauliflower, cabbage, tomato, beans, sunflower and onion are harmed by this pest in India [4], which can lead to a 26–100% yield loss in field conditions [5]. In addition to eating the buds, flowers and pods of legumes, *S. litura* is primarily a defoliator [6].

Diamides have been more well-known in recent years as a result of their noteworthy qualities, which include their high efficacy against target pests, rapid beginning of action and favourable safety profile. Due to these qualities, crop pest management has become quite popular, especially when dealing with caterpillar larvae [7,8]. However, the persistent and widespread use of these pesticides has resulted in a number of problems, such as a decrease in their ability to control pests and the quick evolution of resistance in natural populations. Several field populations, such as *Plutella xylostella* (Linnaeus) [9], *Spodoptera exigua* [10,11], *Adoxophyes honmai* [12], *Tuta absoluta* [13] and *Chilo suppressalis* [14] are notable examples of this resistance evolution.

In many nations, emamectin benzoate is a widely used pesticide that is crucial for controlling lepidopteran pests that are significant to agriculture. Its impressive efficacy against this particular insect group combined with its broad range of activity make it appealing [15,16] (Zhang *et al*., 2014). The chemical group that contains emamectin benzoate (EB) is avermectin. As per Roditakis *et al*. [13], this acts on insect nervous systems as a chloride channel activator, so inhibiting muscle contraction and finally causing death. The resilience to lepidopterans displayed by EB is noteworthy [17].

Some noteworthy examples are *Mythimna separata* [18,19], *Spodoptera frugiperda* [20,21] and *S. exigua*, where resistance was observed [22], (Zhang *et al*., 2014). Because of its ecological selectivity towards a range of beneficial arthropods, applying EB in an integrated pest management (IPM) plan could be seen as a substantial pest control method.

Evaluation of resistance to various pesticides is required for resistance management which calls for credible resistance monitoring techniques and appropriate baseline data inturn helps farmers to go with better IRM strategies.

2. MATERIALS AND METHODS

2.1 Collection of Susceptible Strain of *S. litura*

The original susceptible population of *S. litura* larvae was collected from the ICRISAT

Entomology Laboratory in Patancheru, Hyderabad and was raised in a laboratory setting without being exposed to any insecticides for around six generations. The gathered population was raised in the Department of Entomology laboratory of the College of Agriculture in Rajendranagar, Hyderabad for four more generations on an artificial diet. The F_{10} generation population's third instar larvae were subsequently employed in bioassay investigations.

2.1.1 Collection of *S. litura* population from vegetable crop ecosystem of Rangareddy district

Two major vegetables cultivated mandals *viz.,* Maheshwaram and Chevella were selected and field population of *S. litura* were collected from the vegetable crop ecosystem of these mandals during *Kharif*, 2022. The field collected populations were brought to laboratory and both these populations were reared separately on artificial diet and maintained at 25±2°C temperature and 75±5% relative humidity. The third instar larvae of F_1 generation were used for bioassay studies.

In the vegetable crop ecosystem of Maheshwaram and Chevella, two prominent vegetable-cultivated mandals, a field population of *S. litura* was collected in the Kharif of 2022. Both the field-collected populations and the laboratory-raised ones were raised independently on artificial diets at a temperature of 25±2°C and a relative humidity of 75±5%. For bioassay investigations, larvae from the F_1 generation's third instar were employed.

2.2 Test Insecticides

The commercial formulations of three commonly used insecticides in vegetable crop ecosystem *viz*., emamectin benzoate, flubendiamide and chlorantraniliprole were used for resistance studies as mentioned in Table. 1.

2.2.1 Preparation of stock solution of insecticides

The following formula was used to prepare one per cent stock solution of the test insecticides (Naveed, 2005)

$$\text{Stock solution} = \frac{\text{Required concentration (1\%)}}{\text{\% formulation of test insecticide}} \, x \, 100$$

*Quantity of water taken for the preparation of solution

To obtain a one percent stock solution, the measured amount of emamectin benzoate was diluted in distilled water and the volume was increased to 100 ml in a volumetric flask. Five or six concentrations were obtained by preparing successive serial dilutions. For a whole day at 0°C, prepared pesticide dilutions were kept in the refrigerator without losing their effectiveness. Initially, insecticide was tested at wide concentrations. Based on the recorded mortality, which ranged from 20.00 to 80.00% larval death, narrow range concentrations were investigated. A comparable untreated control group was kept throughout the entire experiment.

Table 1. Details of test insecticides used against *S. litura*

Common name	Group	Source of supply
Chlorantraniliprole 18.5% SC	Diamide	Procured from local market
Emamectin benzoate 5% SG	Avermectin	Procured from local market
Flubendamide 39.5% SC	Diamide	Procured from local market

Table 2. Insecticide concentrations used against the susceptible population of *S. litura*

Insecticides	Population	Concentrations used (in per cent)
Chlorantraniliprole	10th generation	0.0002, 0.0004, 0.0006, 0.0008, 0.0010, 0.0012, 0.0014
Emamectin benzoate	10th generation	0.0003, 0.0005, 0.0007, 0.0009, 0.0011, 0.0013, 0.0015
Flubendiamide	10th generation	0.0005, 0.0006, 0.0007, 0.0008, 0.0009, 0.0010, 0.0011

Table 3. Insecticide concentrations used against field population of *S. litura* collected from Chevella and Maheshwaram mandals of Rangareddy district

Insecticides	Concentrations used (in per cent)
Chlorantraniliprole	0.003, 0.005, 0.007, 0.009, 0.011, 0.013, 0.015
Emamectin benzoate	0.011, 0.014, 0.017, 0.020, 0.023, 0.026, 0.029
Flubendamide	0.012, 0.018, 0.024, 0.030, 0.036, 0.042, 0.048

2.3 Bioassay

For every test pesticide, a total of seven narrow range test concentrations were set based on preliminary mortality data collected using broad range of concentrations. As indicated in Table 2, these test concentrations were set in order to calculate the median lethal concentration (LC_{50}) against susceptible *S. litura* populations. In the same manner, it was thought that *S. litura* had developed resistance as a result of the frequent application of these three test pesticides in the Chevella and Maheshwaram mandals vegetable crop ecosystem. For each of the test insecticides, seven test concentrations were determined based on the preliminary mortality results obtained against those local populations of *S. litura*, as indicated in Tables 3. Bioassay investigations were carried out using conventional leaf disc method on *S. litura* third instar larvae, as reported by Johny and Muralirangan [23]. New castor leaves were sliced into 6-centimeter-diameter leaf discs. After that, these leaf discs were submerged for roughly 30 seconds in a test insecticide solution. To avoid desiccation, the leaves were first allowed to air dry on paper towels before being moved to petri dishes with wet filter paper within. The leaf discs that were submerged in distilled water served as the control group. Each petri plate included ten susceptible *S. litura* larvae in their third instar, which were placed inside and sealed with a lid to represent a single replication. For a total of 40 larvae per concentration, each concentration was repeated four times, using 10 larvae per replication. In order to ascertain the LC_{50} value for the sensitive population for a given test insecticide, seven distinct concentrations of each pesticide, as indicated in Table 2, were set up. Furthermore, ten *S. litura* larvae in their third instar that were taken from the cultures of Chevella and Maheshwaram mandals were also tested for the fixed seven different concentrations listed in Table 3. The LC_{50} values for that specific test insecticide were determined based on the mortality data from the bioassay experiment, which was presumed to be a resistant population of that specific mandal.

2.4 Data Collected

Observations on larval mortality of *S. litura* were recorded at 72 hours after treatment (HAT) by counting dead and live larvae. Larvae were considered as dead when they did not move after repeated probing.

2.5 Data Analysis

The mortality data were corrected by using Abbott’s formula (Abbott, 1925)

Corrected mortality= % Mortality in treatment - % Mortality in control X 100 / 100 - % Mortality in control

The corrected data was subjected to Probit analysis [24] using SPSS software. slope, median lethal concentration (LC_{50}), 95% confidential limits and chi- square ($\chi 2$) values were calculated for each insecticide.

2.6 Calculation of degree of resistance acquired by *S. litura* against test insecticides

The degree of insecticide resistance in field collected populations of *S. litura* were assessed by computing resistance ratios (RR) as follows

RR = LC_{50} value of the field population / LC_{50} value of the susceptible population

3. RESULTS AND DISCUSSION

3.1 Determination of Level of Resistance Acquired by *S. litura* for Certain Commonly Used Insecticides in Vegetable Crop Ecosystem of Rangareddy District

The baseline susceptibility was initially calculated using the unexposed F_{10} generation susceptible third instar *S. litura* population for three selected insecticides. Similarly, the LC_{50} was also calculated for the F_1 generation third instar larvae of field populations of Chevella and Maheshwaram. The resistance ratio was then calculated to determine the folds of resistance acquired by these populations for each of the insecticide.

3.1.1 Dose mortality response of susceptible and field populations of *S. litura* to selected insecticides using leaf disc method

The results of the preliminary study conducted to know the baseline susceptibility of laboratory population and field collected populations of *S. litura* to chlorantraniliprole, emamectin benzoate and flubendamide is summarized in Tables 4, 5 and 6.

3.1.1.1 Dose mortality response of susceptible and field populations of S. litura to Chlorantraniliprole

The mortality response of *S. litura* populations were found to increase with increase in insecticidal concentrations of chlorantraniliprole. The laboratory susceptible population recorded a mortality of 40.0, 50.0, 62.5, 80.0, 85.0, 90.0 and 92.5 per cent at concentrations of 0.0002, 0.0004, 0.0006, 0.0008, 0.0010, 0.0012 and 0.0014 per cent, respectively at 72 hours after treatment (Table 4). The field population of Chevella recorded per cent larval mortality of 20.0, 42.5, 50.0, 55.0, 62.5, 75.0 and 82.5 at concentrations of 0.003, 0.005, 0.007, 0.009, 0.011, 0.013 and 0.015 per cent, respectively. The *S. litura* population of Maheshwaram also recorded mortality in a much similar pattern as that was observed against third instar larva of Chevella population, ranging from 22.5 to 85.00 per cent for the similar concentrations. The comparison of larval mortality of laboratory susceptible population with that of Chevella and Maheshwaram populations clearly showed that laboratory susceptible population recorded higher per cent mortality at lower concentrations while the field populations recorded lower per cent mortality at higher concentrations.

3.1.1.2 Dose mortality response of susceptible and field populations of S. litura to Emamectin benzoate

The laboratory susceptible population of *S. litura* recorded mortality of 27.5, 52.5, 55.0, 75.0, 80.0, 85.0 and 90.0 per cent, respectively at 72 hours after emamectin benzoate administration at 0.0003, 0.0005, 0.0007, 0.0009, 0.0011, 0.0013 and 0.0015 per cent concentrations, respectively (Table 5). A little higher concentration of emamectin benzoate were used against field populations of Chevella and Maheshwaram. The Chevella population recorded per cent larval mortality of 22.5, 32.5, 45.0, 52.5, 57.5, 75.0 and 82.5 at 0.011, 0.014, 0.017, 0.020, 0.023, 0.026 and 0.029 per cent concentrations, respectively. The *S. litura* population of Maheshwaram recorded per cent larval mortality of 27.5, 32.5, 47.5, 55.0, 57.5, 62.5 and 87.5 at similar concentrations that were used against Chevella population.

3.1.1.3 Dose mortality response of susceptible and field populations of S. litura to flubendamide

Mortality response of susceptible *S. litura* population due to application of flubendamide at 0.0005, 0.0006, 0.0007, 0.0008, 0.0009, 0.0010 and 0.0011 per cent concentrations were found to be 22.5, 30.0, 52.5, 65.0, 75.0, 82.5 and 85.0 per cent, respectively at 72 hours after treatment with flubendamide (Table 6). *S. litura* population from Chevella recorded mortality of 40.0, 42.5, 52.5, 55.0, 72.5, 77.5 and 95.0 per cent at concentrations of 0.012, 0.018, 0.024, 0.030, 0.036, 0.042 and 0.048 per cent, respectively while the population of Maheshwaram recorded mortality of 37.5, 47.5, 55.0, 62.5, 67.5, 77.5 and 90.0 per cent, respectively for the similar concentrations.

3.1.2 Determination of baseline susceptibility of field populations and susceptible population of *S. litura* against selected insecticides using leaf dip method

Log dose probit assays were carried out to determine the median lethal concentration for the selected insecticides *viz.,* chlorantraniliprole, emamectin benzoate and flubendamide against *S. litura* collected from Chevella and Maheshwaram mandals as well as for the laboratory susceptible population. The results were presented in Table 7.

3.1.2.1 Toxicity to chlorantraniliprole to susceptible and field populations of S. litura

The results pertaining to toxicity of chlorantraniliprole to susceptible and field populations of *S. litura* showed that the insecticide was highly toxic to laboratory susceptible population with low LC50 value of 0.000328 per cent while it was found to be less toxic to field population of Chevella with LC50 value of 0.006904 per cent. Chlorantraniliprole with LC50 value of 0.007689 per cent was found to be less toxic to population of Maheshwaram when compared to *S. litura* population of Chevella (Table 7).

Table 4. Dose mortality responses of chlorantraniliprole against 10th generation susceptible and field population of *S. litura* from Chevella and Maheshwaram

Susceptible population		Chevella population		Maheshwaram population	
Concentration (%)	**Mortality (%)**	**Concentration (%)**	**Mortality (%)**	**Concentration (%)**	**Mortality (%)**
0.0002	40.0	0.003	20.0	0.003	22.5
0.0004	50.0	0.005	42.5	0.005	32.5
0.0006	62.5	0.007	50.0	0.007	50.0
0.0008	80.0	0.009	55.0	0.009	52.5
0.0010	85.0	0.011	62.5	0.011	55.0
0.0012	90.0	0.013	75.0	0.013	62.5
0.0014	92.5	0.015	82.5	0.015	85.0
Control	00.0	Control	00.0	Control	00.0

Table 5. Dose mortality responses of emamectin benzoate against 10th generation susceptible and field population of *S. litura* from Chevella and Maheshwaram

Susceptible population		Chevella population		Maheshwaram population	
Concentration (%)	**Mortality (%)**	**Concentration (%)**	**Mortality (%)**	**Concentration (%)**	**Mortality (%)**
0.0003	27.5	0.011	22.5	0.011	27.5
0.0005	52.5	0.014	32.5	0.014	32.5
0.0007	55.0	0.017	45.0	0.017	47.5
0.0009	75.0	0.020	52.5	0.020	55.0
0.0011	80.0	0.023	57.5	0.023	57.5
0.0013	85.0	0.026	75.0	0.026	62.5
0.0015	90.0	0.029	82.5	0.029	87.5
Control	00.0	Control	00.0	Control	00.0

Table 6. Dose mortality responses of flubendamide against 10th generation susceptible and field population of *S. litura* from Chevella and Maheshwaram

Susceptible population		Chevella population		Maheshwaram population	
Concentration (%)	**Mortality (%)**	**Concentration (%)**	**Mortality (%)**	**Concentration (%)**	**Mortality (%)**
0.0005	22.5	0.012	40.0	0.012	37.5
0.0006	30.0	0.018	42.5	0.018	47.5
0.0007	52.5	0.024	52.5	0.024	55.0
0.0008	65.0	0.030	55.0	0.030	62.5
0.0009	75.0	0.036	72.5	0.036	67.5
0.0010	82.5	0.042	77.5	0.042	77.5
0.0011	85.0	0.048	95.0	0.048	90.0
Control	00.0	Control	00.0	Control	00.0

The 95 per cent fiducial limits obtained during LC50 value calculation for Chevella (0.004118 to 0.00965%) and Maheshwaram (0.004666 to 0.011691%) populations did not overlap with fiducial limits of susceptible population (0.000120 to 0.000486%) which confirmed that field populations of both locations *viz*., Chevella and Maheshwaram were significantly different from susceptible population. Estimated χ2 values of chlorantraniliprole (0.869, 0.492 and 1.326) for all the three populations were smaller than the table value (9.488) at 5 per cent level of significance, that established the homogeneous nature of population. The slopes of the probit line were found to be 7.173, 4.906 and 4.407 for laboratory susceptible, Chevella and Maheshwaram populations of *S. litura,* respectively and were in accordance to the log concentrations.

3.1.2.2 Toxicity of emamectin benzoate to susceptible and field populations of S. litura

The result on toxicity of emamectin benzoate to laboratory susceptible and field populations of *S. litura* showed that LC50 values for field collected populations were much higher than the LC50 value for laboratory susceptible population. The LC50 value for Chevella population was found to be 0.018310 per cent which was all most similar to that obtained for the Maheshwaram population *i.e.,* 0.018078 per cent. However, the LC50 value for laboratory susceptible population was found to be 0.000518 per cent which was very much less compared to field populations (Table 7).

The results pertaining to 95 per cent fiducial limits of LC50 values of Chevella (0.014132 to 0.022659%) and Maheshwaram (0.012986 to 0.023173%) populations did not overlap with fiducial limits of susceptible population (0.000279 to 0.000694%) confirmed existence of variability between the field populations of both locations *viz*., Chevella and Maheshwaram as well with the susceptible population. Further, the homogeneous nature of a given population was confirmed by the estimated χ2 value for emamectin benzoate (0.401, 0.496 and 1.372) which were found to be smaller than the table value (9.488) at 5 per cent level of significance. The slopes of the probit line were found to be 8.508, 6.660 and 5.866, respectively for three different populations *viz.,* laboratory susceptible, Chevella and Maheshwaram populations and were in accordance with the log concentrations.

3.1.2.3 Toxicity of flubendamide to susceptible and field populations of S. litura

The toxicity of flubendamide to laboratory susceptible populations of *S. litura* was found to be much higher with low LC50 value of 0.000697 per cent. However, the LC50 values for the field populations *viz.,* Chevella and Maheshwaram were found to be 0.019680 per cent and 0.019065 per cent, respectively (Table 7).

The existence of variability among three different populations was confirmed with the results obtained pertaining to 95 per cent fiducial limits of LC50 value. The fiducial limits of Chevella (0.009262 to 0.026532%) and Maheshwaram (0.014635 to 0.022595%) populations did not overlap with the fiducial limits of susceptible population (0.000573 to 0.000794%). The homogeneity of the tested populations was confirmed with the estimated χ2 values *viz.,* 0.241, 2.399 and 3.812 for laboratory susceptible, Chevella and Maheshwaram populations, respectively which were found to be smaller than the table value (9.488) at 5 per cent level of significance. The slopes of the probit line was found to be 8.008, 4.102 and 3.812 for laboratory susceptible, Chevella and Maheshwaram populations, respectively and were in accordance with the log concentrations.

Based on their LC50 values (Fig. 1), among the three insecticides tested chlorantraniliprole (0.006904, 0.007689 and 0.000328%, respectively) was found to be significantly more toxic to all three populations *viz*., Chevella, Maheshwaram and susceptible populations followed by emamectin benzoate (0.018310, 0.018078 and 0.000518%, respectively) and flubendamide (0.019680, 0.019065 and 0.000697%, respectively).

The toxicity of all the three insecticides arranged in decreasing order as follows: chlorantraniliprole > emamectin benzoate > flubendamide. Similar results were also reported by earlier workers. Sattar *et al*. [25] found that emamectin benzoate is the most effective compound against *Helicoverpa armigera* followed by flubendiamide, lufenuron, spinosad, indoxacarb and neem oil. Sabri *et al*. [26] reported moderate toxicity of emamectin benzoate in field collected population from Faisalabad, Pakistan compared to control treatment against different life stages of *S. litura*. Natikar and Balikai [27] revealed that flubendamide was least toxic among nine insecticides that were tested against *S. litura.* Karuppaiah and Srivastava [28] reported highest toxicity of chlorantraniliprole with the lowest LC50 value followed by emamectin benzoate and indoxacarb when tested against 7-day-old *S. litura* larvae using leaf dip method in Sonepat field collected population from Punjab.

The results pertaining to toxicity of all the selected insecticides against two different field populations and the laboratory cultured *S. litura* population clearly indicated development of resistance by field populations of *S. litura* to all the three insecticides. This is evident by the record of higher LC50 values for the field populations compared to laboratory susceptible population. However, calculation of resistance ratio clearly established the level of resistance acquired by a given regional population for a particular insecticide. The development of resistance for a given insecticide is a normal phenomenon that could happen for several reasons which include repeated application of a given insecticide over a long period of time. Indiscriminate usage of an insecticide at higher doses than recommended dosage. Non adoption of rotation of insecticidal sprays having different modes of action *etc*. are some primary reasons

Table 7. Toxicity of selected insecticides to susceptible and field populations of *S. litura*

Insect population	**Insecticide**	**LC50 (%)**	**95% Fiducial limits**		**χ2 value** (Heterogenicity)	**slope ± SEM**
			Lower	**Upper**		
Susceptible population (10th generation)	Chlorantraniliprole	0.000328	0.000120	0.000486	0.869	7.173 ± 1.99
	Emamectin benzoate	0.000518	0.000279	0.000694	0.401	8.508 ± 2.32
	Flubendamide	0.000697	0.000573	0.000794	0.241	8.008 ± 2.07
Chevella population	Chlorantraniliprole	0.006904	0.004118	0.009658	0.492	4.906 ± 1.52
	Emamectin benzoate	0.018310	0.014132	0.022659	0.496	6.660 ± 2.040
	Flubendamide	0.019680	0.009262	0.026532	2.399	4.102 ± 1.306
Maheshwaram population	Chlorantraniliprole	0.007689	0.004666	0.011691	1.326	4.407 ± 1.50
	Emamectin benzoate	0.018078	0.012986	0.023173	1.372	5.866 ± 1.99
	Flubendamide	0.019065	0.014635	0.022595	3.812	3.812 ± 0.65

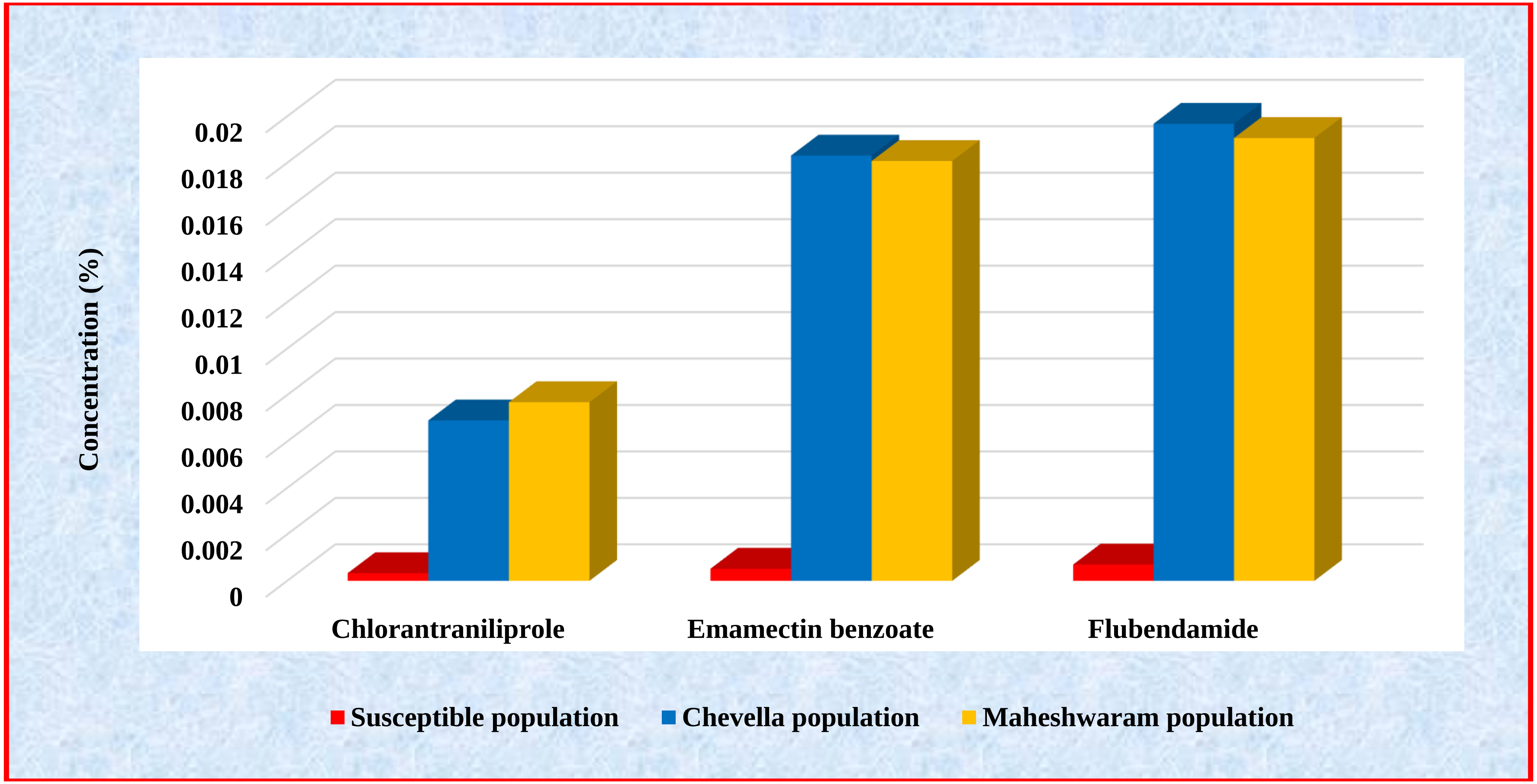

Fig. 1. **LC_{50} of test insecticides against susceptible and field populations of *S. litura***

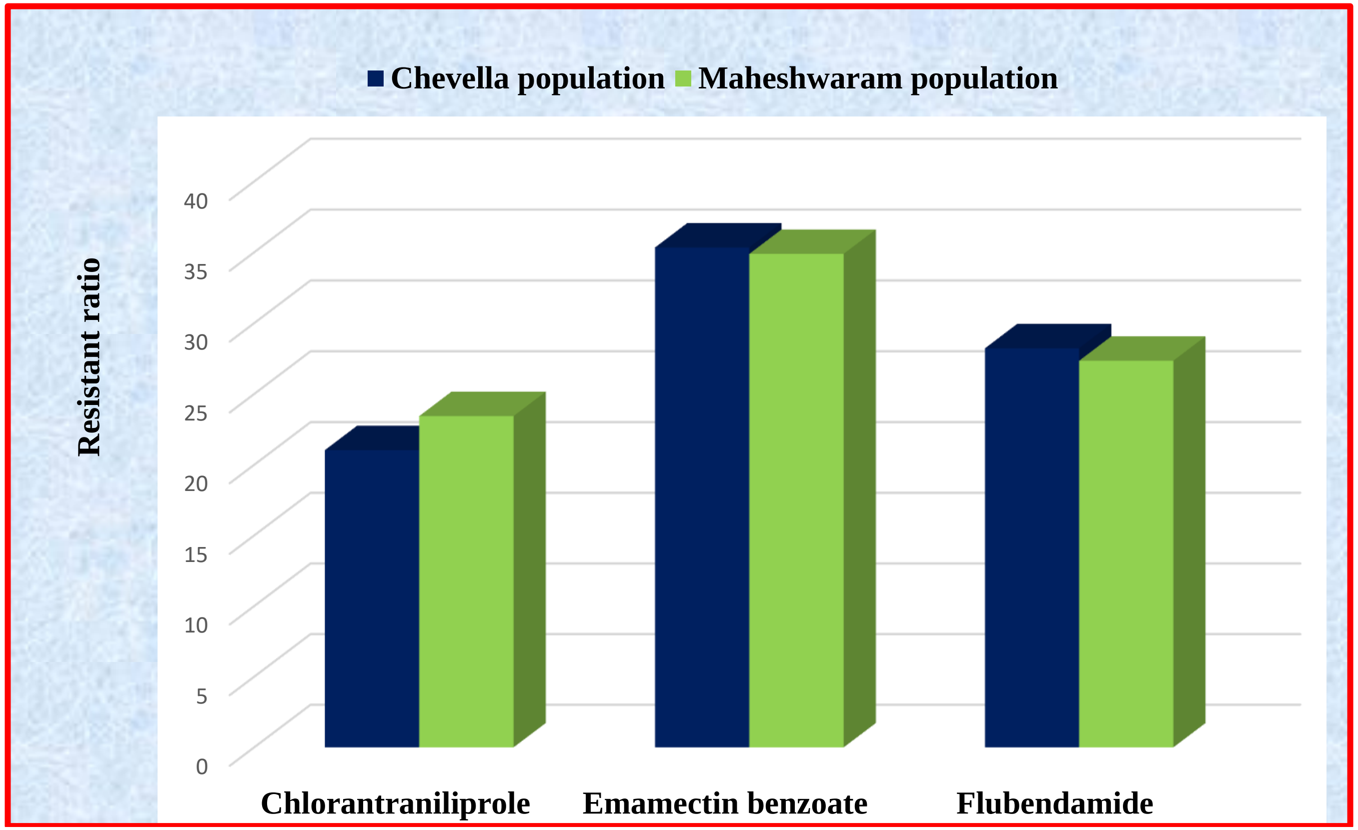

Fig. 2. Resistant ratio of test insecticides against field populations of *S. litura*

along with other minor reasons such as non - adoption of integrated pest management practices especially for insects like *S. litura* having broad host range.

The present results pertaining to development of resistance by *S. litura* for the selected three insecticides *viz.,* chlorantraniliprole, emamectin benzoate and flubendamide were in conformity with the findings of Hafez [29] who reported development of 3.17 and 5.57-folds resistance in field collected strains of *S. littoralis* from Egypt towards flubendamide and emamectin benzoate, respectively compared to laboratory susceptible population. The development of resistance was attributed to survivals of heterozygous population. Similar, development of resistance in *Tuta absoluta* against chlorantraniliprole and flubendamide was also reported by Roditakis *et al*. [13] in Sicily (Italy) and Crete (Greece) due to application history of insecticides related to diamide group in Crete (52%) and Sicily (64%). They further assumed cross resistance as the key factor for development of resistance by *T. absoluta* even against the newly launched insecticides because of similar mode of action. Su *et al.* [30] reported that all the field populations of *S. litura* collected from 12 different provinces from China showed less susceptibility to chlorantraniliprole than the laboratory susceptible population. They further reported that *S. litura* populations from two provinces out of 12 showed more tolerance against chlorantraniliprole. It was later found that *S. litura* populations of those two provinces were frequently challenged by application of huge insecticidal sprays and concluded that the development of resistance in *S. litura* might be due to heterogeneity, environment dissimilarities and different insecticide application history.

3.1.3 Degree of resistance acquired by field populations of *S. litura* against selected insecticides

The resistance ratios in field population of *S. litura* to different insecticides were calculated by taking into account of LC50 value of susceptible population and field population of Chevella and Maheshwaram and presented in Table 8 and 9 (Fig. 2). The results revealed that Chevella and Maheshwaram populations of *S. litura* developed highest resistance of about 35.34 and 34.90-fold, respectively against emamectin benzoate. However, the Chevella and Maheshwaram populations developed only moderate level of resistance up to 28.23 and 27.35-fold, respectively against flubendamide. Least level of resistance *viz.,* 21.05 and 23.44-fold was developed by Chevella and Maheshwaram populations against chlorantraniliprole.

The order of resistance ratios both in Chevella and Maheshwaram was as follows: emamectin benzoate > flubendamide >chlorantraniliprole.

These findings are in consistent with those of Stavrakaki *et al*. [31] who conducted resistant studies in *T. absoluta* in a field population from Crete (London) against emamectin benzoate and reported reduced efficacy. They also reported development of high level of resistance up to 60 folds in laboratory selected population exposed continuously to emamectin benzoate for eight consecutive generations. Similar studies were conducted by Muraro *et al.* [21] in the field collected population of *S. frugiperda* and reported development of resistance up to 2340-fold after ten generations of selection pressure against emamectin benzoate. The development of resistance by field population of *S. litura* against 21 insecticides was studied by Wang *et al.* (2019) from Huizhou, Guangdong Province, China and reported highest resistance up to 234.1-fold was developed against metaflumizone and 183.3 against emamectin benzoate in *S. litura*. Development of highest resistance (220-fold) by field population of *S. exigua* against ememectin benzoate was reported by Ishtiaq *et al.* [22] According to bioassay conducted by Zaka *et al*. [32], emamectin benzoate- selected strain of *S. litura* developed a resistance ratio that was 911 times greater when compared to the susceptible strain. Similarly, Zhang *et al.* [33] evaluated eight insecticides against *S. exigua* populations and found resistance ratios in the range of 3.00 to 37.4-fold against emamectin benzoate in different populations. Che *et al*. [10] investigated the resistant status of nine insecticides in 16 field population of *S.exigua* compared with susceptible population resulting resistant ratio of emamectin benzoate (4 to 348-fold). Zhou *et al.* [34] evaluated resistance to emamectin benzoate in Taian, Zhangqiu and Anqiu populations of *S. exigua* and reported resistance ratios ranging from 3.77 to 4.93.

In the present study the level of resistance development ranged between 21.05- 35.34 against all the three selected insecticides. The field populations of *S. litura* tested in the present study were collected from the major vegetable growing areas of Rangareddy district where the insecticides, chlorantraniliprole, emamectin

Table 8. Resistance ratio of selected insecticides against Chevella field population compared with susceptible population of *S. litura*

Insecticide	LC50 of Chevella population	LC50 of Susceptible population	Resistance Ratio (RR)
Chlorantraniliprole	0.006904	0.000328	21.05
Emamectin benzoate	0.018310	0.000518	35.34
Flubendamide	0.019680	0.000697	28.23

**Resistant ratio = LC50 of field population / LC50 of susceptible population*

Table 9. Resistance ratio of selected insecticides against Maheshwaram field population compared with susceptible population of *S. litura*

Insecticide	LC50 of Maheshwaram population	LC50 of Susceptible population	Resistance Ratio (RR)
Chlorantraniliprole	0.007689	0.000328	23.44
Emamectin benzoate	0.018078	0.000518	34.90
Flubendamide	0.019065	0.000697	27.35

**Resistant ratio = LC50 of field population / LC50 of susceptible population*

benzoate and flubendamide were being used widely for control of lepidopteran pests including *S. litura* for more than a decade. *S. litura* being polyphagous is a serious problem in the vegetable crop ecosystem the reason being the availability of wide range of host plants throughout the year. Due to high economic value of the vegetables, farmers apply chemical insecticides intensively to protect the crop from pest damage. Muraro *et al*. [21] studied the patterns of resistance development by *S. frugiperda* in Brazil and reported that at the recommended field rate of emamectin benzoate application the heterozygous population had a high rate of survival. These survival populations were favouring the evolution of resistance. Thus, they confirmed that the resistance is functionally dominant under field condition which was the primary reason that led to field failures in specific Brazilian regions. They further reported that insecticidal application history could be the second important factor for development of resistance by *S. frugiperda* through cross-resistance. Thus, in order to prevent *S. frugiperda* in Brazil from developing resistance to emamectin benzoate, it was recommended that insecticides with various modes of action be rotated. Biradar *et al.* [35] reported higher levels of resistance in the populations of Mysuru and Haveri of *P. xylostella* when it was subjected to the selective pressure of emamectin benzoate because it is often usage in those regions.

Roditakis *et al*. [13] reported high percentage usage of insecticides belonging to diamide group in the sampled farms from Italy and Greece which was the primary reason for the development of high levels of cross resistance against all insecticides belonging to diamide group. They further recommended rational use of diamides in pest management programmes to avoid any further development or expansion of resistance against insect pests in both the countries. Wang *et al.* [36,37] reported high levels of field-evolved resistance to chlorantraniliprole in Chinese populations of *P. xylostella* due to over usage of chlorantraniliprole and found field-evolved resistance to chlorantraniliprole showed strong cross-resistance to flubendiamide. They further suggested that both the compounds should not be alternated in resistance management strategies. The further opined that continuous and intensive use of these insecticides against *P. xylostella* might be the reason for development of high level of resistance against *S. litura*. Similarly, high fold of resistance development was reported by Wang and Wu [36,37] due to intensive use of chlorantraniliprole against *P. xylostella* from Guangdong Province of southern China.

Hence, application of insecticides having different mode of action along with practicing of integrated pest management practices is the key to delay or avoid resistant development against insecticides [38].

4. SUMMARY AND CONCLUSIONS

The baseline susceptibility was initially established for three selected insecticides *viz.,* chlorantraniliprole, emamectin benzoate and flubendamide against 10th generation third instar larvae of *S. litura* by using leaf disc method. Similarly, LC_{50} values were also determined for the F_1 generation third instar larvae of *S. litura* from Chevella and Maheshwaram. *S. litura* population collected from Chevella and Maheshwaram mandals exhibited higher LC_{50} to all the three selected insecticides as compared to the susceptible population. Irrespective of populations, among the three insecticides tested chlorantraniliprole exhibited higher toxicity by recording lower LC_{50} values of 0.006904, 0.007689 and 0.000328 per cent followed by emamectin benzoate (0.018310, 0.018078 and 0.000518%, respectively) and flubendamide (0.019680, 0.019065 and 0.000697%, respectively) against Chevella, Maheshwaram and susceptible populations, respectively. The decreasing order of toxicity of selected insecticides as chlorantraniliprole> emamectin benzoate> flubendamide.

In the present study, field populations of *S. litura* from Chevella and Maheshwaram developed resistance against to all the three selected insecticides with resistance ratios ranged from 21.05 to 35.34. Irrespective of locations, high level of resistance in *S. litura* larvae was recorded to emamectin benzoate (35.34 and 34.90-fold, respectively) followed by moderate level of resistance to flubendamide (28.23 and 27.35-fold, respectively) and low level of resistance to chlorantraniliprole (21.05 and 23.44-fold, respectively) in Chevella and Maheshwaram populations, respectively. The order of resistance levels of selected insecticides as follows: emamectin benzoate> flubendamide> chlorantraniliprole. The field populations of *S. litura* tested in this study were collected from major vegetable growing areas of Rangareddy district, where the farmers have been using chlorantraniliprole, emamectin benzoate and flubendamide widely for the management of several lepidopteran insect pests including *S. litura.* Continuous and intensive use of above selected insecticides might be the reason for acquiring resistance in field populations of *S. litura*. Among all tested insecticides, chlorantraniliprole was highly toxic as evidenced by lower LC_{50} values followed by emamectin benzoate and flubendamide against all tested populations of *S. litura*. The degree of resistance acquired by field population of *S. litura* from both Chevella and Maheshwaram mandals of Rangareddy district was high against emamectin benzoate.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/3435