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Original Research Article

Baseline susceptibilityand resistance monitoring for Novaluran 10% EC against *Spodoptera frugiperda(J. E. Smith)*

ABSTRACT

A Study was undertaken to assess the susceptibility offall armyworm (FAW), *Spodoptera frugiperda(J. E. Smith)*toNovaluran (10% EC) bydiet overlay bioassay method.Novaluran 10% EC was tested *in vitro* against *S. frugiperda*, obtained from four important Maize-producing tracts in Tamil Nadu. The susceptible populationwasobtained from FAW laboratory at TNAU which was inthe150th generation and further, reared up to 157thgeneration.The LC_{50} and LC_{95} values of Novaluran10% ECto the susceptible population decreased from 0.832 to 0.746 ppm and 2.134 to 1.451 ppm, respectively. The susceptibility index of Novaluran was 1.115 and 1.470. The F157 population of *S. frugiperda*was used to conduct the preliminary discriminating dosage in Novaluran10% ECand was 0.746 ppm. Resistance monitoring studies of *S. frugiperda*from Coimbatore, Perambalur, Salem, and Theni districts revealed that the LC_{50}values ofNovaluran10% ECranged from 0.792 and 0.930 ppm for Theni and Coimbatore, respectively. Novaluran 10% EC had the highest resistance ratio of 1.246 fold in the Coimbatore field population and the lowest resistance ratio of 1.061 fold in the Theni field population when compared with theTNAU FAW laboratory susceptible population of *S. frugiperda*

Keywords*:* Insecticide susceptibility; discriminating dose; *S. frugiperda*; Novaluran 10% EC; resistance ratio;resistance monitoring; susceptibility index

1. INTRODUCTION

The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith),(Lepidoptera: Noctuidae) is a voracious insect pest endemic to the Western Hemisphere, mainly in South AmericaPogue (2002) [1]. It is one of the most quickly spreading and invasive maize pests in Africa and Asia(Tambo et al.,2021;Ramasamy 2022) [2-3]. *S.frugiperda* has become a pest species due to biological traits like polyphagy, hidden larval feeding habits, high reproductive capability, adult dispersal, and many generations per year(Liet al 2020; Bird et al 2021)[4-5]. *S. frugiperda* is an economically important pest that infects maize and other Gramineaefamily crops (Andrews, 1988) [6]. Fall armyworm is a polyphagous insect that causes substantial damage to cereal and vegetable crops (Goergen et al., 2016; Roger et al., 2017) [7-8]. Moths have migratory behaviour as well as a more confined dispersion habit. Before oviposition, they can travel beyond 500 km (300 miles) (Prasanna et al., 2018)[9].FAW was initially detected in West Africa in late 2016 (Goergen et al., 2016) and has since spread to 44 African countries (Bhusal et al., 2019)[7,10]. Sharanabasappa and Kalleshwaraswamy (2018) reported the first appearance of this novel invasive pest FAW in India [11]. It feeds on at least 186 host plants and is a major pest of economically important crops such as corn, sorghum, and rice (Casmuz et al. 2010) [12].FAW has evolved resistance to 29 insecticides with six distinct mechanisms of action (Chao et al., 2019) [13].In every farming system where pesticides are frequently employed, resistance isa major concern, and monitoring the target pest's susceptibility is critical for successful integrated pest management (IPM) and insecticide resistance management (IRM). Insecticide use is literally expanding in maize fields in recent days. As a result, the goal of this study was to monitor the fall armyworm resistance development to Novaluran10% EC in populations obtained from several maize production districts of Tamil Nadu. Studies on the resistance levels of the freshly built FAW in Tamil Nadu settings will help stronger and more effective IPM decision management systems. In this regard, attempts were made to determine the resistance levels of *S. frugiperda*against Novaluran10% EC in maize.

2. MATERIAL AND METHODS

2.1 Maintenance of Insect Culture

Fourth and fifth instar larvae field populations of *S. frugiperda* were collected from four diverse geographical locations *viz.,*Coimbatore, Perambalur, Salem, and Theni in Tamil Nadu, India (Fig. 1 and Table 1). These larvae were reared in an artificial dietin individual feeding boxes at TNAU FAW laboratory. They were separated from the containers after pupation and placed into adult emergence cages with 10 per cent sugar solution to aid in oviposition. The egg masses that resulted were grown inanartificial diet and the population was kept under controlled circumstances (25°C, 70% relative humidity, and a 14:10 h light/dark photoperiod) without selection pressure (no pesticide exposure).In the study, the field-collected populationwill be served as a resistant strain of FAW (RS). Further,thesusceptible strain of FAW (SS) received fromtheFAW laboratory without selection pressure upto 157 generations under similar conditions at the Department of Agriculture Entomology, Tamil Nadu Agricultural University, Coimbatore was utilized to compare the median fatal dose with a resistant strain.

Table 1. Background data for field populations of *Spodoptera frugiperda* collected from different sites

Collected Location	Coordinates	Map Reference No.	Host plant
Perambalur –Tamilnadu	11.33º N, 78.81º E	1	*Zea mays*
Salem – Tamilnadu	11.62º N, 78.58º E	2	*Zea mays*
Coimbatore – Tamilnadu	11.01º N, 76.93º E	3	*Zea mays*
Theni – Tamilnadu	10.09º N, 77.64º E	4	*Zea mays*

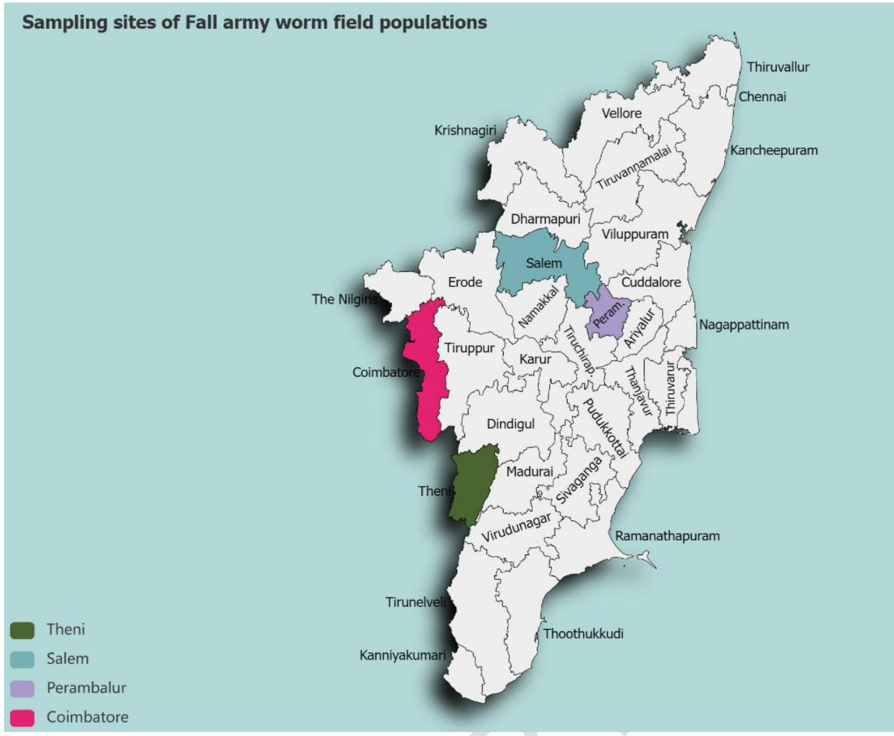

Fig. 1. Sampling sites of *S. frugiperda* field populations in Tamil Nadu

2.3 Diet overlay bioassay

The Second instar of *S. frugiperda*larvae was subjected to a diet overlay bioassay method described by Ahmad andGull (2017) [14]. 1 mL of liquefied diet was placed on 24 well bioassay plates and dried for 30 minutes. Serial dilutions of the active component of each proposed insecticide in mg/L were prepared using distilled water. Insecticide concentrations (100µL each) were poured onto the surface of the diet in individual wells. By rotating and shaking the wells, the insecticide solution was evenly spread throughout the diet. For control, the diet was treated with distilled water. After the insecticide solution had dried (45minutes),10 larvae per replication were released.For each concentration, three replicateswere maintained. Each treatment maintained the same number of larvae and untreated as a control. Larvae were maintained at a constant temperature of 25 °C (-2 °C) with a photoperiod of 14 hours before and after treatment. Larval fatalities were recorded after 24 hours, 48 hours, and 72 hours. When larvae were probed with a probe and did not move, they were deemed dead. The POLOPLUS application was used to examine all of the bioassay data.

2.4 Discriminating Dose Fixation

The median lethal concentration (LC_{50}) was determined using preliminary bioassay data from the 150th susceptible population developed from FAW lab at the Department of Entomology, TNAU. From the 151st, no selection pressure (or pesticide exposure) was applied until the Fn generation. Based on the doses indicated by the preliminary range finding test, bioassays were performed for the susceptable population. A discriminating dose was determined based on the LC_{50} and LC_{95} value obtained for the 'n' generation for susceptible population.

2.5 Statistical Analysis

Finney's probit analysis was used to establish the median lethal doses (LC_{50}) of the pesticide selected, which were validated in POLOPLUS software version 2.0. Susceptibility indices were calculated using the LC_{50} and LC_{95} values obtained from the last generation that was not exposed to pesticides. The Susceptibility Index (SI) is the ratio of the first generation LC_{50} or LC_{95} to the final generation LC_{50} or LC_{95}. Regupathy and Dhamu (2001) [15] determined the rate of resistance drop (R) and the number of generations necessary for a ten-fold fall in LC50 value (G).

$$\mathbf{R} = \frac{\mathbf{Log(Final\ LC50) - Log\ (initial\ LC50)}}{\mathbf{n}}$$

$$\mathbf{G = 1/R}$$

$$\mathbf{ID\ value} = \left[\frac{\mathbf{Slope\ of\ last\ Generation}}{\mathbf{Slope\ of\ first\ generation}} - \mathbf{1}\right] \mathbf{x\ 100}$$

$$\mathbf{RR} = \frac{\mathbf{LC50\ of\ resistant\ Strain\ \ \ (RS)}}{\boldsymbol{LC50\ of\ susceptibility\ strain\ (RR)}}$$

2.5 Monitoringthe Insecticide Resistance

A diluted insecticide based on the concentration of the discriminating dose of Novaluran (0.746 ppm) was applied to the surface of artificial diet in 24 wells using the diet overlay bioassay method against the larval population collected from four fields in Coimbatore, Salem, Perambalur, and Theni.

$$\mathbf{RP = (100 - CM)SE}$$

The technique given by (Abbott,1925) [16] was used to calculate the corrected mortality (CM) and standard error (SE).

3. RESULTS AND DISCUSSION

The log concentration probit mortality lines (lcpm) were built for the population of fall armyworm moths obtained from maize fields and raised up to F157 generations without insecticide exposure in FAW laboratory, Department of Agricultural Entomology, Tamil Nadu Agricultural University using baseline data for test insecticide of Novaluran 10% EC.Table3shows the LC_{50} and LC_{95} values of Novaluran 10% EC against *S.frugiperda* determined by diet overlay bioassay for F151, F152, F153, F154, F155, F156, and F157 generations.

3.1 Baseline susceptibility

The median LC_{50} and LC_{95} values for the F151 population against Novaluran were 0.832 ppm and 2.134 ppm, respectively. The median LC_{50} and LC_{95} values for the F157 population for Novaluran was 0.746 ppm and 1.451 ppm. The $LC_5$0 and LC95 values were observed to be lowering with following generations and stabilizing for F156 and F157 generations, indicating that susceptibility increased with succeeding generations.

The calculated LC_{50} and LC_{95} values ofnovaluran revealed that susceptibility steadily increased with successive generations from F151 to F157 (LC_{50}: 0.832 to 0.746 ppm) and(LC_{95}: 2.134 to 1.451 ppm). After F157 generation, the susceptibility index ofnovaluranwas 1.115 and 1.470ppm(Table 3).Resistance drop (R) of novaluranwas -0.009. A negative R value suggested that susceptibilityincreased with successive generations. The number of generations needed for a 48-fold drop in LC_{50} was 108 (Table 2).Based on the baseline toxicity values obtained for the F157th generation of fall armyworm moth reared in insecticide-free conditions, a preliminary discriminating dose (DD) of 1.451 ppm novaluran was determined. Based on the existing range data, a tentative discriminating dosage of 1.45 was determined to be served for detecting novaluran resistance in field populations of Tamil Nadu, India, including Coimbatore, Perambalur, Salem, and Theni.

According to Vinothkumar Bojan et al.(2023), the LC_{50} value of novaluran 10% EC is 0.91ppm in field collected population in Coimbatore,India[17]. In Israel, Rami Horowitz et al. (2022) found that the LC_{50} value of novaluran 5% SG is 0.06ppm in susceptible population[18].According to Rebeca Gutiérrez-Moreno (2019), the Puerto Rican population displayed extraordinary field-evolved resistance to triflumuron (20-fold), with an LC_{50} of 0.08ppm [19].Thirawutetal.(2023) revealed that in Southeast Asian nations, the LC_{50} value of a field population of 5 locations against Lufenuron 5% EC is TM2019 (2.359), SN2019 (2.267), SP2021 (4.558),TL2021 (1.034),KC2022 (0.259),WS2022 (0.209)and the resistance co-efficient values indicated that *S. frugiperda* developed low resistance toonly one pesticide (lufenuron) among the other pesticides[20].

Table 2. Baseline susceptibility of *S. frugiperda* to Novaluran 10% EC by Diet overlay bioassay method

Generation	Chisquare (Σ2)	Slope	LC50 (ppm)	Fiducial Limit LL	Fiducial Limit UL	LC95 (ppm)	Fiducial Limit LL	Fiducial Limit UL
F151	0.114	4.204	0.865	0.786	0.952	2.125	1.444	3.126
F152	0.224	4.235	0.847	0.769	0.934	2.088	1.423	3.062
F153	0.153	4.275	0.783	0.705	0.870	1.911	1.354	2.696
F154	0.445	4.400	0.774	0.697	0.859	1.850	1.334	2.565
F155	0.316	4.624	0.768	0.695	0.850	1.755	1.310	2.351
F156	0.807	5.619	0.749	0.685	0.819	1.498	1.215	1.845
F157	0.923	6.037	0.746	0.683	0.806	1.451	1.162	1.813

LL – Lower Limit; UL – Upper Limit

Table 3. Susceptibility Index of *S. frugiperda*to Novaluran 10% EC

Generation	LC50 (ppm)	LC95 (ppm)	Susceptibility Index LC50	Susceptibility Index LC95	Rateof Resistance Decline R	Rateof Resistance Decline G	Slope function I/D %
F151	0.865	2.125	1.159	1.464	-0.009	-108.903	43.571
F157	0.746	1.451	1.000	1.000			

Table 4. Resistance Ratio of Novaluran 10% ECto different locations of *S. frugiperda*

Location	N	Regression Equation	LC50 (ppm)	Fiducial Limit LL	UL	LC50of susceptible Population (ppm)	Resistance Ratio
Coimbatore	180	y=5.108+ 3.465x	0.930	0.827	1.047	0.746	1.246
Salem	180	y=5.131+3.373x	0.908	0.812	1.091	0.746	1.217
Perambalur	180	y=5.282+4.954x	0.878	0.800	0.953	0.746	1.176
Theni	180	y=5.494+4.874x	0.792	0.722	0.868	0.746	1.061

3.2 Resistance Ratio

The bioassay was carried out against*S.frugiperda* field populations collected in from different District ofTamil Nadu (Coimbatore, Perambalur, Salem, and Theni). For the test insecticides (Novaluran 10% EC), log concentration probit mortality (lcpm) lines were fitted to resistance populations acquired across sites. The median lethal concentration (LC50) values for each region's F1 *S.frugiperda* generation were computed.

The LC50 values in ppm for the Coimbatore, Salem, Perambalur, and Theni populations in Novaluran 10% EC were 0.930, 0.908, 0.878, and 0.792, respectively. The resistance ratios (RRs) were determined using the susceptible population's LC50 (0.746 ppm) and revealed a 1.176 (Perambalur), 1.217 (Salem), 1.246 (Coimbatore), and 1.061 (Theni) fold increase in resistance as compared to the susceptible population (Table 4).

4. CONCLUSION

The current study unveiled that populations of the *S. frugiperda*, gathered from distinct maize cultivating regions in Tamil Nadu namely Perambalur, Salem, Coimbatore, and Theni, exhibited variations in their sensitivity to Novaluran. These differences were attributed to factors like changes over time, geographical diversity, varying response to the chemical's toxicity, the dosage employed, and the manner in which the test insecticide was used. In comparison to the population from Salem, Perambalur, and Theni, the Coimbatoresample shown greater resistance to Novaluran 10% EC.

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