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Baseline susceptibility of selective insecticides against *Spodoptera frugiperda* (JE Smith) on maize in Tamil Nadu

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Abstract
The fall armyworm (FAW*)* *Spodoptera frugiperda* (JE Smith) is an invasive pest of maize in India. This pest reached epidemic proportions, causing enormous damage on the maize crop in several Indian states. The reliance on pesticides to control fall armyworm could lead to development of resistance in many of the Indian maize growing areas. Study was undertaken to assess the susceptibility of fall armyworm to three insecticides *viz*., chlorantraniliprole 18.5 SC, flubendiamide 480 SC and spinetoram 11.7 SC was determined by leaf disc bioassay. The LC_{50} and LC_{95} values of field population towards chlorantraniliprole 18.5 SC, flubendiamide 480 SC and spinetoram 11.7 SC were 6.93 and 16.40 μgmL^{-1}; 121.36 and 180.69 μgmL^{-1} and 0.046 and 0.321 μgmL^{-1} respectively. The susceptibility of field collected populations were compared with FAW population maintained in the laboratory without exposure to insecticides for 25 generations. Resistance ratio (RR_{50}) of the test insecticides *viz*., chlorantraniliprole 18.5 SC (2.24-fold), flubendiamide 480 SC (2.44-fold) and spinetoram 11.7 SC (1.64-fold) implied that flubendiamide 480 SC was showing more resistance compared to chlorantraniliprole 18.5 SC and Spinetoram 11.7 SC. Spinetoram 11.7 SC was more potent compared to other two insecticides based on relative potency ratio (REP).

Keywords: Baseline insecticide susceptibility, chlorantraniliprole 18.5 SC, flubendiamide 480 SC and spinetoram 11.7 SC, Resistance ratio, Spodoptera frugiperda

1. Introduction

Spodoptera frugiperda (JE Smith, 1797) [28], (Lepidoptera: Noctuidae), also known as the fall armyworm (FAW), is native to the tropical and subtropical regions of America (Sparks, 1979) [21]. It is a polyphagous pest with wider host range, including 353 plants from 76 different families, primarily Poaceae, Asteraceae, and Fabaceae (Montezano *et al*., 2018) [19]. The main hosts *viz*. maize and sorghum suffer the most damage (Casmuz *et al*., 2010; Montezano *et al*., 2018) [6, 19]. Its invasiveness and ranking as one of the most harmful economic pests could be attributed to its superior biological traits, which include the capacity to survive in a variety of habitats, strong ability for migration, and high fecundity (Jing *et al*., 2021) [16]. FAW has now spread across the world (Anonymous 2021) [1] covering 107 countries. It was first discovered to have invaded West Africa in 2016 (Anonymous 2016) [2], reported later in Southeast Asia and more recently in Australia (Goergen *et al*., 2016 [12], Kalleshwaraswamy *et al*., 2019 [29], Shylesha *et al*., 2018) [20].

In the Indian subcontinent, FAW was first noticed in maize fields of Karnataka during May 2018 (Ganiger *et al*., 2018) [10]. The first report of the fall armyworm in Tamil Nadu was in sugarcane (Srikanth *et al*., 2018) [22]. The food security of India has then, been in danger due to the invasion of fall armyworm. To address the food security and global economic concerns, synthetic insecticides have continued to play an important role in FAW control programmers. Farmers rely heavily on synthetic pesticides for the immediate control of the fall armyworm threat. In Tamil Nadu, only ad-hoc recommendations were made initially for controlling this devastating pest. Concealed mode of feeding necessitated the need for multiple sprays and higher doses which in turn has resulted in development of resistance globally to various classes of chemical insecticides and, more recently, against transgenic BT crop (Yu, 1982; Giraudo *et al*., 2015; Flagel *et al*., 2018) [24, 11, 9]. It has been reported that FAW has developed resistance to 29 insecticides belonging to groups of six different modes of action (Chao *et al*., 2019) [7]. Studies on resistance levels of the recently established FAW under Tamil Nadu conditions would support for stronger and effective IPM decision management systems. In this context, attempts were made on determining the resistance levels of *S. frugiperda* against chlorantraniliprole 18.5 SC, flubendiamide 480 SC and spinetoram 11.7 SC.

2. Materials and Methods

2.1 Mass rearing of *Spodoptera frugiperda*

Egg masses and larvae were collected from the maize fields of Tamil Nadu Agricultural University, Coimbatore and reared on TNAU semi synthetic diet in the FAW laboratory, Department of Agricultural Entomology. Egg masses collected were released into freshly prepared semi synthetic diet and reared. Once the larvae reached 3rd instar, individual larvae were placed into distinct containers with semi synthetic diet to prevent cannibalism. After pupation, they were collected from the containers and placed in adult cages provisioned with honey solution to facilitate efficient oviposition. The collected egg masses were continued to be reared following the same procedure and the F1 population was maintained without selection pressure (no exposure to insecticides), under controlled conditions (25±1 °C, 70±10% relative humidity, and a 14:10 h light/dark photoperiod) during all phases of development. This population was used as the resistant strain of FAW (RS) in the study. Susceptible strain of FAW (SS) which had been maintained in the laboratory without selection pressure for 25 generations under identical conditions at the Department of Agricultural Entomology, Tamil Nadu Agricultural University, and Coimbatore was used in the experiment to compare the median lethal dosage with that of the resistant strain.

2.2 Test insecticides used

The insecticides used in the present investigation were commercial formulations of chlorantraniliprole 18.5 SC, flubendiamide 480 SC and Spinetoram 11.7 SC (the details of which are presented in Table 1). Insecticide stock solutions were prepared and serial dilutions were made by dissolving the insecticide in distilled water containing 0.5% Triton X-100. Distilled water containing 0.5% Triton X-100 alone was used as control. Preliminary range finding tests were done, where the larvae were exposed initially to a wide range of concentrations and subsequently narrowed down to 5 or 6 concentrations which correspond to mortality ranging from 20 to 80 per cent (White and Loschiavo, 1995) [30].

Table 1: Insecticide formulations tested against fall armyworm

S. No	Chemical	Trade name	Formulation	Group	MoA	Company
1.	Chlorantraniliprole	Coragen	18.50 SC	Diamides	RyR modulator	Dupont Chemicals (India) Limited
2.	Flubendiamide	Fame	39.35 SC			Bayer Crop Science India
3.	Spinetoram	Makeba	11.70 SC	Spinosyns	nAChR allosteric modulator	Dow Agrosciences

MoA: mode of action, nAChR, Nicotinic acetylcholine receptor, SC: Suspension Concentrate

2.3 Leaf disc bioassay

Fresh and healthy maize leaves were collected from the pot culture or fields which were pesticide free. Leaf discs of about 4 to 5 cm were cut on either side of midrib and were immersed in the test concentrations for about a minute. Leaf discs were removed and placed on the filter paper to drain the excess water; the setup was then left for a short while until the leaf surfaces dried. Six welled tissue culture plates were used, into which agar-agar (10g in 1 lit) of 1cm depth was poured. The treated leaves were placed on the agar medium after solidification to help in retaining moisture. Ten larvae were used per replication and each treatment was replicated thrice. Third instar larvae were pre starved for 4 to 5 hours and individual larvae were released into each well to prevent cannibalism. The tissue culture plates were closed with lid having holes in it. The larvae were considered dead if they showed growth retarded symptoms like feeding cessation, reduced body size or if they were not able to return to upright position when prodded (moribund). The mortality of the larvae was observed at 24, 48 and 72 hours after treatment.

2.4 Statistical Analysis

The mortality data collected from bioassays were corrected using the formula given by Abbot (1925) [3]. Probit analysis was performed with the help of the software Polo Plus v.2.0 (LeOra software ©2002-2022) (Russell *et al*., 1977) [31] to determine the log concentration probit mortality lines (LCPM), slope, standard error, LC_{50}, LC_{95} and their 95% fiducial limits. Resistance ratio RR was used to determine the intensity of resistance of a strain or population of insects to a particular insecticide. Resistance ratio was calculated as the ratio of LC_{50} of Resistant Strain (RS) to LC_{50} of susceptible strain (SS). Relative potency (REP) is the ratio between two chemicals having equally effective doses (Finney, 1971) [8]. REP ratios of test insecticides against fall armyworm were calculated by dividing LC_{50} of least toxic chemical to LC_{50} of each chemical used in the experiment.

3. Results and Discussion

3.1 Baseline susceptibility

The LC_{50} values of the three test chemicals in the leaf disc bioassay carried out with the field collected Coimbatore population of *S. frugiperda* (RS) showed that spinetoram 11.7 SC (0.046 µgmL^{-1}) was the most toxic to the FAW RS population followed by chlorantraniliprole 18.5 SC (6.93 µgmL^{-1}) and flubendiamide 480 SC (121.36 µgmL^{-1}) (Table 2). The bioassay experiments on *S. frugiperda* susceptible F_{25} population (SS) maintained without insecticidal pressure, revealed that the LC_{50} values against chlorantraniliprole 18.5 SC, flubendiamide 480 SC and spinetoram 11.7 SC were, 3.09 µgmL^{-1}; 49.61 µgmL^{-1} and 0.028 µgmL^{-1} respectively (Table 3).

Similar study by Zhao *et al.*, (2020) [27] revealed that the LC_{50} values of chlorantraniliprole ranged between 0.849 mg/L to 3.446 mg/L when tested on eight field collected FAW populations. Results of Vinothkumar *et al*., (2021) [23] showed that the LC_{50} values for chlorantraniliprole was 5.63 µgmL^{-1} for third instar larvae which was in line with the findings of this study. The results of Beuzelin *et al*., (2021) [32] indicated wide variations in LC_{50} values as compared with the current study (0.022 to 0.084 ppm). Similar variations were also reflected in the works of Zhang *et al*. (2021) [26]; Kulye *et al*. (2021) [18] and Hardke *et al*. (2011) [14].

The LC_{50} value of flubendiamide (121.36 µgmL^{-1}) obtained in the present study was not in accordance with experiments carried out by Kulye *et al*. (2021) [18], (0.040 to 0.841); Zhang *et al*. (2021) [26], (0.009 to 2.5 µgmL^{-1}) and Hardke *et al*. (2011) [14], (0.930 µgmL^{-1}). But the findings of Vinothkumar *et al*. (2021) [23], who reported LC_{50} value of flubendiamide as 162.99 µgmL^{-1} was in accordance with the present study and the lesser variation recorded, which could be attributed to the similarity of geographic conditions. The current study presented LC_{50} value of spinetoram as 0.046 µgmL^{-1} and was in line with the LC_{50} value of 0.02 µgmL^{-1} obtained by Vinoth Kumar *et al*., (2021) [23] and Zhao *et al*. (2020) [27], (0.179 to 0.475 mgL^{-1}). Study by Moreno *et al*. (2021) [33] showed moderate variation with LC_{50} range of 0.003 to 0.02 µgmL^{-1}. The reasons for such differential susceptibility of insecticides across different locations might be due to different insecticide usage pattern, climate and type of host.

Table 2: Acute toxicity of test insecticides against field collected *Spodoptera frugiperda* population (resistant strain RS)

S No	Chemical	Slope± SE	Chi square	LC_{50} (µgmL^{-1})	Fiducial data		LC_{95} (µgmL^{-1})	Fiducial data	
					LL	UL		LL	UL
1.	Chlorantraniliprole 18.5 SC	4.394±1.194	0.035	6.93	6.27	7.68	16.40	12.40	33.45
2.	Flubendiamide 480 SC	9.516±2.143	0.669	121.36	116.15	127.40	180.69	160.67	231.72
3.	Spinetoram 11.7 SC	1.940±0.457	2.817	0.046	0.037	0.061	0.321	0.170	1.309

SE: Standard Error, LL: Lower Limit, UL: Upper Limit

Table 3: Acute toxicity of test insecticides against laboratory reared *Spodoptera frugiperda* population (susceptible strain SS)

S. No	Chemical	Slope±SE	Chi square	LC_{50} (µgmL^{-1})	Fiducial data		LC_{95} (µgmL^{-1})	Fiducial data	
					LL	UL		LL	UL
1.	Chlorantraniliprole 18.5 SC	2.654±0.564	1.021	3.09	2.61	3.63	12.91	8.79	27.90
2.	Flubendiamide 480 SC	3.935±0.860	1.916	49.61	44.55	55.63	129.87	98.57	229.35
3.	Spinetoram 11.7 SC	1.941±0.454	0.127	0.028	0.022	0.035	0.194	0.109	0.684

SE: Standard Error, LL: Lower Limit, UL: Upper Limit

3.2 Resistance ratio

The Resistance ratios (RR) were worked out by taking into account the LC_{50} of susceptible population maintained in the laboratory (SS) and field population from Coimbatore (RS). The RR obtained in the present investigation were 2.24-fold for flubendiamide 480 SC; 2.44-fold for chlorantraniliprole 18.5 SC and 1.64-fold for spinetoram 11.7 SC (Table 4). In correlation with this study, resistance ratio was found to be higher for flubendiamide than for the anthracitic diamides like chlorantraniliprole and cyantraniliprole as per the findings of Bolzan *et al*. (2018) [34].

The resistance ratio in case of chlorantraniliprole discreetly varied from that of Kulye *et al*. (2021) [18] who observed a resistance ratio of 6.84-fold where the field population was compared with the population maintained since fall armyworm entered India (2018). Wide variation of RR of 160 fold in chlorantraniliprole 18.5 SC was reported by Moreno *et al*. (2018) [35]. The resistance ratio of flubendiamide 480 SC was 500-fold (Moreno *et al*., 2018) [35] and 7.36-fold (Kulye *et al*., 2021) [18] all of which imply very low level of resistance development in the population tested in the current experiment.

From the results of the present study, spinetoram 11.7 SC showed lower RR of 1.64-fold among the three insecticides. The resistance ratio of spinetoram was recorded as 14-fold by Moreno *et al*. (2018) [35]. A recent study in Brazil showed huge variation in resistance ratio of 1844-fold. They reported cross-resistance with in spinosyns and resistance to spinetoram was identified as autosomal and incompletely recessive (Lira *et al*., 2020) [15]. Lower RR for spinetoram in the present study clearly indicated that the resistance development against spinetoram in the Coimbatore field population is extremely low.

Table 4: Resistance ratio of test insecticides against *Spodoptera frugiperda*

S. No	Chemical	Susceptible LC_{50}	Resistant LC_{50}	Resistant ratio RR_{50}
1.	Chlorantraniliprole 18.5 SC	3.09	6.93	2.24
2.	Flubendiamide 480 SC	49.61	121.36	2.44
3.	Spinetoram 11.7 w/w SC	0.028	0.046	1.64

Table 5: Relative potency ratios of selected insecticides against *Spodoptera frugiperda* resistant and susceptible populations

S. No	Insecticide	LC_{50} (Susceptible Population)	REP (Susceptible Population)	LC_{50} (Field Population)	REP (Field Population)
1.	Chlorantraniliprole 18.5 SC	3.09	16.05	6.93	17.50
2.	Flubendiamide 480 SC	49.61	1.00	121.36	1.00
3.	Spinetoram 11.7 SC	0.028	1771.78	0.046	2638.26

3.3 Relative potency of selected insecticides

The REP values were calculated for chlorantraniliprole and spinetoram, since, flubendiamide was the least toxic chemical among the three in case of both resistant and susceptible populations. The REP values for the resistant strain were 16.05 and 1771.78 for chlorantraniliprole and spinetoram respectively. Similarly, the values were 17.50 and 2638.26 for chlorantraniliprole and spinetoram respectively in the case of susceptible population. The present investigation revealed that spinetoram was more potent insecticide among the three followed by chlorantraniliprole 18.5 SC and flubendiamide 480 SC. The above results were in accordance with that of Kulye *et al*. (2021) [18] who reported emamectin benzoate followed by spinetoram was more potent.

4. Conclusion

The present study was aimed at determining the susceptibility of *S. frugiperda* towards chlorantraniliprole 18.5 SC, flubendiamide 480 SC and spinetoram 11.7 SC in both field-collected population and susceptible population. Among the insecticides tested, FAW populations was found to be showing more resistance against flubendiamide 480 SC compared to chlorantraniliprole 18.5 SC and spinetoram 11.7 w/w SC. Spinetoram 11.7 SC continued to be highly potent against FAW. The presence of resistance alleles in FAW populations conferring low-to-moderate levels of resistance to test insecticides highlights the need for a continued monitoring. Effective IRM strategies need to be formulated, taking into account the rotation of dioxide applications with insecticides having different modes of action in appropriate windows of crop growth.

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