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Monitoring of target site mutation in insecticide binding receptors in *Spodoptera frugiperda* population of different locations in Tamil Nadu

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Abstract
Fall armyworm (FAW), *Spodoptera frugiperda*, is a major pest of maize that originated in America and invaded worldwide. The intensive use of insecticides and crops expressing *Bacillus thuringiensis* (*Bt*) proteins for the control of FAW has led to many cases of resistance. Target-site mutations are one of the most common mechanisms of resistance, and assessing their frequency is crucial for managing this insect pest against insecticide resistance. In this study, the diagnostic methods revealed that there were no mutations in acetylcholinesterase (AchE), conferring resistance to organophosphates and carbamates. In voltage-gated sodium channels (VGSC) and ryanodine receptors (RyR) targeted by pyrethroids, and diamides, no mutations were detected. No indels in the ATP-binding cassette transporter C2 (ABCC2) associated with *Bt*-resistance were observed in samples. *In vitro* insect Bioassay was carried out with a field recommended dose of the test chemicals *viz*., Profenofos, cypermethrin, chlorantranipole along with Bt strain HD1. Results showed that a cent per cent larval mortality was recorded in all treatments after 72 hours of treatment which showed that fall armyworm Coimbatore population maintained in our laboratory as susceptible one. The molecular methods established show robust results in FAW samples collected across a geographical range and can be used to support decisions for sustainable FAW control and applied resistance management.

Keywords: Fall armyworm, insecticide resistance, *Bt* resistance, target-site mutations, resistance management

1. Introduction
The Fall armyworm (FAW), *Spodoptera frugiperda* (Lepidoptera: Noctuidae) is a migrating polyphagous pest that is endemic to America's sub-tropical and tropical regions but has already spread globally (Goergen *et al*., 2016) [12]. It infests maize, cotton, rice, soybean, sorghum, and vegetables, and is one of the most economically devastating insect pests. Insecticide spraying has become more common as a result of over-infestation and significant economic loss. Insecticide resistance is usually conferred by the insensitivity of the target receptor and/or pharmacokinetic processes modifying the rate or the properties of the insecticides delivered to the target site. Amino acid substitutions/indels at the VGSC (T929I, L932F, and L1014F), AChE (A201S, G227A, and F290V), RyR (I4790M and G4946E) and ATP-binding cassette subfamily C2 transporter (ABCC2) (GC insertion and GY deletion) have been linked to resistance in *S. frugiperda* to pyrethroids, carbamates and organophosphates, diamides and *Bt* proteins (*e.g.*, Cry1F), respectively. Fall armyworm populations have developed resistance to a range of pesticide classes such as organophosphates, pyrethroids, carbamates and benzoylureas due to widespread and indiscriminate insecticide application (Carvalho *et al*., 2013; Chen *et al*., 2019; Diez and Omoto, 2001; Yu, 1991, 1992 and Yu *et al*., 2003) [6, 9, 10, 20, 21, 22].

2. Materials and Methods
2.1. Laboratory bioassay of synthetic pesticides against FAW
2.1.1. FAW culture
Insects were collected from fall armyworm infested maize fields from Eastern Block, Tamil Nadu Agricultural University, Coimbatore. Insect cultures of *S. frugiperda* were maintained in the Insect bioassay laboratory in the Department of Plant Biotechnology, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore with TNAU diet (modifying CIMMYT diet (Tefera *et al.*, 2011) [19] and used for *in vitro* insecticidal study.

Larvae were maintained on an artificial diet under laboratory conditions (25±1 °C; 75±5% RH; 16/8 hours of light/ dark).

2.1.2. Insecticidal assay

Three synthetic insecticides obtained from different sources and one spore crystal mixture of the reference *Bt* strain were used for insecticidal assay and its mode of action was given in Table 1. The *in-vitro* leaf surface coat bioassay was conducted to test the insecticidal activity of synthetic pesticides and spore crystal mixture of *Bt* strain against the neonates of *S. frugiperda*. Each synthetic insecticide was thoroughly mixed with water (20 ml) for 5–10 minutes according to the manufacturer's instructions. Young maize leaves (top 1-2) were collected from fifteen days old maize plants and were cut into~5 x 2 cm size leaf bits. The leaf disc was treated with a quantified (25 µg/ml) spore crystal mixture and synthetic pesticides in the amount of 20 µl (ten µl on each side) and left to dry in the open air. Maize leaves eaves that were treated with methanol and water were used as a negative control. Each treated leaf was placed in a Petri plate (90 x 15 mm) containing one layer of moist Whatman filter paper no 1. The ready-to-hatch egg masses were collected and placed in a Petri plate the day before, and the next day morning,~12 h old larvae were employed for the bioassay. Using a fine camel hair brush, pre-starved neonates were released on each leaf without causing any physical damage. Each treatment consisted of hundred neonate larvae with five replications. The experiment was conducted in a controlled environment with a temperature of 25±1°C and relative humidity of 75±5 per cent. After the 24th, 48th, and 72nd hours of treatment, observations on larval mortality were obtained, and cumulative mortality after 72 hours was represented in percentage.

Table 1: List of insecticides with it field recommended dosage and target site used in insecticide assay against *Spodoptera frugiperda.*

Sl. No	Insecticide	Trade Name	Dose (ml lit^{-1})	Target site
1	Cypermethrin 25% EC	cymbush	2 ml lit^{-1}	Acetylcholinesterase (AchE)
2	Profenophos 50% EC	profex	2 ml lit^{-1}	Voltage-gated sodium channel (VGSC)
3	Chlorantraniliprole 18.5% SC	coragen	0.4 ml lit^{-1}	Ryanodine receptor (RyR)
4	HD1, *Bt* strain	-	60 ng/ µl	ATP-binding cassette transporter subfamily C2 (ABCC2)

2.1.3. Statistical analysis

The bioassay was carried out in five replications in a completely randomized design (CRD). The impact of synthetic insecticides and spore crystal mixture on insect mortality was studied using a one-way analysis of variance (ANOVA). Duncan's multiple range test (DMRT) was used and performed in Statistical Package for Social Sciences (SPSS version 16.0. Chicago, SPSS Inc, USA) to compare the treatment means at a 5% significance level.

2.2. Molecular characterization of target site mutation in insecticide binding receptors

2.2.1. Survey for collection of FAW

The survey was carried out with FAW samples collected from maize fields in seven different locations of Tamil Nadu that including Coimbatore, Salem, Trichy, Tirunelveli, Madurai, Tiruvannamalai, Cuddalore and two laboratory populations. Fourth to sixth instar FAW larvae were collected individually and maintained on a semi-synthetic diet during transport. The collected samples were preserved at -20 ºC (Sanyo, Japan) individually in microfuge tubes for further molecular studies.

2.2.2. Genomic DNA extraction

The genomic DNA was extracted from field-collected larvae from seven locations in Tamil Nadu and also from two lab populations. The genomic DNA was isolated from FAW larvae using the conventional DNA isolation method with Cetyl Trimethyl Ammonium Bromide (CTAB) buffer (Doyle and Doyle, 1990) [8]. The DNA extraction buffer contained 100 mM TrisHCl, 10mM EDTA, 1.4 M NaC1 and 2 per cent CTAB, with pH adjusted to 8 units and 6.2 per cent β-Mercaptoethanol (added prior to use). Individual insects were homogenized with 200 µl CTAB buffer in an Eppendorf tube and incubated in the water bath at 65°C for an hour. The tubes were then cooled to room temperature and centrifuged at 12,000 rpm at 4 °C for 15 minutes. The supernatant was transferred to a fresh tube to which an equal volume (200 µl) of chloroform: isoamyl alcohol (24:1 v/v) was added. This was gently shaken for five minutes and centrifuged at 12,000 rpm for 10 minutes at 4°C. The top clear aqueous layer containing the DNA was transferred to a fresh tube and incubated overnight at -20 °C with an equal volume of Ice-cold isopropanol. The samples were finally centrifuged at 12,000 rpm for 10 minutes to pellet the DNA. The pellets were washed with 150 µl of 70 per cent ethanol by centrifuging at 12,000 rpm for 5 minutes and air-dried. The DNA pellet was finally dissolved in 25 µl of nuclease-free water, labelled and stored at – 20 °C until further use.

2.2.3. PCR analysis

DNA isolated from FAW larvae from different locations and lab populations was assessed by PCR amplification. DNA samples were diluted with 1X TE buffer in order to obtain a working solution of 50-100 ng/ µl (working aliquot) for performing PCR. Primers were synthesized by Bio serve Biotechnologies Private Limited, Hyderabad, India. The list of primers, amplicon size and temperature profile administrated for the genetic characterization of FAW were given the Table 2. PCR amplification for all segments was carried out with 25 µl reaction mixture (12.5µl of 2X Thermo Scientific PCR Master Mix, 1µl forward primer, 1 µl reverse primer, 9.5 µl nuclease free water and1µl DNA template) using a thermocycler.

Table 2: List of primers, amplicon size and temperature profile of primers used for the genetic characterization of FAW.

Target	Mutation	Primer name	Primer Sequence 5' → 3'	Annealing temperature	References
Acetylcholinesterase (Ache)	A201S /G227A	Sf_A201S_G227A_F	TTTGATACCCCTGATGTACC	53	Boaventura *et al.* (2020) [4]
		Sf_A201S_ G227A_R	AATGAAACCGAAACTGCTC		
Voltage-gated sodium channel (VGSC)	L1014F	Sf_L1014_F	TCTTCCTGGCTACAGTCG	50	Boaventura *et al.* (2020) [4]
		Sf_L1014_R	GACAGTAACAGGGCCAAG		
	L932F /T929I	Sf_L932_T929_F	TAATGGGTAGGACAATGG	53	Boaventura *et al.* (2020) [4]
		Sf_L932_T929_R	AATCCACGTAATTTTTCC		
ATP-binding cassette transporter subfamily C2 (ABCC2)	GC insertion	Sf_ABCC2_F	TGGAGGCCGAAGAGAGACA	50	Banerjee *et al.* (2017) [3]
		Sf_ABCC2_R	AGGAGTTGACTGACTTCATGTACCT		
Ryanodine receptor (RyR)	G4946E	Sf_G4946_F	GTGATGGGCAACTTCAAC	50	Boaventura *et al.* (2020) [4]
		Sf_G4946_R	TTTTCCGTTATGCGTGAC		
	I4790M	Sf_taq_I4790_F	ACGACGATGCACTAGAAG	60.6	Boaventura *et al.* (2020) [4]
		Sf_taq_I4790_R	CACCTTGAGATGATAGTACC		

2.2.4. Sequencing and analysis

The unpurified PCR products (20µl) were sent for the double-pass (Forward and Reverse) sequencing facility available at the Biokart Pvt Ltd., Bangalore, India. The raw sequences thus received from the firm were aligned and edited with Bioedit software (version 7.25). For sequence analysis, several software like EXPASY (Nucleotide to Protein Translation Tool), CLUSTAL OMEGA (Multiple Sequence Alignment Tool), EMBOSS Needle (Pairwise Sequence Alignment Tool) available in online were used. After analysing, the sequences were checked for homology with the already existing sequences in the National Centre for Biotechnology Information (NCBI) website.

3. Results

3.1. *In vitro* insecticidal activity

There were significant differences among the synthetic insecticides in causing mortality to larvae at 24 h (SED = 3.36; CD (0.05) = 7.009; CV=9.13), 48 h (SED = 5.169; CD (0.05) = 10.78; CV=11.98), and 72 h (SED = 2.648; CD (0.05) = 5.525; CV=5.66) after treatment. Profenophos 50% EC caused 99 Per cent mortality, followed by cypermethrin 25% EC (83% mortality), *Bt* strain, HD1, (81% mortality), and chlorantraniliprole 18.5% SC (80% mortality) after 24 hours of treatment. Profenophos 50 EC and chlorantraniliprole 18.5% SC caused the highest mortality of 100 Per cent in 48 hours after treatment while cypermethrin 25% EC caused 93 Per cent mortality in 48 hours of treatment respectively, whereas *Bt* strain, HD1 of 89 Per cent mortality 72 h after treatment while chlorantraniliprole 18.5% SC produced 89 Per cent and 100 Per cent of larval mortality after 48 and 72 hours of treatment respectively (Table 3, Fig. 1).

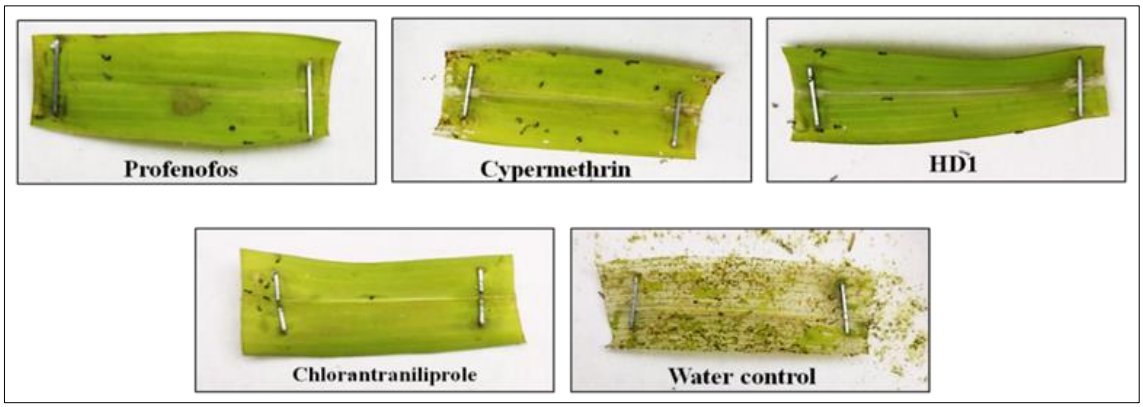

Fig 1: Leaf damage by FAW in leaf surface coating with synthetic insecticides and *Bt* strain HD1 at 25 µg/ml dose

Table 3: Effect of synthetic pesticides and spore crystal mixture of *bt* strain against neonates of *S. frugiperda*

S. No	Treatments	Larval mortality (%) *Spodoptera frugiperda*		
		24 hrs	48 hrs	72 hrs
1	Chlorantraniliprole 18.5%SC	80.00±3.53 (63.43)^{b}	100.00 ±0.00 (90.00)^{a}	100.00 ±0.00 (90.00)^{a}
2	Cypermethrin 25%EC	83.00± 2.73 (65.64)^{b}	93.00 ±7.58 (74.65)^{b}	100.00 ±0.00 (90.00)^{a}
3	Profenophos 50%EC	99.00±2.23 (84.26)^{a}	100.00±0.00 (90.00)^{a}	100.00 ±0.00 (90.00)^{a}
4	HD1 *Bt* strain	81.00±4.18 (64.15)^{b}	89.00±8.21 (70.63)^{b}	100.00 ±0.00 (90.00)^{a}
5	Water*	5.00±5.00 (12.92)^{c}	5.00±0.00 (12.92)^{c}	5.00 ±0.00 (12.92)^{b}
	SED	3.36	5.169	2.648
	CD (0.05)	7.009	10.78	5.525
	CV (%)	9.13	11.98	5.66

* Negative control

Values are arc sin transformed, and represented as Mean ±SD and values followed by the same letter in a column are not significantly different (p<0.05)

3.2. Molecular characterization of target site mutation by different insecticides on neonate larvae of *Spodoptera frugiperda*

3.2.1. Ryanodine receptor

The BLAST results of the retrieved sequence showed homologous with Ryanodine receptor sequences of *S. frugiperda*, *S. exigua* and *S. litura*. The similarity results show that the FAW sequences of G4946E Primers and I4790M primers displayed the highest homology of 99.90% with *S. frugiperda* strain Sus (MK226188). PCR samples were analysed for all 9 samples for the presence of an amino acid substitution at position G4891 (corresponding to G4946 in *P. xylostella* RyR (Fig.2a and 2b) and substitution at I4790 (numbering according to *P. xylostella* RyR (Fig.3a and 3b) shown to confer diamide resistance, but no amino acid substitution was found.

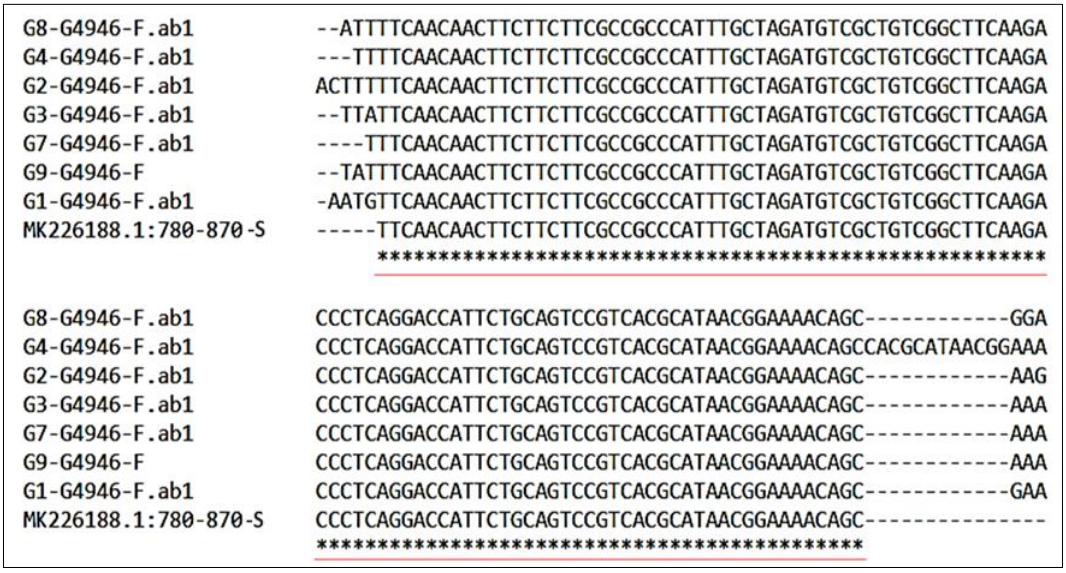

Fig 2a: Multiple Sequence Alignment of PCR sequenced samples harbouring potential mutation G4946E region with the *S. frugiperda* strain Sus (MK226188).

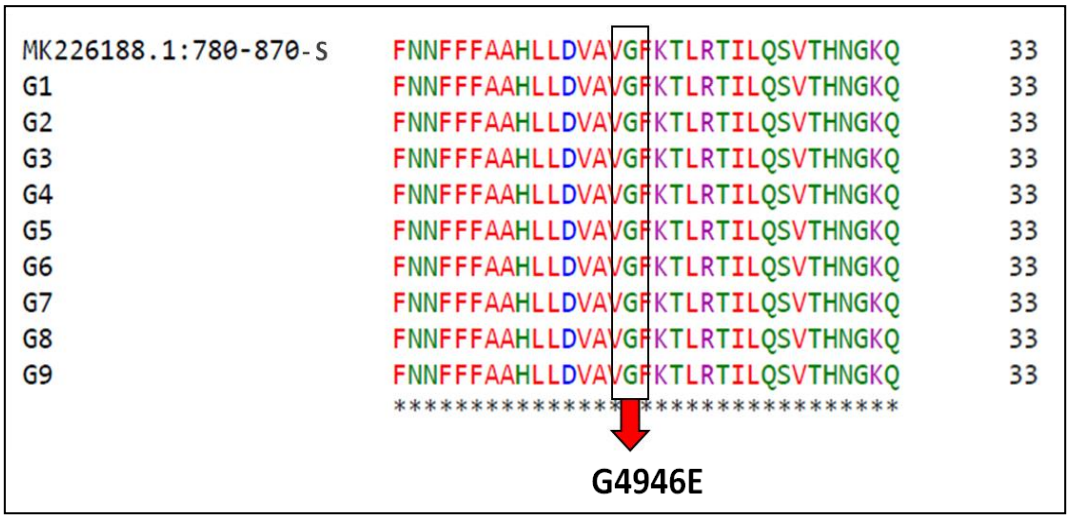

Fig 2b: Multiple Amino Acid Alignment of PCR sequenced samples harbouring potential mutation G4946E region with the *S. frugiperda* strain Sus (MK226188).

Fig 3a: Multiple Sequence Alignment of PCR sequenced samples harbouring potential mutation I4790M region with the *S. frugiperda* strain Sus (MK226188).

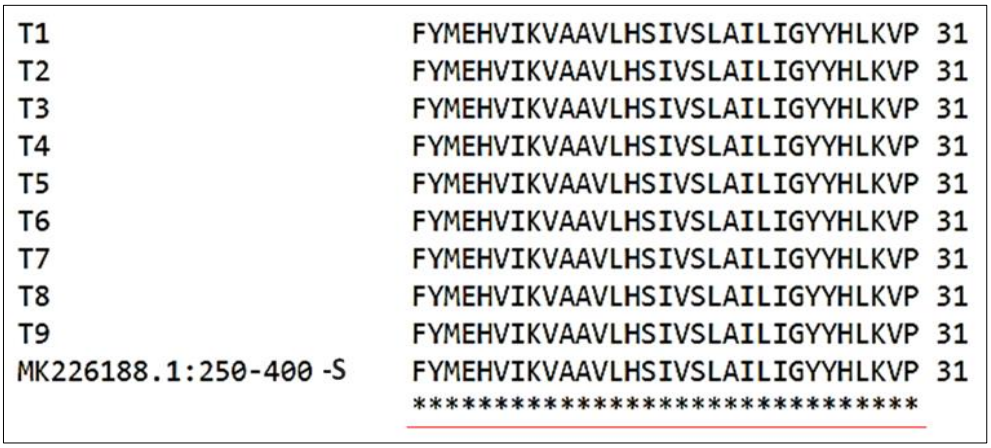

Fig 3b: Multiple Amino Acid Alignment of PCR sequenced samples harbouring potential mutation I4790M region with the *S.frugiperda* strain Sus (MK226188).

3.2.2. Voltage Gated Sodium Channel

The BLAST results of the retrieved sequence showed homologous with Voltage Gated Sodium Channel (VGSC) receptor sequences of *S. frugiperda*, *S. exigua* and *S. litura*. The similarity results show that the FAW sequences of L1014 and L932F/T929I displayed the highest homology of 99.98% with *S. frugiperda* isolate NaChFAW_S insecticide receptor (KC435026.1). Multiple sequence alignment of VGSC primer amplified PCR samples and the partial sequence of *S. frugiperda* organophosphate resistant strain (KC435026.1) were performed for target-site identification. Sequences were compared for the presence of T929I, L932F and L1014F (Fig.4a,5a and 6a) target-site mutations in the VGSC numbered according to *Musca domestica* sodium channel. On the one hand, amino acid alignment of the VGSC (Fig.4b, 5b and 6b) from consensus amino acid sequences obtained from 9 FAW samples, *S. frugiperda* resistant samples revealed no target-site mutation linked to organophosphate resistance in the VGSC.

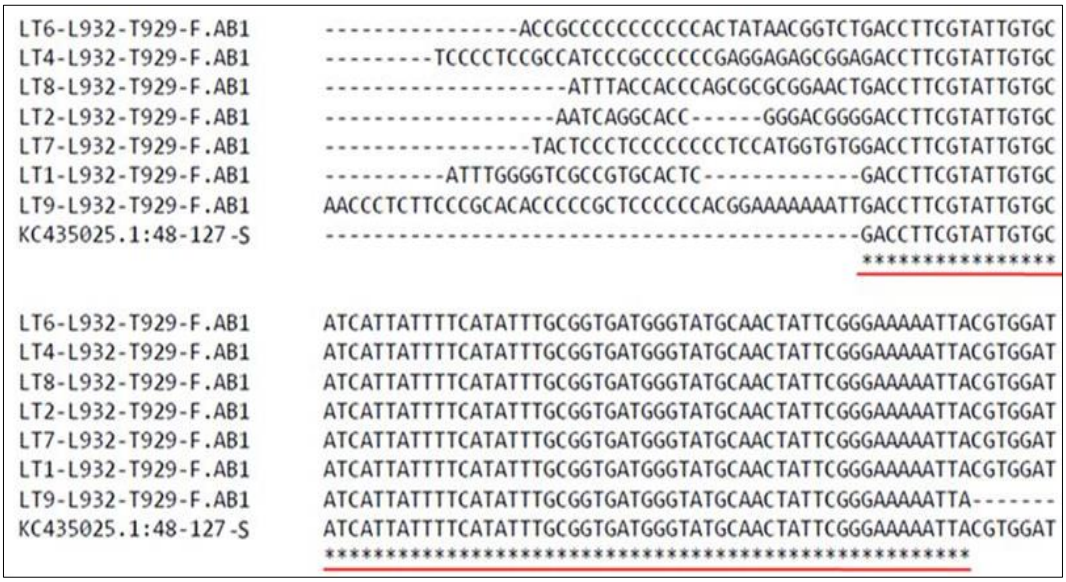

Fig 4a: Multiple Sequence Alignment of PCR sequenced samples harbouring potential mutation L932F/T929I region with the *S. frugiperda* strain Sus (KC435025.1)

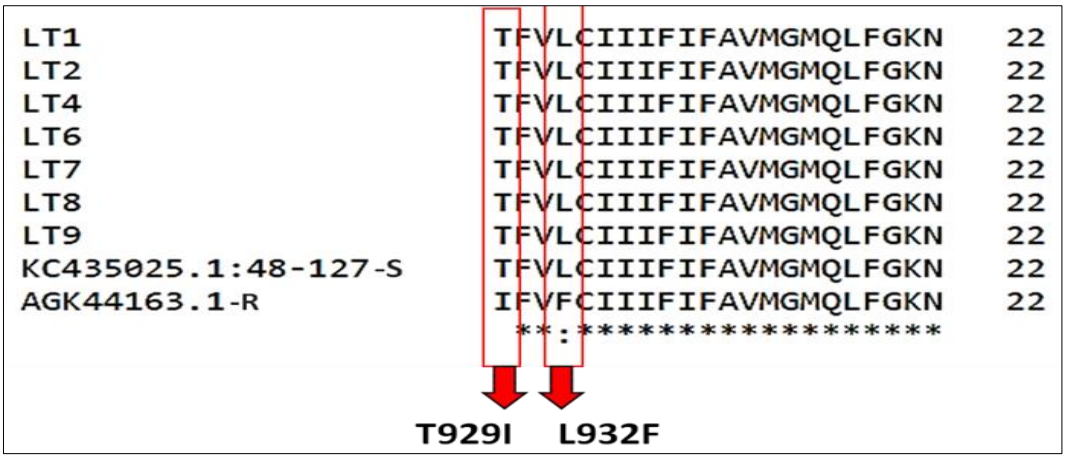

Fig 4b: Multiple Amino Acid Alignment of PCR sequenced samples harbouring potential mutation L932F/T929I region with the *S. frugiperda* strain Sus (KC435025.1).

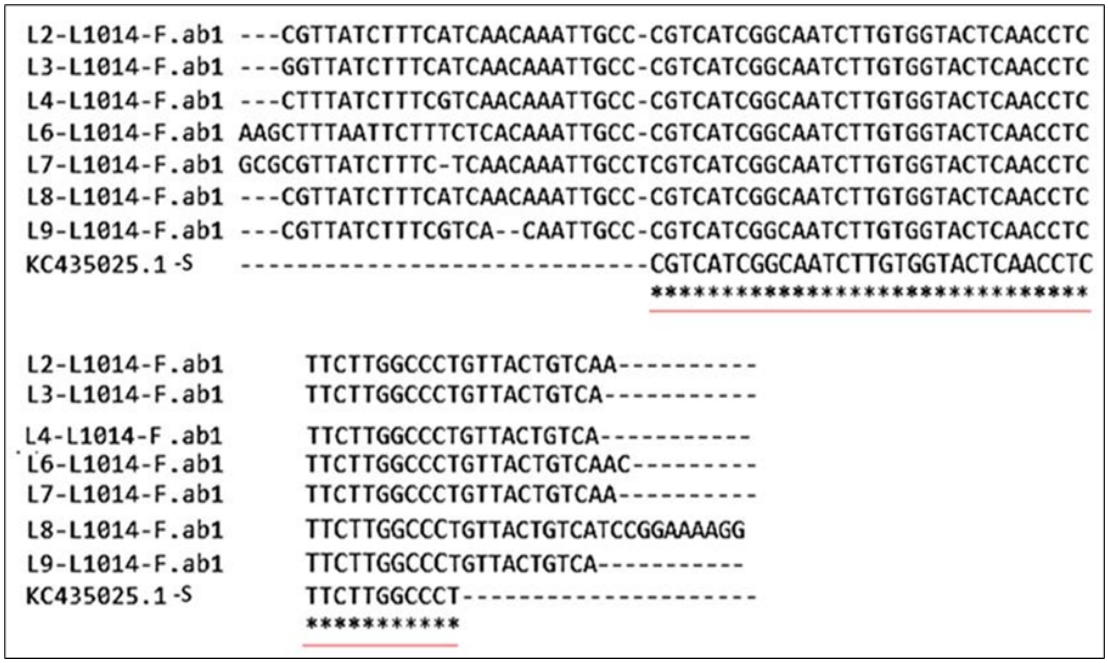

Fig 5a: Multiple Sequence Alignment of PCR sequenced samples harbouring potential mutation L1014 region with the *S. frugiperda* strain Sus (KC435025.1).

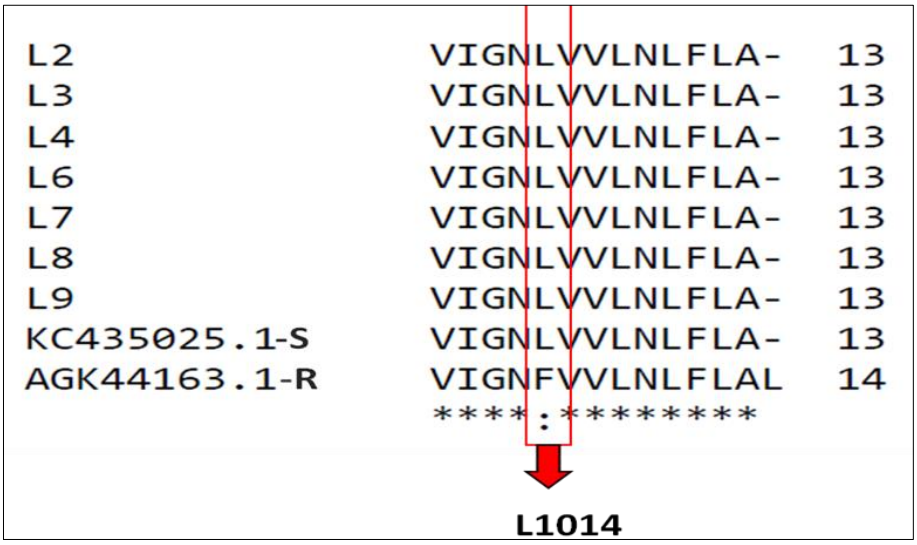

Fig 5b: Multiple Amino Acid Alignment of PCR sequenced samples harbouring potential mutation L1014 region with the *S. frugiperda* strain Sus (KC435025.1).

3.2.3. Acetylcholine receptor

The BLAST results of the retrieved sequence showed homologous with Acetylcholine (AchE) receptor sequences of *S. frugiperda*, *S. exigua* and *S. litura*. The similarity results show that the *Spodoptera* sequences of A201S /G227A displayed the highest homology of 99.97% with *S. frugiperda* isolate AceFAW_S insecticide receptor (KC435023.1). Multiple sequence alignment of A201S /G227A primer amplified PCR samples and the partial sequence of *S. frugiperda* pyrethroid resistant strain (KC435023.1) were performed for target-site identification. Sequences were compared for the presence of A201S /G227A target-site mutations in the VGSC numbered according to *Musca domestica* sodium channel. On the one hand, amino acid alignment of the AchE from consensus amino acid sequences obtained from 9 FAW samples, *S. frugiperda* resistant samples revealed no target-site mutation linked to pyrethroid resistance in the AchE (Fig.5a and 5b).

3.2.4. ABCC2 Receptor

The BLAST results of the retrieved sequence showed homologous with ABCC2 receptor sequences of *S. frugiperda*, *S.exigua* and *S.litura*. The similarity results shows that the *Spodoptera* sequences of ABCC2 displayed the highest homology of 99.40% with *S. frugiperda* isolate Sf_Cor ATP-binding cassette transporter subfamily C2 (ABCC2) (MN399980.1). Multiple sequence alignment of all PCR sequenced samples with *S. frugiperda* isolate Sf_Cor ATP-binding cassette transporter subfamily C2 (ABCC2) (MN399980.1). These alignment results do not show any GC insertion in the particular region (Fig.7).

Fig 6a: Multiple Sequence Alignment of PCR sequenced samples harbouring potential mutation L1014 region with the *S. frugiperda* strain Sus (KC435025.1).

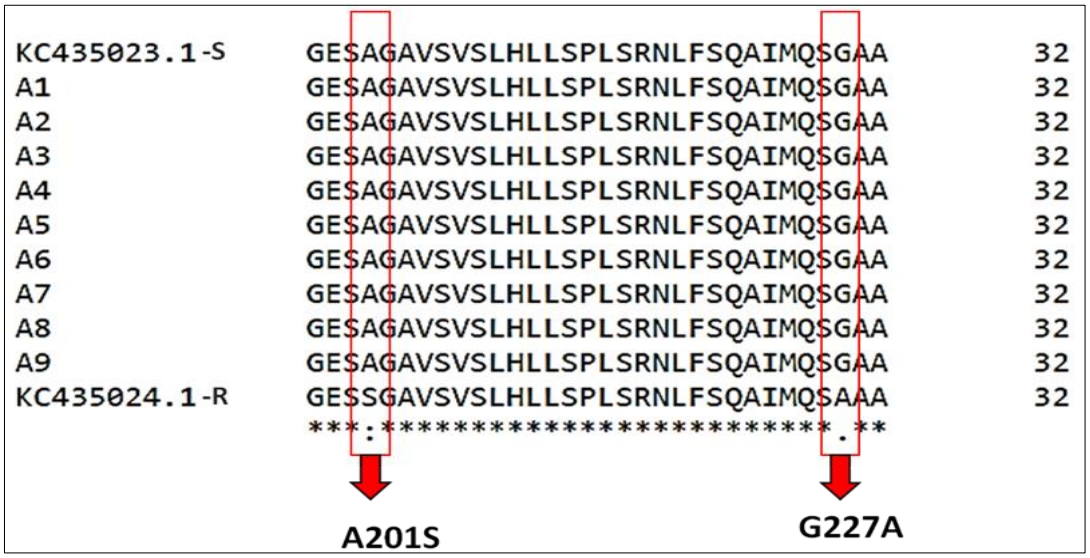

Fig 6b: Multiple Amino Acid Alignment of PCR sequenced samples harbouring potential mutation L1014 region with the *S. frugiperda* strain Sus (KC435024.1).

Fig 7: Multiple Sequence Alignment of PCR sequenced samples harbouring potential mutation GC insertion with the *S. frugiperda* Sf_Cor ATP-binding cassette transporter subfamily C2 (ABCC2) (MN399980.1).

4. Discussion

One of the most challenging issues in the management of fall armyworm is the development of insecticide resistance. Fall armyworm invasions can be monitored and prevented by identifying resistance-related genes. To identify the different target sites of receptors of detoxification genes *viz*., Voltage Gated Sodium Channels (VGSC), Acetylcholinesterase, Ryanodine receptor and ATP binding cassette transporter were used against FAW in this study. The target site mutations in different detoxification genes were analysed in the Coimbatore population of fall armyworm. The target site mutation linked with the VGSC receptor was analyzed using L1014 and L932F/T929I primers and the sequence results were compared with a susceptible strain of *S. frugiperda* isolate NaChFAW_S insecticide receptor (KC435026.1). The results revealed that the Coimbatore strain had the highest homology of 99.98 per cent with susceptible strain, which indicated that resistant mutation is not prevalent. The L1014F mutation was first discovered in pyrethroid-resistant house fly strains and is the most widely reported kdr-type mutation in a diversity of arthropod species, conferring 10–30 fold pyrethroid resistance (Davies and Williamson 2009) [7]. This mutation was also related to sensitivity to specific pyrethroids, according to functional analysis of insect channels, when injected into oocytes of *Xenopus laevis* (Araujo *et al*., 2011) [2]. T929I is a super kdr mutation that was first discovered in the pyrethroid-resistant diamondback moth, *P. xylostella*, and has since been found in human head lice, *Pediculosis capitis*, maize weevil, *Sitophilus zeamais*, and tomato leafminer, *Tuta absoluta* (Araujo *et al*., 2011; Haddi *et al*., 2012; Lee *et al*., 2000; Schuler *et al*., 1998) [2, 13, 17, 18]. The L932F mutation has only been found in human

head lice, where it is frequently linked to the T929I mutation and the peach potato aphid *Myzus persicae* was also linked to the L1014F mutation (Fontaine *et al*., 2011; Lee *et al*., 2000) [11, 17]. But these mutations were absent in the FAW population of Coimbatore.

Similarly, Acetylcholinesterase (AchE) target mutation was analyzed by using A201S/G227A primers. The sequence results of the sample from the lab population showed the highest homology of 99.97 per cent with *S. frugiperda* isolate AceFAW_S insecticide receptor (KC435023.1) and the results revealed that no target-site mutation was linked in the AchE for the samples analyzed. Andrews *et al*. (2004) reported that, in laboratory research work bioassays, the organophosphates resistant strain showed modest resistance (20-fold) to the organophosphate chlorpyrifos compared to the SUS strain. The existence of amino acid alterations A201S, G227A, and F290V at three sites implicated earlier in OP resistance in different insect species was discovered after cloning a major part of the *ace-1* gene encoding the target protein of organophosphate and carbamate insecticides. The A201S mutation was found in the OP strain with the lowest frequency (17.5%) of the three. The cotton aphid, *Aphis gossypii*, was the first insect to report this mutation, which is linked to insensitivity to a wide variety of carbamates and organophosphates (Andrews *et al*., 2004) [1]. It was later discovered in organophosphate-resistant rice stem borer, *Chilo suppressalis*, oriental fruit fly, *Bactrocera dorsali*, and diamondback moth, *P. xylostella* (Hsu *et al*., 2004; Jiang *et al*., 2007; Lee *et al*., 2008) [14, 15, 16].

To identify the target site of Ryanodine receptor (RyR), G4946E and I4790M primers were used and the amplified PCR products were sequenced. The sequence data was compared with *S. frugiperda* susceptible strain (MK226188), and it showed 99.90% homology. The phthalic (flubendiamide) and anthranilic acid diamides (*e.g*., chlorantraniliprole) chemotypes of diamide insecticides were shown to be affected differently by point mutations leading to amino acid substitutions, particularly G4946E and I4790M in the lepidopteran RyR (numbering according to the *P. xylostella* RyR). Pests with a methionine at position 4790 are less susceptible to phthalic diamides. Resistance ratios of 160 to 500 times have been recorded to chlorantraniliprole and flubendiamide in Puerto Rico, although the mechanisms of resistance have not been well investigated (Boventuara *et al*., 2020) [4]. The results of present study did not have mutation in target sites of Ryanodine receptor.

Similarly, changes in the expression level and mutations reduce Cry toxin binding to their specific midgut receptors, which is a well-known *Bt* toxin resistance mechanism. The ABC transporter subfamily C2 (ABCC2) had been implicated in the insertion of Cry toxins into the midgut membrane of lepidopteran species in numerous investigations. Many lepidopteran pests, notably *S. frugiperda*, had been connected to mutations in the ABCC2 transporters, which have been associated to Cry1-type resistance (Bravo *et al*., 2007) [5]. The ABBC2 receptor sequence of FAW population maintained in our lab was compared with FAW isolate Sf_Cor ATP-binding cassette transporter subfamily C2 (MN399980.1). The alignment results did not show any GC insertion in the particular region which indicated that the population might be susceptible to *Bt* Cry1F toxin.

Based on the earlier reports, three insecticides targeting different target sites *viz*., Profenofos, cypermethrin, chlorantranipole along with *Bt* strain HD1 were used for insect bioassay to identify the insect resistance in FAW population maintained in laboratory. *In vitro* insect Bioassay was carried out with a field recommended dose of the test chemicals. Results showed that a cent per cent larval mortality was recorded in all treatments after 72 hours of treatment which showed that fall armyworm Coimbatore population maintained in our laboratory as susceptible one.

5. Conclusion

Based on our PCR amplified results described in this study, the field efficacy of potential insecticides and *Bt* proteins are efficient against FAW and there is no mutation in target site mutation. This study gives a new approach to pest management of FAW and reduce the risk of economic losses, resistance management strategies will need to be implemented at regional levels in the newly invaded countries and can be supported by using the presented diagnostic tools to detect and monitor the early presence of resistance alleles in the field.

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