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S Ramesh Babu
Agricultural Research Station, Maharana Pratap University of Agriculture and Technology (MPUAT), Borwat Farm, Banswara, Rajasthan, India

RK Kalyan
Agricultural Research Station, Maharana Pratap University of Agriculture and Technology (MPUAT), Borwat Farm, Banswara, Rajasthan, India

Sonika Joshi
Agricultural Research Station, Maharana Pratap University of Agriculture and Technology (MPUAT), Borwat Farm, Banswara, Rajasthan, India

CM Balai
Krishi Vigyan Kendra (KVK), Faloj, Dungarpur, Rajasthan, India

MK Mahla
Department of Entomology, Rajasthan College of Agriculture, MPUAT, Udaipur, Rajasthan, India

P Rokadia
Agricultural Research Station, Maharana Pratap University of Agriculture and Technology (MPUAT), Borwat Farm, Banswara, Rajasthan, India

Correspondence
S Ramesh Babu
Agricultural Research Station, Maharana Pratap University of Agriculture and Technology (MPUAT), Borwat Farm, Banswara, Rajasthan, India

Report of an exotic invasive pest the fall armyworm, *Spodoptera frugiperda* (J.E. Smith) on maize in Southern Rajasthan

S Ramesh Babu, RK Kalyan, Sonika Joshi, CM Balai, MK Mahla and P Rokadia

Abstract
The fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) is a polyphagous Lepidopteran pest recently invaded in India on maize, sorghum and sugarcane crop causes severe economic losses. This pest has been identified by morphological and DNA barcoding methods. The present study revealed that the percentage damage was between 10 to 40% in different hybrids, inbred lines of maize and sweet corn. FAW larvae found during the vegetative stage of the crop *i.e.* knee height stage and continues upto cob formation stage. Dark brown grown larvae having inverted 'Y' shape on the head with remarkable elevated distinct four dark coloured black spots (Pinacula) arranged in square on the abdominal segment in 8th segment whereas trapezoidal pattern on 9th abdominal segment. This pest has been reported for the first time on maize in Southern Rajasthan and the proper monitoring should be carried out in this region which will be useful to the farmers for the timely management of FAW.

Keywords: *Spodoptera frugiperda*, DNA barcoding, FAW, Southern Rajasthan

1. Introduction
An invasive pest, the fall armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), is an economically destructive, highly mobile important pest which are native to tropical and subtropical America [1]. Recently, this pest has invaded and showing rapid spread in many of the countries including Africa continent which becomes a serious pest causing severe damage to maize crop and other graminaceous crops [2] and threatens food security of the millions of people. FAW is a polyphagous Lepidopteran pest which has a host range of 353 plant species from 76 plant families, namely Poaceae, Asteraceae and Fabaceae from Brazil [3]. Over 30 countries have identified the pest within their borders including the island countries [1]. The yield losses in maize ranging from 8.3 M to 20.6 M tons per year from 12 maize producing countries [4].
Fall armyworm attack occurs from maize crop from emergence to tasseling, silking and cob formation stage. This pest can reduce corn grain yield up to 34% and estimated at U$400 million annually in Brazil [5]. In India, recently FAW has been reported in the state of Karnataka [6-9] Andhra Pradesh, Madhya Pradesh, Maharashtra, Tamil Nadu, Telangana, Gujarat and Chhattisgarh [10-12]. In the present study, we are reporting for the first time in Southern Rajasthan on winter maize crop in Banswara and Dungarpur districts which were confirmed by the morphological and molecular method. This is a new invasive pest in this zone, infestation has been reported in some of the popular hybrids, inbreds of maize and sweet corn. Therefore, the detailed study has to be conducted for the presence of FAW in different hosts as well as management of this pest with the appropriate insecticides to the benefit of the farmers.

2. Materials and Methods
2.1 Collection of larvae
Eggs and larvae were collected from the maize fields during January to April, 2019 at Agricultural Research Station, Borwat Farm, Banswara. This station is located between 73°2' to 75°E' longitude and 23°11' to 24°23' N latitude at an altitude of 660 m above mean sea level, in Humid Southern Plain Zone of Rajasthan. Larvae were also collected from the infected maize fields from Dungarpur district (Punawara Village).

The collected eggs and larvae were maintained in the laboratory at 25±2^{0}C and 60-70% humidity for the emergence of FAW larvae and adults and also for the any parasitoids emergence. The adults and larval specimens were being maintained at ARS, Banswara, Rajasthan.

2.2 DNA extraction, amplification and sequencing:

Larvae were collected from the maize fields were preserved in 95% ethanol at -20^{O}C until further use. A portion of larval tissue was dissected, air-dried for few minutes and rinsed with molecular grade water to remove the excess ethanol in the sample. Total genomic DNA was extracted using DNASure Tissue mini kit (Nucleo-pore, Genetix Brand, India), following the manufacturer's instructions. The intact genomic DNA was visualized in 1.2% agarose gel (PureGene, Genetix Biotech India PVT. Ltd., New Delhi). The concentration of DNA sample was adjusted to 50 ng/µl and stored at -20°C for further use. The PCR reaction was carried out for the amplification of Cytochrome oxidase subunit I (*COI*) gene which is of ~700 by using universal primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA3') [13]. PCR (C1000Touch^{TM} Thermal cycler of Bio-Rad, U) was performed with initial denaturation for 4 min at 94°C, followed by 35 cycles of 30 sec denaturation at 94°C, 45 sec primer annealing at 47°C, 45 sec initial extension at 72°C and a final extension of 20 min at 72°C [14]. The PCR amplification was performed for 50µL containing 25 µL Dream Taq PCR Master Mix (2X) (Thermo Fisher, Scientific, UK), 2µL of template DNA, 10 pmol of each forward and reverse primer and final volume was made by using nuclease free water. The amplified PCR products were separated by electrophoresis in a 1.2% agarose gel containing ethidium bromide (0.5µg/µL) for 60 min at 80 V (BIO-RAD, USA) and visualized in gel documentation system (Gel Doc^{TM} EZ Imager, BIO-RAD, USA). The PCR products were purified by using Gene JET PCR purification Kit (Thermo Fisher, Scientific, UK) and sequenced by using ABI PRISM 3730xl Genetic Analyzer develop by Applied Biosystems, USA (Agile Life science Technologies India Pvt. Ltd, Pune). The obtained sequences were aligned Bio Edit sequence alignment editor (version 7.0.5.3) and homology were confirmed by using NCBI-BLAST (BLASTn, http:// www.ncbLn1m.nih.gov) and also from the Barcoding of Life Data system (BOLD; http://www.boldsystems.org/) to confirm the identity of the sequence. The sequence was deposited in the Genbank of National Center for Biotechnology Information (NCBI), USA and accession numbers were obtained according to Sharanabasappa *et al.* (2018) and Shylesha *et al.*, 2018. The sequences also submitted to the Insect Barcode Informatica (IBIn) of National Bureau of Agricultural Insect Resources, Bengaluru, India.

3. Results and Discussion

3.1 Morphological identification

Fig 1: Egg mass and black-headed neonate larvae emerging out of egg mass

Eggs are laid in groups which are dome shaped brownish yellow in colour and laid under the surface of the leaves, inner side of the whorls and near the stem. Eggs are covered with greyish coloured scales by the female adults (Fig 1). The similar observations are also reported by Shylesha *et al.*, 2018. Larvae were identified based on the important distinguished morphological characters with the previous reports [16, 17, 8]. Young larvae are light greenish in colour with dark black head and the grown up larva is dark brown with reddish brown head marked with inverted 'Y' shape on the head with the elevated distinct dark coloured black spots (Pinacula) on the whole body which bears spines (long primary setae).

The arrangement of the dorsal pinacula *ie* the four black spots arranged in square on the 8th abdominal segment and large spots especially on 9th segment have a typical arrangement in a trapezoidal pattern and also seen from 1 to 7th abdominal segments. Reddish brown pupae having a typical cremaster with 2 spines (Fig 2). These observations are conformity with the others report on the incidence [16, 17, 8].

Fig 2: Four spots on 8th Abdominal Segment arranged in square shape and Inverted Y shape on the head

Fig 3: FAW (*Spodoptera frugiperda*) pupae with typical cremaster with spines

Morphological characters of male and female moths were observed based on earlier reports on identification markings of *S. frugiperda* by Pogue, 2002 and EPPO, 2015. Forewings are greyish brown with contrasting markings like reniform indistinct spot at the junction of M3 and CuA1 veins as reported by Sharanabasappa *et al.* (2018), with a small v-shaped mark and a distinct white patch at the apex (Fig 4). Forewings of female are ground color brown i.e. with a mottled colouration of grey and brown, with brown markings and without white patch near apical margin of the wing [8].

Fig 4: FAW (*Spodoptera frugiperda) male moth* with conspicuous white spot on tip of forewing and female moth

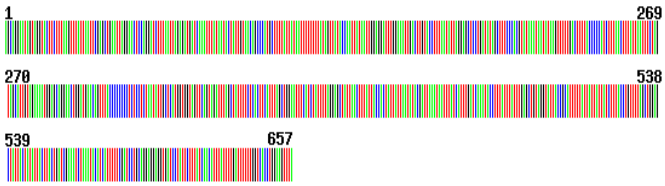
3.2 Molecular identification

The universal primers were used to amplify the target fragment (Mitochondrial Cytochrome Oxidase Subunit I (*COI*) gene) of ~650 bp in size from the genomic DNA of *Spodoptera frugiperda*. The search analysis in the BLAST and BOLD revealed that the insect species belongs to *S. frugiperda*. The *COI* gene sequence of *S. frugiperda* from Banswara and Dungarpur maize fields is of 657 and 664 bp in size, respectively (Genbank Accession No: MK633906 and MK591010). These sequences were showed 98-100% identical with the sequences from India (Vijayawada: GenBank MH899611 and Tirupati: GenBank MH899610) and other countries (Dominica Republic: GenBank MK3182971; Kenya: GenBank MH190445; South Africa: GenBank MF593258). Similarly, the Barcode of life Data base identification showed that the present study sequences belong to *S. frugiperda* with 100% similarity (BIN ID: BOLD ACE4783). The similar *COI* gene fragment was also reported by Shylesha *et al.*, 2018 and Mahadeva Swamy *et al.*, 2018.

***Spodoptera frugiperda* sequence (Gene accession no: MK633906)**

AGCAGGAATAGTAGGTACTTCTTTAAGTTTATTAATT
CGAGCTGAATTAGGAACTCCAGGATCTTTAATTAGA
GATGATCAAATTTATAATACTATTGTAACAGCCCAT
GCTTTTATTATAATTTTTTTTATAGTTATACCAATTAT
AATTGGAGGATTTGGAAATTGACTTGTACCTTTAATA
TTAGGAGCTCCTGATATAGCTTTCCCACGTATAAATA
ATATAAGTTTTTGACTTTTACCCCCATCTTTAACTTTA
TTAATTTCTAGTAGCATTGTAGAAAATGGAGCAGGA
ACTGGATGAACAGTTTACCCCCCCCTCTCCTCTAATA
TTGCTCATGGTGGTAGTTCAGTAGATTTAGCTATTTT
CTCACTTCATTTAGCTGGAATTTCATCTATTTTAGGA
GCTATTAACTTTATTACCACTATTATTAATATACGAT
TAAATAATTTATCATTTGATCAAATACCTTTATTTAT
TTGAGCTGTAGGTATTACCGCATTTTTATTATTATTA
TCTTTACCTGTTTTAGCTGGAGCTATTACTATATTAC
TTACTGATCGAAATCTAAATACATCATTTTTCGATCC
TGCAGGAGGAGGTGATCCTATTCTTTATCAACATTTA
TTTTGATTTTTTGGTCACCTGGAAGTTTA

DNA barcode image of *Spodoptera frugiperda* (NBAIR Accession NO: SPINRA292-19)



3.3 Symptoms and nature of damage

The percentage damage was in between 10 to 40% in different hybrids, inbred lines of maize and sweet corn. FAW larvae found during the vegetative stage of the crop *i.e.* knee height stage and continues upto cob formation stage. About 49% infestation level of FAW during reproductive stage was observed by Sonali Deole and Nandita Paul, 2018. Damage observed almost all the plant parts of the crop. During the vegetative stage, newly emerged larvae gregariously feeds resulted in white elongated patches [6] and scrapping the epidermis of the leaves resulting in clusters of pinhole like damage or small, round which becomes elongated and shows window panes in later stage of the crop (Fig. 5).

Fig 5: FAW damaged maize plants and damage on maize leaves

In the feeding site of FAW, moist/dry saw dust like frass found on maize which can be easily spotted sign of FAW larvae feeding (Fig.6). Similar symptoms on sugarcane were also observed by Chormule *et al.*, 2019. Later instars larvae fed inside the leaf whorls resulted in series of small/elongated big holes across the maize leaves. The typical symptom like

heavily skeletonized leaves and windowed whorls during vegetative stage occurs due to the continuous feeding of the FAW larvae. Late instars larvae can damage the entire central portion of the crop.

Fig 6: Maize plants infested with FAW larvae along with saw dust like frass

The late instars larvae can feed on reproductive stage of the crop especially tassels and developing cobs (Fig.7). The matured larvae can feed on cob or kernels of maize which ultimately reduces the yield and quality of maize produce [19]. Most of the larval stages were found inside the maize crop under protected conditions.

Fig 7: FAW (*Spodoptera frugiperda*) larvae on the reproductive stages (tassel and cob formation) of maize crop

3.4 Natural enemies

FAW larvae were collected and reared on maize plants and observed for the emergence of parasitoids. Solitary and gregarious parasitoids were observed, collected and stored. The confirmation of the parasitoid species will be done in further studies. NPV infected like larvae were also collected from the maize fields and further confirmation is needed.

4. Conclusion

The present study confirms the occurrence of FAW *S. frugiperda* in Southern Rajasthan. FAW can occur throughout the year which can feed on different hosts during off-season also. It is a unique quarantine pest so, there will be continuous monitoring is needed to tackle this pest in India. This pest occurs in winter season on maize crop only and there will be chances of further spread in other crops during *kharif* season in this zone. The timely management practices will be developed which can be ecologically sound and fit into the IPM programme.

5. Acknowledgments

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