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Research Article

Studies on new invasive pest *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and its natural enemies

A. N. SHYLESHA[1*], S. K. JALALI[1], ANKITA GUPTA[1], RICHA VARSHNEY[1], T. VENKATESAN[1], PRADEEKSHA SHETTY[1], RAKSHIT OJHA[1], PRABHU C. GANIGER[2], OMPRAKASH NAVIK[1], K. SUBAHARAN[1], N. BAKTHAVATSALAM[1], CHANDISH R. BALLAL[1] and RAGHAVENDRA, A[1].

[1]ICAR-National Bureau of Agricultural Insect Resources, H. A. Farm Post, Bellary Road, Hebbal, Bengaluru - 560024, Karnataka, India
[2]All India Coordinated Research Project on Small Millets, Univeristy of Agricultural Sciences, GKVK, Bengaluru - 560065, Karnataka, India
[*]Corresponding author E-mail: shyleshaan.nbaii@gmail.com; anshylesha@gmail.com

ABSTRACT: Occurrence of *Spodoptera frugiperda* (J. E. Smith) (Insecta: Lepidoptera: Noctuidae), commonly known as fall armyworm, in southern India is reported along with associated natural enemies. Severe damage was noticed in Chikkaballapur, Hassan, Shivamogga, Davanagere and Chitradurga during July-August 2018. The incidence ranged from 9.0 to 62.5 percent at various locations, maximum incidence was recorded in Hassan district followed by Chikkaballapur, Davanagere, Shivamogga and Chitradurga. Morphology and molecular based taxonomic tools were used for the identification of this pest. The GenBank accession number MH704433 of Chikkaballapur population was released on 1st August, 2018 and Barcode obtained from BOLD System-ID: AGIMP054-18. The survey also revealed natural parasitism by egg parasitoids *viz*., *Telenomus* sp. (Hymenoptera: Platygastridae) and *Trichogramma* sp. (Hymenoptera: Trichogrammatidae), gregarious larval parasitoid, *Glyptapanteles creatonoti* (Viereck) (Hymenoptera: Braconidae) solitary larval parasitoid, *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae), and a solitary indeterminate larval-pupal (Hymenoptera: Ichneumonidae: Ichneumoninae) parasitoid. *Spodoptera frugiperda* is the first host record for *G. creatonoti* across the globe. *Glyptapanteles creatonoti*, being a well established parasitoid of various noctuids in India and Malaysia, was capable of parasitizing *S. frugiperda*. Besides these, other commonly found bioagents *viz*., *Forficula* sp. (Dermaptera: Forficulidae) and entomopathogenic fungus *Nomuraea rileyi* (Farl.) Samson was also collected in large numbers. We report the natural enemy complex of *S. frugiperda* for the first time from India. The electro physiological response of Indian population of *S. frugiperda* male adults to pheromone was established. The studies to manage this pest by any/all means are in progress.

KEY WORDS: Karnataka, maize, new pest

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INTRODUCTION

The fall armyworm is native to the tropical region of the western hemisphere from the United States to Argentina. *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) is considered the most important pest of corn in Brazil, the third largest corn producer in the world after the USA and China. In this country alone, costs to control the fall armyworm on corn exceed 600 million dollars annually. Till 2015, this pest has not been reported in any other part except in America. In 2016, it was recorded in Africa causing serious damage on maize crop (Goergen *et al.,* 2016). In September 2017, the UK Aid and CABI published a report commissioned by the UK Department for International Development called: "Fall Armyworm: Impacts and Implications for Africa", according to the which the Fall Armyworm (FAW) could potentially cause corn yield losses in a range from 8.3 million to 20.6 million tonnes in 12 African countries per annum (if no control methods are put in place). The value of these losses is estimated at between US$2,481 million and US$6,187 million. Now in 2018, this notorious pest has entered India.

MATERIALS AND METHODS

Surveys conducted

Based on the surveys conducted from July to August 2018 by ICAR-NBAIR team, *Spodoptera frugiperda* was recorded from many locations in Karnataka on maize crop. Surveys were conducted in five districts of Karnataka *viz*., Chikkaballapur, Hassan, Shivamogga, Davanagere and Chitradurga in 15 different locations to record the incidence of *S. frugiperda* where the maize crops were between the age of 15−60 days. The specimens examined for the present study are deposited in the ICAR- National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. Images of parasitoid and predators were taken with a Leica M 205 A stereozoom microscope with Leica DC 420 inbuilt camera using automontage software (version 3.8).

Abbreviations used

BMNH: British Museum of Natural History (= Natural History Museum), London, UK.

ICAR-NBAIR: ICAR-National Bureau of Agricultural Insect Resources, Bengaluru, India.

IITA**:** International Institute of Tropical Agriculture, Africa.

RESULTS AND DISCUSSION

The incidence ranged from 9.0 to 62.5 percent at various locations with maximum incidence recorded in Hassan district followed by Chikkaballapur, Davanagere, Shivamogga and Chitradurga. The larvae were found to be infected with entomopathogenic fungus, *Nomuraea rileyi* (Farl.) Samson and other natural enemies like egg, larval and larval-pupal parasitoids that were obtained after rearing them in the laboratory. The populations from different regions of Karnataka were collected and studied for both morphological and molecular characterization as well as natural parasitization.

Nature of damage (Figs 1A−F)

After the eggs hatch, the young larvae feed on the opened leaves by scraping and skeletonizing the upper epidermis leaving a silvery transparent membrane (Fig. 1A).

Fig. 1. Damage symptoms of *Spodoptera frugiperda* in the maize field: A, young larva leaving silvery transparent membrane; B, larva feeding inside whorl with faecal matter; C&D, tassel feeding by larvae; E & F, feeding symptoms.

Later on the larvae enters into the whorl and start feeding between the leaves (Fig. 1B). Usually within a whorl, one or two larvae are present as a result a lot of faecal matter gets accumulated within the whorl leading to the characteristic symptom of damage (Fig. 1B). The older larvae feed on the developing primordial shoot, thus resulting in dead heart symptoms. Tassel feeding was also noticed in many fields (Figs. C, D). Larval feeding causes characteristic large feeding areas on the open leaves in the later stages (Fig. 1F). If the crop is affected in the early stage (upto 25 days of sowing) the mortality of the plants will be very high. High rainfall combined with overcast skies for more than a week is optimal for the increase in fall armyworm activity.

Host range

Based on literature survey this is considered as a serious polyphagous pest of voracious nature with a wide host range of approximately more than100 recorded plant species in 27 families (Goergen *et al.,* 2016). This pest prefer plants from Gramineae family including many economically important plants such as maize, millet, sorghum, sugarcane, rice, wheat, etc. There are reports on its infestation on other field crops like cowpea, groundnut, potato, soybean, cotton, etc.

Morphological characterization and sexual dimorphism of *Spodoptera frugiperda*

Material examined (voucher): Five adult females and four males dry mounted; India: Karnataka: Chikaballapur; feeding on *Zea mays* L.; 09.vii. 2018; coll. A. N. Shylesha.

*DNA voucher specimen details***:** One male dry mounted on card (right fore, mid and hind legs removed); India: Karnataka: Chikaballapur; feeding on *Zea mays* L.; 30.vii. 2018; coll. A. N. Shylesha; GenBank Accession No. MH704433 and BarCode from BOLD System ID AGIMP054-18.

Life stages (Figs 2A−K)

Egg (Figs 2A, B): Egg laying occurs on the inner side of the whorl and also on the under surface of the leaf in a mass of (observations from field) deposited in layers (89 eggs arranged in layers/patch as shown in Fig. 2B). The eggs are dome shaped brownish yellow coloured and loosely covered with pale yellowish coloured frass. On higher magnification, shining reticulated surface can be easily noticed. The egg diameter and height ranges as 0.49−0.51 and 0.35−0.37, respectively.

Larval stages (Figs 2C−F): First instar larva is greenish in colour with black head while the final instars are with dark grey head and dull grey body with white subdorsal and lateral white lines. The mature larva is with a white inverted on the head and with distinct black spots on the

body. Arrangement pattern of black spots is square on 8th and trapezoidal on 9th segment (Fig 2E).

Pupa (Fig. 2G): Pupa is reddish brown in color and pupation occurs in the soil.

Adult (Figs 2H−K): Sexual dimorphism is clearly evident in the adult moths. Adult male forewing (Figs 2H & I) is greyish brown with reniform indistinct spot, faintly outlined in black, with a small v-shaped mark (Fig. 2I, marked with red coloured circle); light brown orbicular spot, somewhat oval and oblique in shape (Fig. 2I, marked with green coloured circle) and white patch at the apical margin of the wing (Fig. 2I, marked with blue coloured circle). Adult female forewing (Figs 2J & K) is with a mottled colouration of grey and brown, with brown markings and without white patch near apical margin of the wing as seen in male.

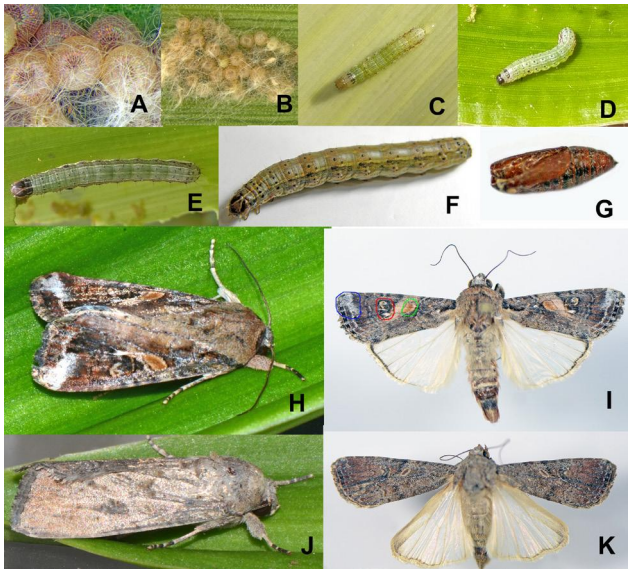

Fig. 2. Life stages of *Spodoptera frugiperda*: A &B, eggs; C−F, larval instars; H, adult male in habitus; I, adult male (dorsal view); J, adult female in habitus; K, adult female (dorsal view).

Molecular characterization of *Spodoptera frugiperda*

DNA extraction: Field collected larvae of *S. frugiperda* were placed in 1.5 ml micro centrifuge tubes separately. Genomic DNA was isolated by using DNA extraction kit (QIAGEN DNeasy blood and tissue kit Cat. 69504, Germany).

COI amplification

DNA extraction was performed on single specimen using Qiagen DNeasy® kit, following the manufacturer's protocols. The remaining individuals of same species were kept as voucher specimens at −70°C in ICAR-NBAIR, Bengaluru. DNA thus obtained was subjected to PCR amplification of a 658 bp region near the 5' terminus of the *COX1* gene following standard protocol (Hebert *et al*., 2003). Primers used for amplification of *CO1* gene were: forward primer (LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer (HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'). Polymerase Chain Reaction were carried out in flat capped 200 µL volume PCR tubes obtained from M/s Tarsons, Kolkata, India. 50 µL reaction volume contained: 5 µL GeNeiTM Taq buffer, 1 µL GeNeiTM 10 mM dNTP mix, 1 µL (20 pmol/µL) forward primer, 1 µL (20 pmol/µL) reverse primer, 1 µL GeNeiTM Taq DNA polymerase (1 U/µL), 5 µL DNA (50 ng/µL), and 36 µL sterile water. Thermo cycling consisted of an initial denaturation of 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 45°C for 1 min, extension at 72°C for 1 min. PCR was performed using a BioRad C1000™ Thermal Cycler. The amplified products were analysed on 1.5% agarose gel electrophoresis as standard protocol. The amplified products were sequenced by M/s Chromous, Bengaluru. Each specimen PCR sample was bi-directionally sequenced and checked for homology, insertions and deletions, stop codons, and frame shifts by using NCBI-BLAST and ORF finder. The COI generated consensus sequences have been deposited in NCBI GenBank database (Benson *et al*., 2005).

Sequence retrieval from BOLD database

In order to compare our isolated sequence with available database sequences, we referred to BOLD (http://v4.boldsystems.org/) to download COI sequences of *S. frugiperda*. The Chikaballapur population showed 100% resemblance with the sequences submitted from Canada (GenBank: GU095403.1) and Costa Rica (GenBank JQ547900.1).

Spodoptera frugiperda sequence (Chikaballapur) with GenBank accession No. MH704433; released on 01/08/2018:

AACATTATATTTTATTTTTGGAATTTGAGCAGGAATAGTAGGTACTTCTTTAAGTTTATTAATTCGAGCTGAATTAGGAACTCCAGGATCTTTAATTGGAGATGATCAAATTTATAATACTATTGTAACAGCCCATGCTTTTATTATAATTTTTTTTATAGTTATACCAATTATAATTGGAGGATTTGGAAATTGACTTGTACCTTTAATATTAGGAGCTCCTGATATAGCTTTCCCACGTATAAATAATATAAGTTTTTGACTTTTACCCCCATCTTTAACTTTATTAATTTCTAGTAGCATTGTAGAAAATGGAGCAGGAACTGGATGAACAGTTTACCCCCCCCTCTCCTCTAATATTGCTCATGGTGGTAGTTCAGTAGATTTAGCTATTTTCTCACTTCATTTAGCTGGAATTTCATCTATTTTAG-

GAGCTATTAACTTTATTACCACTATTATTAATATAC-
GATTAAATAATTTATCATTTGATCAAATACCTT-
TATTTATTTGAGCTGTAGGTATTACCGCATTTT-
TATTATTATTATCTTTACCTGTTTTAGCTGGAGCT-
ATTACTATATTACTTACTGATCGAAATCTAAATA-
CATCATTTTTCGATCCTGCAGGAGGAGGTGATC-
CTATTCTTTATCAACATTTATTT

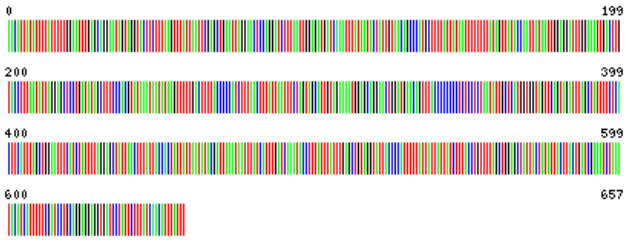

Fig. 3. Barcode image of *Spodoptera frugiperda*.

BOLD-ID: AGIMP054-18

Morphological characterization/details of associated natural enemies and compatible approaches for management of *Spodoptera frugiperda*

I. Egg parasitoid: *Telenomus* sp. (Hymenoptera: Platygastridae) (Figs 4A−C)

Material examined: Two females mounted on card; India: Karnataka: Chikaballapur: Konagona koppa; 01.viii. 2018; ex egg mass of *Spodoptera frugiperda* (J. E. Smith); coll. Omprakash Navik.

II. Egg parasitoid: *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) (Figs 4D−F)

Material examined: Two females mounted on slide; India: Karnataka: Chikaballapur: Konagona koppa; 01.viii. 2018; ex egg mass of *Spodoptera frugiperda* (J. E. Smith); coll. Omprakash Navik.

III. Larval parasitoid: *Glyptapanteles creatonoti* (Viereck) (Hymenoptrea: Braconidae) (Fig. 4G & H)

Brief diagnosis: Adult female with general body colour black except first tergite yellowish brown at base (extreme apex black), lateral membranous margins of two basal tergites and third tergite laterally yellowish brown. Legs yellowish brown except apical third of hind tibiae and hind tarsi. Hind coxae smooth and shining. Propodeum smooth and shining with slight excavation. Ovipositor sheath longer than the long hind tibial spur.

Material examined: Five females dry mounted on card; India: Karnataka: Chikaballapur; 09.vii. 2018; ex larva of *Spodoptera frugiperda* (J. E. Smith); coll. S. K. Jalali.

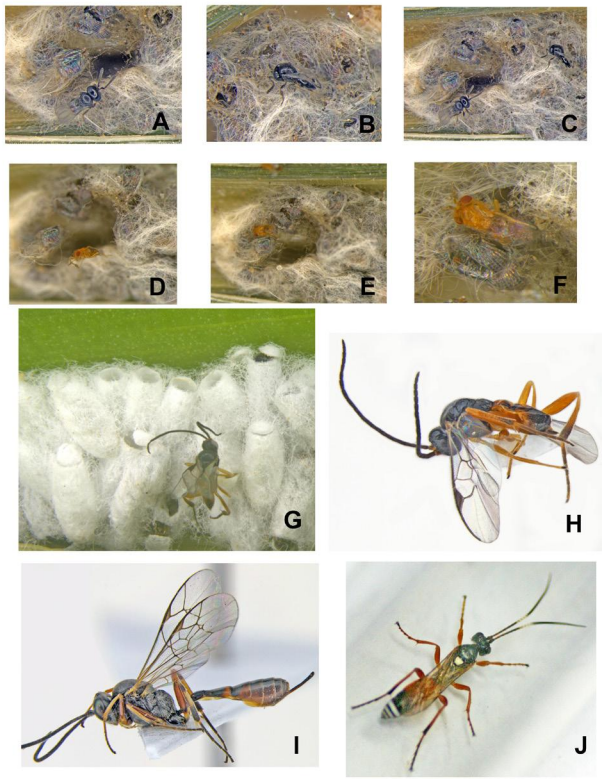

Fig. 4. Parasitoid complex of *Spodoptera frugiperda*: A−C, parasitized eggs of *S. frugiperda* with *Telenomus* sp.; D−F, parasitized eggs of *S. frugiperda* with *Trichogramma* sp.; G, cocoons of larval parasitoid *Glyptapanteles creatonoti*; adult female wasp of *G. creatonoti*; I, larval parasitoid *Campoletis chlorideae* Uchida; J, indet. ichneumonid larval-pupal parasitoid

Label data of voucher specimens examined (BMNH, London)

Apanteles creatonoti Vier.; G. Nixon det.1950; 04724 Malaya Kuala Lumpur; 18.12.1943; Entom. Div., Agric. Dept.; ex. 04723 Larva; COM. DIST. EST.; Coll No. 11490; BMNH.

Apanteles creatonoti Vier.; G. E. J. Nixon det. 1959; *Deiopera pulchella*; Phillippines, Laguna, Los Banos, 150 ft alt; 186; 20.vi.1934; Feng Yung Tsan; per LB Uichaneo; BMNH.

Comments: This parasitic wasp species was identified as *Glyptapanteles creatonoti*. All the specimens matched with the voucher specimens obtained from BMNH, London in 2013−14 and with the description provided by Wilkinson (1928). *Spodoptera frugiperda* is the first host record for *G. creatonoti* across the globe. Our studies show that *G. creatonoti*, being a well established parasitoid of various noctuids in India and Malaysia, was capable of parasitizing *S. frugiperda*.

IV. Larval parasitoid: *Campoletis chlorideae* Uchida (Hymenoptera: Ichneumonidae) (Fig. 4I)

Material examined: One female mounted on card; India: Karnataka: Hassan; 02.viii. 2018; ex larva of *Spodoptera frugiperda* (J. E. Smith); coll. Omprakash Navik.

V. Larval-pupal parasitoid: Indeterminate Ichneumonidae (Hymenoptera: Ichneumoninae) (Fig. 4J)

*Material examined***:** One female mounted on card; India: Karnataka: Chikaballapur; 09.vii. 2018; ex pupa of *Spodoptera frugiperda* (J. E. Smith); coll. S. K. Jalali.

Comments: This species belongs to subfamily Ichneumoninae, probably from the tribe ?Heresiarchini. This subfamily is one of the most difficult to identify especially for the Oriental region where no global work has been published. Also being singleton, the identity is kept in abeyance until detailed studies are undertaken after examination of multiple specimens.

V. *Forficula* sp. (Dermaptera: Forficulidae) (Figs 5A–F)

*Brief diagnosis***:** The genus *Forficula* L. is characterized by combination of the following characters: fourth antennal segment shorter than the third (Fig. 5A); first tarsal segment twice as long as third, second tarsal segment widened and dilated into a heart-shaped lobe (Fig. 5B); mesosternum quadratic about as wide as long (Fig. 5C); male forceps strongly depressed, dilated in basal portion (Fig. 5D) and tegmina normally developed, not shortened (Fig. 5E). Female as in Fig. 5F. For detailed diagnosis refer Steinmann (1993).

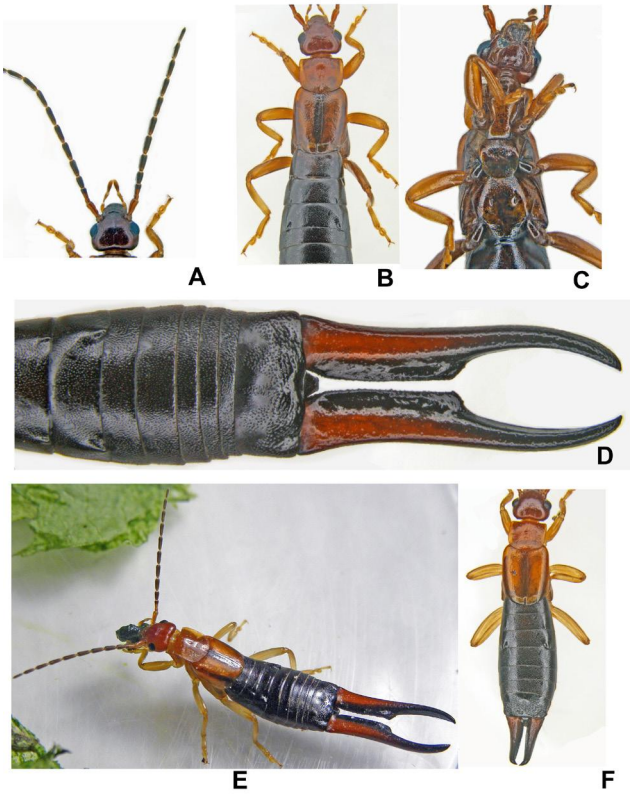

Fig. 5. *Forficula* sp.: A, male head with antennae; B, male head, mesosoma and metasoma in part (dorsal view); C, male head and mesosoma (ventral view);D, male metasoma in part with pincers; E, male in habitus; F, female (dorsal view).

Material examined: Four males and two females dry mounted; two male genitalia slide mounted; India: Karnataka: Doddaballapura; 09.vii. 2018; feeding larvae of *Spodoptera frugiperda* (J. E. Smith) in maize whorls; coll. A. N. Shylesha.

Comments: Forficula sp. was observed feeding on *S. frugiperda* larvae in some of the fields which were free from insecticidal spray.

VI. Entomopathogenic fungus: *Nomuraea rileyi* (Farl.) Samson (Fig. 6)

Material collected: India: Karnataka: CoA: Hassan: Karakare, 02.viii.2018; Shivamogga: Sogane, 03.viii. 2018; Chickaballapur: D. Husuru, 08.viii. 2018; ex infected larvae of *Spodoptera frugiperda* (J. E. Smith); coll. Omprakash Navik.

Fig. 6. *Nomuraea rileyi* infected larvae

Comments: Larvae infected with *Nomuraea rileyi* were collected from some of the fields. Further studies to evaluate *N. rileyi* for management of *S. frugiperda* are in progress.

VII. Electrophysiological response of male *Spodoptera frugiperda* to pheromone

Antennal response of adult *S. frugiperda* males to (*Z*)-*9-Tetradecen-1*-ol *acetate* (as major component) was established by electroantennography. 5 µg of pheromone when exposed to adult male antennae caused over 3 mv response

(Fig. 7). This confirms the physiological response of the antennal neurons to pheromone. Further field evaluation of the lure is under process.

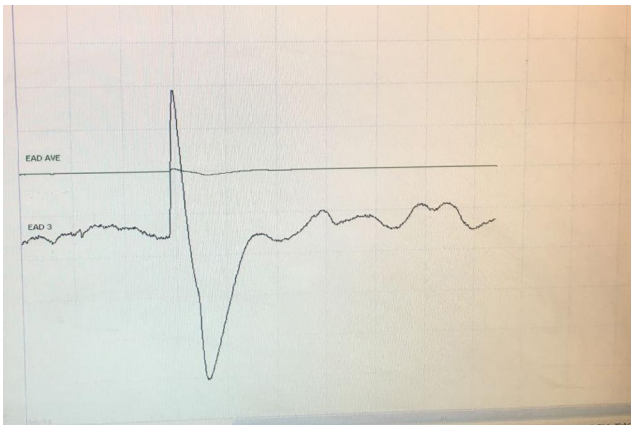

Fig. 7. EAG response trace of *Spodoptera frugiperda* male.

CONCLUSION

Potential threat

As mentioned by some of the pioneers and experienced team of scientists in the detection and subsequent spread of fall armyworm into the new regions of globe, IITA (2018) has expressed serious concern on this invasion into India "Its occurence with high incidence in the South Indian state of Karnataka is likely to be soon followed by spreads to the Tamil Nadu and Andhra Pradesh states that are major regions for hybrid maize seed production in India. Further expansion of FAW to countries adjacent to India such as Bangladesh, Nepal, Pakistan, and beyond will put the maize production of the whole Asian continent seriously at risk with dire economic consequences."

From India, Sharanabasappa and Kalleshwara Swamy (2018) observed the incidence of fall armyworm, *S. frugiperda* in mid-May in the maize fields at the College of Agriculture, University of Agricultural and Horticultural Sciences (UAHS), Shivamogga, Karnataka. University of Agricultural Sciences (UAS-B) reported the pest incidence to the Karnataka State Department of Agriculture (Ganiger *et al*., 2018). ICAR- NBAIR team released pest alert at national level (ICAR-NBAIR, 2018). At this juncture, the prime emphasis at ICAR-NBAIR is on documenting the spread and identifying potential natural enemies for biological control options and exploring other compatible management strategies.

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