Indian Journal of Entomology, 81(4): 635-639 (2019) DoI No.: 10.5958/0974-8172.2019.00137.8

FIRST RECORD OF FALL ARMY WORM *SPODOPTERA FRUGIPERDA* (J. E. SMITH) FROM NEPAL

Ajaya Shree Ratna Bajracharya*, Binu Bhat, Premnidhi Sharma, Pathour R. Shashank**, Naresh M. Meshram** and Tahseen Raza Hashmi**

Entomology Division, Nepal Agricultural Research Council, Lalitpur, Nepal
**Division of Entomology, ICAR- Indian Agricultural Research Institute, New Delhi 110012
*Email: ajayabajracharya@yahoo.com (corresponding author)

ABSTRACT

The fall army worm *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a destructive invasive insect pest of cereal crops native to American continent. It is a polyphagous insect with host range of more than 353 plant species and maize is a preferred host. This pest is a strong flier and can fly up to 500 km before oviposition. Present study reports *S. frugiperda* as a first record from Nepal (Nawalpur district) observed in May 2019 along with its morphological and molecular diagnosis.

Key words: *Spodoptera frugiperda*, maize, Nawalpur, Nepal, first record, description, diagnosis, mtCo1

The fall army worm (FAW) *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is a destructive invasive pest of cereal crops native to tropical and subtropical regions of Americas. It has very wide host range and more than 80 crop species had been recorded as host and maize is a preferred host (Prasanna et al., 2018). Apart from maize it can cause major damage to other cultivated crops including sorghum, rice, wheat, finger millet, sugarcane, cabbage, beet, groundnut, soybean, onion, cotton, tomato, potato and many fodder grasses. Previous studies had specified that 353 plants were recorded as larval host of *S. frugiperda* belonging to 76 plant families: major host families are Poaceae (106 species), Asteraceae (31 species) and Fabaceae (31 species) (Debora, 2018).

This pest is strong flier with migratory and localized dispersal habit and can fly up to 500 km before oviposition (Prasanna et al., 2018). It is known for travelling 1600 km from southern U. S. state Mississippi to southern Canada in 30 hr with assistance of proper wind pattern (Rose et al., 1975). Yield loss of maize, sorghum, rice and sugarcane by *S. frugiperda* infestation in all African countries were estimated at 20.15, 7.45, 56.15 and 51.05%, respectively causing predicted economic loss accounting to US$ 13,383 million (Abrahams et al., 2017). Maize yield reduction due to feeding of fall army worm larvae is 34 % in Brazil (Cruz et al., 1999). *Spodoptera frugiperda* was confined to American continents till 2015. The pest was reported for the first time out of American continents in 2016 from Benin, Nigeria, Togo and Sao Tome and Principe of Africa (Goergen et al., 2016) and in 2018 it spread into 43 African countries (Prasanna et al., 2018). This pest was reported for the first time in Asia in May 2018 from Shivamogga of Karnataka, India (Sharanbasappa et al., 2018; Mahadeva Swamy et al., 2018). Subsequently, this pest had also been reported from Sri Lanka, Bangladesh, Myanmar, Thailand, Vietnam, China, Taiwan, Korea and Japan. In the present study, the *S. frugiperda* has been recorded for the first time in Nepal from Nawalpur district (N 27°42'16.67", E 084°22'50.61") on 9th May, 2019. This article highlights the identification of *S. frugiperda* based on external morphology of larvae and adult moths, and genitalia of male and female moths. The article also provides the molecular evidence to support the morphological diagnosis.

MATERIALS AND METHODS

Maize fields from Gaindakot area of Nawalpur district (N 27°42'16.67", E 84°22'50.61") were found with typical insect damage symptoms of papery windows and ragged holes in leaves along with extensive whorl damage producing large amount of faecal matter. Larvae with different external morphological characters previously not observed in Nepal were found damaging maize leaves and whorl. These larvae were brought to the Entomology Division, Nepal Agricultural Research Council (NARC) and reared up to adults in rectangular plastic rearing boxes with dimensions of 18.7 x 12.6 x 7.8 cm at laboratory condition. Maize leaves of variety Manakaman-1 were provided as food to larvae. The adults were allowed to mate in rearing cage made of insect proof net in wooden frames with dimensions of 47 x 47 x 77 cm (l x b x h). Larvae emerging from laid eggs were collected and reared in plastic rearing cages on maize leaves.

The larvae hatched from eggs as well as field collected larvae were studied for external morphology. Similarly, adults emerging from field collected larvae were used for study of morphology including genitalia following Brambila (2009). Observations on morphology were made with a stereomicroscope (Bestscope BS-3040T) and photographed with digital camera (Bestscope, BUC5C-500C, 5MP). These morphology details of larvae and adults were compared with earlier descriptions (Passao, 1991; Sparks et al., 1914; Brambila, 2009; Brambila, 2013; EPPO, 2015; Diedrich Visser. 2017; Sharanabasappa et al., 2018; Ganiger et al., 2018).

Total genomic DNA, from the tissue of larvae of the specimens was extracted with DNASure® Tissue Mini Kit, following the manufacturer protocol. The extracted DNA was stored at -20°C for further processing. Molecular based identification was done with diagnostic PCR reaction, using universal sets of mtCOI primer (LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAAACTTCAGGGTGACC AAAAAATCA-3') (Folmer et al., 1994). The concluding volume of the PCR mixture was 25 µl consisting of 12.5 µl of Thermo Scientific maxima hot start PCR master mix, 8.5 µl of molecular grade water, 1 µl each forward primer LCO1490 and reverse primer HCO2198 and 2 µl of genomic DNA. Ventri® 96- well thermal cycler (Applied Biosystems® Life Technologies) was used for the amplification of the samples, under the following cycling protocol: 4 min. hot start at 94°C, 35 cycles of denaturation for 30 sec at 94°C, annealing for 60 sec at 47°C, elongation for 50 sec at 72°C and a final extension was carried out at 72°C for 8 min. The products were checked on 1% agarose gel and visualized under UV using Alphaview® software version 1.2.0.1. The amplified products were sequenced at AgriGenome Pvt. Ltd. (Cochin). After getting the sequence of the amplified product preliminary determination was acquired through sequence assessments using the web based Basic Local Alignment Search Tool (BLAST) algorithm of NCBI (National Center for Biotechnology Information).

Obtained sequence was analysed with 13 consensus sequences of *S. frugiperda* including outgroup, downloaded from NCBI GenBank database. The mtCOI sequences in FASTA format were brought together into the sequence alignment application of MEGA 6 (Tamura et al., 2013) and multiple sequence alignments attained with the Clustal W (Jeanmougin et al., 1998) algorithm using default parameters. The sequences were submitted to NCBI for GenBank accessions. Sequence divergences were estimated using the Kimura 2-Parameter distance model (Kimura,1980) and graphically demonstrated in a maximum likelihood (ML) tree by the program MEGA 6 (Tamura et al., 2013). Tree robustness was evaluated by bootstrapping with 1000 replicates with *Busseola fusca* as outgroup.

RESULTS AND DISCUSSION

Eggs were generally laid in group with overlapping two or more layers and covered with hairs from abdomen of female (Fig. 1a). The eggs are creamy white and wider than height having reticulate ribs on the surface (Fig. 1b). The first instar larvae were whitish which later changed into greenish with black head (Fig. 2a). White longitudinal stripes on dorsal surface could be seen at early stage and pinnacula on the body were prominent with hairs. Pinkish lines were observed above the spiracles especially on posterior abdominal segments. Grown up larvae measured 30-35 mm long and varied from brown, gray, yellowish, pinkish and greenish with granulated texture all over the body (Fig. 2b). Prothoracic plate similar to head and head with reticulate pattern. Ecdysial line on the head forming 'V' shape which continues with middorsal stripe of prothoracic shield forming inverted 'Y' shaped whitish marking (Fig. 2c). Dorsal pinacula on 8th and 9th abdominal segments were larger than corresponding spiracles and pinacula on the other abdominal segments. Pinnacula on 8th abdominal segments were arranged in square shape and trapezoid on 9th abodominal segment (Fig. 2d). Three creamy yellow stripes, one middorsal and two subdorsal were found running down form prothorax to last abdominal segment in parallel manner. Mandibles of the larvae were serrated with conspicuous teeth (Fig. 2e). All above mentioned larval characters resembled with larvae of *S. frugiperda* (Passao, 1991; Sparks et al., 1914; Brambila, 2009; Brambila, 2013; EPPO, 2015; Diedrich Visser. 2017; Sharanabasappa et al., 2018; Ganiger et al., 2018). Pupae were typical of Noctuidae, obtect type with two spines on cremaster (Fig. 2f).

Adult male moth with distinct markings (Fig. 3a) on the forewing whereas marking on female forewing

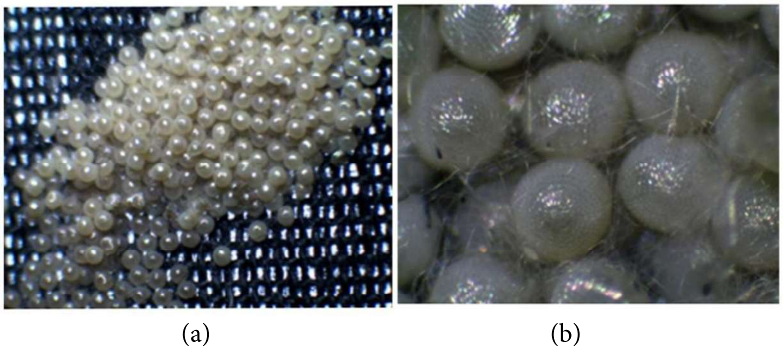


Fig. 1. *Spodoptera frugiperda*: (a) Egg mass, (b) Eggs showing reticulate ribs and hairs

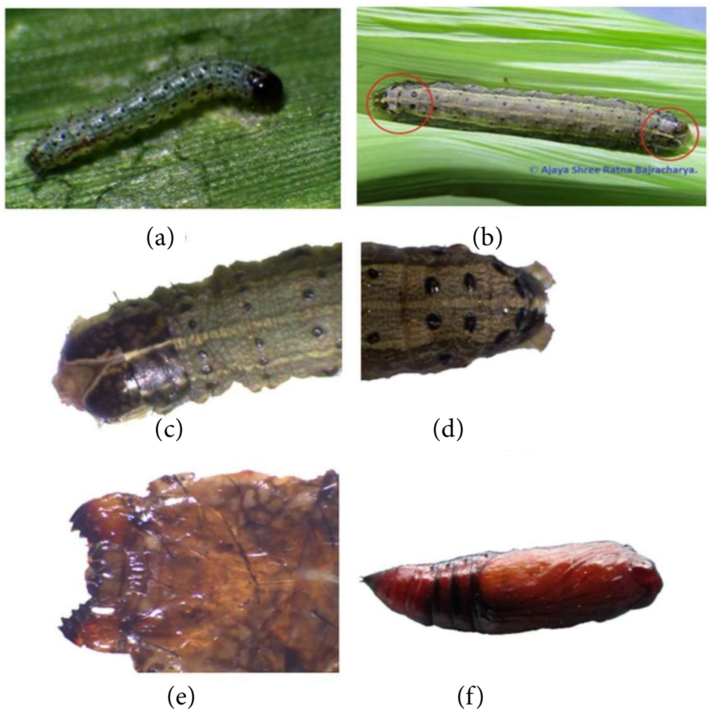
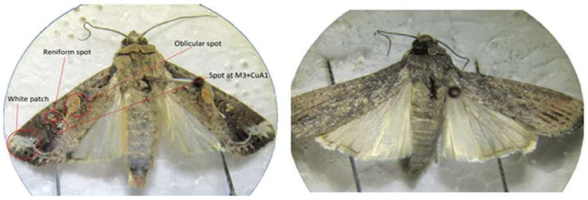


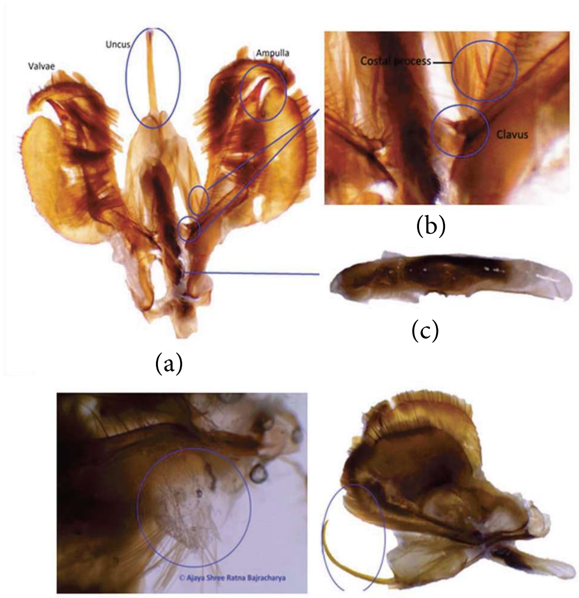
Fig. 2. *Spodoptera frugiperda* larvae and pupa, (a) First instar larva, (b) Grown larva, (c) Inverted 'Y' on head, (d) Pinacula pattern on 8th and 9th abdominal segments, (e) Mandibles of larva, (f) Pupa.

variable (Fig. 3b). The forewing of male had greyish brown to rusty brown with white patch near apical margin. Transverse white and brown lines were not distinct as in native species, *Spodoptera litura*. Oblicular spot in males were brown, oval and oblique. Reniform spot in males were indistinct, partially outlined in black, with a small, sideways 'v' shaped white marking. A small conspicuous white spot at junction of M3 and CuA1 vein was seen. Hindwing in both male and female white with black line on outer margin (instead of inner margin) and anal and inner margins. Male genitalia with valvae broad and quadrate (Fig. 4a); uncus curved at apical half, slender and pointed at apex (Fig. 4a, e); ampulla slightly curved (Fig. 4a). Similarly, clavus short; costal process narrow, elongate, straight, inclined, hair structure at tip (Fig. 4b); coremata with single lobe (Fig. 4d), aedeagus well developed (Fig. 4c). Female genitalia with ductus bursae completely sclerotized; appendix bursae partially sclerotized; corpus bursae bulbous, length less than twice width with striate convolutions and signum was in basal half of corpus bursae (Fig. 5). All these characters were similar to *S. frugiperda* according to previous findings (Brambila, 2013;



(a) (b)

Fig. 3. Adult moths of *S. frugiperda*, (a) Adult male with encircled identification markings on forewing, (b) adult female.



(a) (b) (c) (d) (e)

Fig. 4. Male genitalia of *S. frugiperda*: (a) Male genitalia (b) Clavus and costal process enlarged (c) Aedeagus, (d) Coremata (e) Uncus of male genitalia.

EPPO, 2015; Sharanabasappa et al., 2018; Ganiger et al., 2018).

A phylogenetic tree (ML tree) based on the mitochondrial cytochrome oxidase I gene clearly support the morphological identification of *S. frugiperda* (Fig. 6). The obtained sequence of *S. frugiperda* (Accession number- MN011579), collected from Nepal is settling down with the reported sequences of *S. frugiperda* from database. The molecular analysis confirms and support the morphological identification of *S. frugiperda*, collected from Nawalpur district of Nepal.

All external and internal morphological characters of larvae and adult moths were similar to *S. frugiperda* according to previous finding of various authors as mentioned. Similarly, molecular sequence of *mtCOI* DNA also resembled with *S. frugiperda* from various

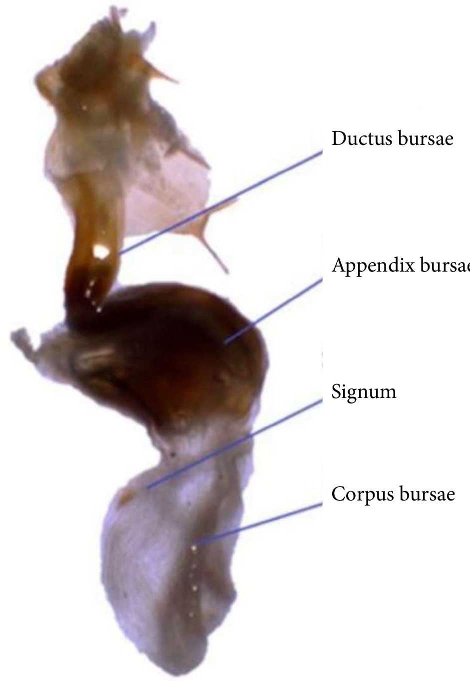


Fig. 5. Female genitalia of *S. frugiperda*

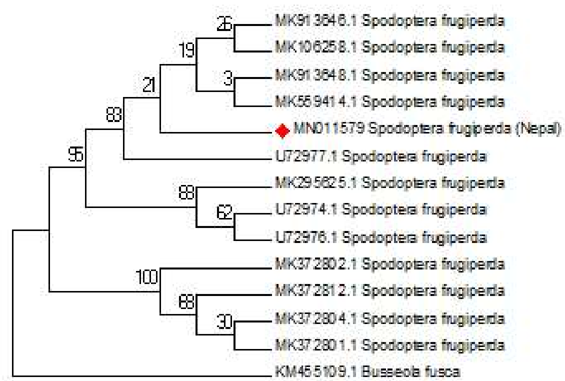


Fig. 6. Phylogram of the evaluated *S. frugiperda*

parts of the world. Thus, it was confirmed that insect infesting maize at Gaindakot area of Nawalpur district (N 27°42'16.67", E 84°22'50.61") in Nepal gets confirmed as *S. frugiperda* and its introduction in Nepal established as the first record.

Maize is second most important staple food crop in Nepal and cultivated in 891,583 ha with production of 2,231,517 mt. Among cultivated area 18.95% fall in plain areas and 81.04% in hills and mountains (Statistical Information, 2017). Around 670,000 mt of maize was still deficit in Nepal especially for feed industries (Govinda et al., 2015). *Spodoptera frugiperda* has potential to cause damage in maize reducing yield up to 51% in Africa (Prasanna et al., 2018) and 34% yield reduction recorded in Brazil (Cruz et al., 1999). In short period of time after first detection from Nepal it has spread into various parts of the country even reporting from 1,700 masl from Kabre of Dolakha district. Nepal is with diverse climatic conditions, from alpine to tropical, within short aerial distance, due to its altitudinal variation. The *S. frugiperda* could cause huge damage in maize affecting food security and feed industry in Nepal. Thus, IPM based solutions need to developed to protect maize from the alien fall army worm.

REFERENCES

Abrahams P, Beale T, Cock M, Corniani N, Day R, Godwin J, Murphy S, Richards G, Vos J. 2017. Fall armyworm status: Impact and control option in Africa, Preliminary Evidence note, UK Aid, CABI.

Brambila J. 2009. Steps for the dissection of male *Spodoptera* moths (Lepidoptera: Noctuidae) and notes on distinguishing *S. litura* and *S. littoralis* from native *Spodoptera* species. USDA-APHIS- PPQ.

Brambila J. 2013. Identification notes for *Spodoptera litura* and *Spodoptera littoralis* (Lepidoptera: Noctuidae) and some native *Spodoptera* moths. USDA-APHIS- PPQ.

Cruz E, Figueiredo M L C, Oliveira A C, Carlos A V. 1999. Damage of *Spodoptera frugiperda* (Smith) in different maize genotypes cultivated in soil in three different levels aluminum saturation. International Journal of Pest Management 45(4): 293-296.

Debora M. 2018. Host plant of *Spodoptera frugiperda* (Lepidoptera:Noctuidae) in the Americas. African Entomology 26(2): 286-300.

Diedrich Visser. 2017. Fall armyworm- an identification guide in relation to other common caterpillars a South African perspective. Presented and produced by Diedrich Visser, Agricultural Research Council - Vegetable and Ornamental Plants (ARC-VOP) Roodeplaat, Pretoria.

EPPO. 2015. OEPP/EPPO Bulletin. Diagnostics. PM 7/124(1). *Spodoptera littoralis, Spodoptera litura, Spodoptera frugiperda. Spodoptera eridiana*. 45(3):410-444.

Folmer O, Black M B, Hoer Wr, Lutz R, Vrijenhoek R C. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3(5): 294-299.

Ganiger P C, Yeshwanth H M, Muralimohan K, Vinaya N, Kumar, A R V, Chandrasekhar K. 2018. Occurrence of the new invasive pest, fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), in the maize field of Karnataka, India. Current Science 115(4): 621-623.

Goergen G, Kumar P L, Sankng S B, Tagola A, Tamo M. 2016. First report of outbreak of fall armyworm *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), a new alien invasive pest in west and central Africa. PloS One 2016. https://doi.org/10.1371/journal.pone.0165632

Govind K C, Karki T B, Shrestha J, Achhami B B. 2015. Status and prospects of maize research in Nepal. Journal of Maize Research and Development 1(1): 1-9.

Jeanmougin F, Thompson J D, Gouy M, Higgins D G, Gibson T J. 1998. Multiple sequence alignment with Clustal X. Trends in biochemical sciences 23(10): 403-405. PMID: 9810230.

Kimura M. 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16(2): 111-120.

Mahadeva Swamy H M, R Asokan, C M Kalleshwaraswamy, Sharanabasappa, Y G Prasad, M S. Maruthi, P R Shashank, Naorem Ibemu Devi, Anusha Surakasula, S Adarsha, A Srinivas, Srinivasa Rao, Vidyasekhar, M Shali Raju, G Shyam Sunder Reddy, S N Nagesh. 2018. Prevalence of "R" strain and molecular diversity of fall army worm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in India. Indian Journal of Entomology 80(3): 544-553.

Passao S. 1991. Color identification of economically important *Spodoptera* larvae in Honduras (Lepidoptera: Noctuidae). Insecta Mundi 5(3-4): 185-196.

Prasanna B M, Huesing J E, Eddy R, Peschke V M (eds.). 2018. Fall armyworm in Africa: A guide for integrated pest management, First Edition. Mexico, CDMX: CIMMYT. 108 pp.

Rose A H, Silverside R H, Lindquist O H. 1975. Migration flight by aphid, *Rhopalosiphum maidis* (Hemiptera: Aphididae) and a noctuid, *Spodoptera frugiperda* (Lepidoptera: noctuidae. Canadian Entomology 107: 567-576.

Sharanabasappa, Kalleshwaraswamy C M, Asokan R, Mahadeva Swamy H M, Maruthi M S, Pavithra H B, Kavita Hedge, Shivaraya Navi, Prabhu S T, Goergen G. 2018. First report of the fall armyworm, *Spodoptera frugiperda* (J E Smith) (Lepidoptera: Noctuidae), an alien invasive pest on maize in India. Pest Management in Horticultural Ecosystem 24(1): 23-29.

Sparks A N, Liu T X. A key to common caterpillar pests of vegetables. Texas Agricultural Extension Service. The Texas A&M University System. B-6110, 7-01.

Statistical Information. 2017. Statistical Information on Nepalese Agriculture (2015/16). Government of Nepal. Ministry of Agriculture Development, Monitoring Evaluation and Statistics Division, Agri Statistics Section, Singhdurbar, Kathmandu, Nepal.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30(12):2725–9. https://doi.org/10.1093/molbev/ mst197 PMID: 24132122 14.

(Manuscript Received: August, 2019; Revised: September, 2019; Accepted: September, 2019; Online Published: September, 2019)