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Original Article

First report of morphometric identification of *Spodoptera frugiperda* J.E Smith (Lepidoptera: Noctuidae) an invasive pest of maize in Southern Sindh, Pakistan

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Abstract

Invasive species always pose a serious threat to agriculture and cost billions of dollars in terms of reduced production and productivity. The recent preliminary survey in a few districts of southern Sindh, Pakistan showed an apprehension of the entry of one more new invasive pest belonging to genus *Spodoptera* is known as Fall Armyworm (FAW), *S. frugiperda* (J.E. Smith) in April 2019. The identification for confirming this alien pest was made employing morphological characters. The males were dissected to study genitalia. The microscopic study revealed that male genitalia of the collected species had a single lobe of coremata which is the main identification of FAW. The wing characteristics showed that the males had dark gray and brown shaded mottled forewings with conspicuous triangular white spots at the tip of the wings. The forewings of females were less distinctly marked except few faint grayish brown markings. There were six larval instars and the best identifying feature of the FAW in mature larval stage (5^{th}-6^{th} instar) was a set of four large rectangular spots on the upper surface of the second last segment of body and the face of larvae with obvious mark of inverted white colored "Y" shape. In morphometric, we measured the head capsule of each larval instar ranging 0.3-2.6mm in length and the larvae about 2-33.08mm. The pupa was reddish brown in color, 14-18mm in length and about 4.5mm in width. The present study confirmed the presence of *S. frugiperda* which requires an immense attention to make an integrated pest management strategy against it.

Keywords: Fall armyworm, Invasive species, Male genitalia, Maize, Sindh

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Introduction

Maize (*Zea mays* L.) is the third vital crop in the cereals and a main staple food of many countries of the world. In Pakistan, it is cultivated on 1.34 million hectares with the production of 6,134 million tones (PBS, 2018). The maize and its by products are utilized for many purposes including human food, livestock fodder, poultry feed and fuel which contribute largely (about 98%) in total country's production. Though, it is cultivated throughout Pakistan, but mainly grown in Punjab and Khyber Pakhtunkhwa provinces (Naz et al., 2003). The crop is attacked by a number of insect species, but 12 species have been reported as key pests of maize which cause heavy damage from sowing to harvesting and even damage continues until storage of seed (Siddiqui and Marwaha, 1993). The severity of pest attack often observes due to bad cultivation practices, susceptible varieties/cultivars, poor infrastructure of godowns and harsh environmental conditions (Arabjafari and Jalai, 2007).

Besides, invasive alien species pose a serious threat to agriculture and cost billions of dollars in terms of reduced production and productivity. This is mainly due to increased trans-boundary movement of agricultural commodities, anthropogenic activities, and climate change (Paini et al., 2016). Our recent preliminary survey showed a panic of the entry of one more new invasive pest of genus *Spodoptera* which is known as Fall Armyworm (FAW), *S. frugiperda* J.E. Smith (Noctuidae: Lepidoptera) into the Southern region of Sindh province. Basically, it is native to the tropical and subtropical regions of the United States of America (USA), where it is a serious pest of maize but also known to attack more than 100 other host plants including grasses. FAW had not been reported until 2015 apart from the USA, but its severe incidence from African countries (Goergen et al., 2016*)* particularly from Ghana during 2017 (Cock et al., 2017) got the special attention of the world. Subsequently, it spread to most of sub-Saharan Africa and confirmed, where it caused heavy damage on very large-scale, specifically to maize crop and to lesser extent on sorghum and other crops (Prasanna et al., 2018). The pest is reported to cause 34% reduction in grain yield and annual loss up to 400 million US$ in Brazil (Lima et al., 2010). Most recently, the occurrence of the FAW on maize in different districts of Karnataka state of India has also been confirmed (Kalleshwaraswamy et al., 2018). Being a neighbouring country, Pakistan has a pivotal threat of this invasive pest on its agro-ecosystem. As, our country is predominantly a tropical state and favours a high rate of multiplication of this pest round the year. Further, its high pestiferous nature poses a formidable challenge to Pakistan's agriculture warranting immediate action before it could assume a serious proportion. In addition, it is reported to cause severe damage to grasses of economically important (i.e. sugarcane, sorghum and rice), horticultural crops (i.e. cabbage, beet, tomato, potato and onion), cotton, pasture grasses, peanut, soybean, alfalfa and millets (Chapman et al., 2000; Pogue, 2002). Thus, the present situation demands an intensive survey, collection, and identification of this pest to report its presence on maize and other crops. Keeping such a significant importance of this invasive pest, this study has been carried out which could confirm the presence of this alien pest in Sindh province of Pakistan on maize.

Material and Methods

Location

Insect species were collected in April 2019 from different maize fields of district Hyderabad, (25.4281° N, 68.5307° E), Tando Allahyar (28.5998° N, 77.0913° E) and Matiyari (25.5011° N, 68.5202° E), as these districts are main area for maize cultivation in Sindh province. The collected insect species were reared at the Department of Entomology, FCPT, SAU, Tando Jam for further detailed study. Identification of FAW was done through male genitalia examination, adult wing patterns and morphometric larvae.

Dissection of male genitalia of FAW

For proper identification of insect species, male genitalia were dissected as previously mentioned by Clark (1941) and compared with earlier findings (Pogue, 2002; EPPO, 2015 and Kalleshwaraswamy et al., 2018). The adult moths were placed in a killing jar having potassium cyanide for completely killing. A 10% Potassium Hydroxide (KOH) and 90% distilled water were mixed with the help of graduated cylinders and pipette to prepare a proper solution. After preparing the solution, the dissected abdomen was placed into a glass test tube. The specimen was kept for 24 hours in preparation for maceration of extra tissues to obtain genitalia for microscopic examination. Next day, the dissected abdomen was rinsed thoroughly to wash away any remaining KOH

solution from the specimen. After rinsing, the abdomen was placed on a microscopic glass slide and few drops of glycerin were added for more clear visibility. Later, the dissection and separation of extra tissues were performed with the help of fine probe and forceps.

Wing pattern of FAW

Few specimens from the insect killing jar were placed on a poly thermal sheet and insect wings were stretched gently and finally pinned with insect pins. Ten samples were taken for studying both male and female wing patterns thoroughly.

Morphometric larval instars of FAW

The egg batches of FAW were taken and placed on a Petri dish (10 x 15 mm) for studying larval instars. At emergence of the first larval instar, each larva was kept separately in a glass vial and fresh leaves of corn were fed to these. Five samples of each larval instar were taken for morphometric study containing length of head capsule and total larval body. All procedures for observing male genitalia were executed by Stereo Zoom microscope with DSLR Camera mounted (Model SZM405; HT Company United Kingdom). In addition, the morphometric study of all larval instars, pupa, and adult moths (male and female) was performed with the help of images taken by Fujifilm Camera (10 MP, Model S8100FD, Indonesia). The illustrations were further improved using Adobe Photoshop graphic software (ver. 7.0) and Corel Draw (ver. 12). All measurements were done in a software generated program image-scope 9.0 (H9D).

Results

The male genitalia of FAW

The male genitalia were dissected in the light of pertinent literature and structure confirmed the presence of FAW feeding on maize. The microscopic study revealed that male genitalia of the collected species had a single lobe of coremata which is the main identification of FAW and juxta was concave at base and with dorsal process (Fig. 1 A). In addition, the genitalia were with right and left valves, both broad and quadrate in shape with curved ampulla and pointed at top. At the base of both valves, the clavus was short with costal process narrow, straight, elongate, and inclined (Fig. 1 B).

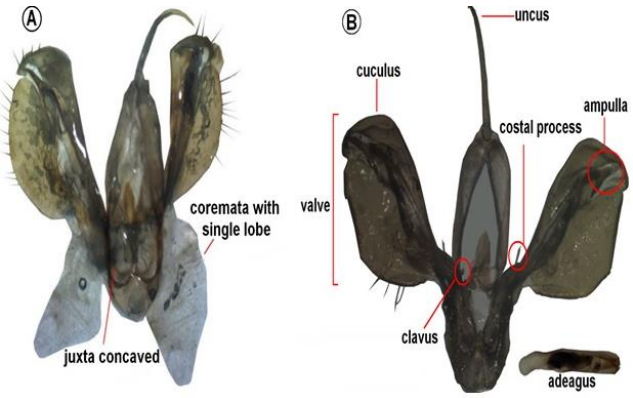


Figure-1. Dissected male genitalia of *S. frugiperda* (a) with coremata (b) without coremata

Wing pattern and measurement of adult FAW

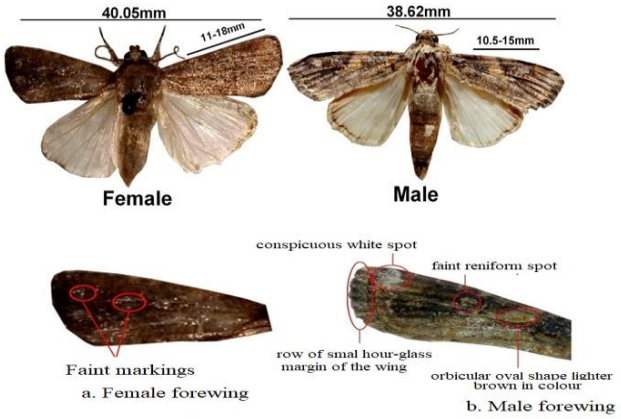
The morphological characteristics of male and female wings of FAW were studied in detail. Males and females were bit different from each other in wing pattern, particularly in forewings. The body length of adult moths was not much different (16.3±0.39 mm; 16.52±0.25mm male and female). Similarly, the total wingspan of adult moths was almost similar in size (38.15±0.42mm; 39.92±0.50 mm male and female). However, the forewing length was higher in female 17.83 ± 0.25mm as compared to male 15.24 ± 0.42 mm. The overall length of male genitalia was 1.95±0.07 mm and pupa 14.14±0.31 mm (Table 1).

Table-1. Morphometric of male and female (mm) and pupa

Body features	Male	Female
Male genitalia length	1.95±0.07	-
Body length	16.3±0.39	6.52±0.25
Single wing length (Forewing)	15.24±0.38	17.83±0.25
Total wingspan (width)	38.15±0.42	39.92±0.50
Pupal length	14.14±0.31	14.14±0.31

The main difference in male and female was their distinct forewings and similarly the pattern of forewing also showed dissimilarity in two species (*S. frugiperda* and *S. litura*) of similar genus i.e. *Spodoptera*. The males have brown shaded mottled and dark gray forewing with contrasting markings and clearly visible white spots in triangular shape near the center and at the tip of the forewing. The contrasting transverse lines in forewing of male further included light brown orbicular spot, v-

shaped markings and row of small sandglass shapes marking near apical margin (Fig. 2). The hind wings of both male and female were with less distinctly silver-white marking and a narrow border of golden color. The female forewings were dimly marked except for a few faint markings, appearing in a



uniform grayish brown to a fine mottling brown and gray.

Figure-2. Male and female adult moths (single forewing a. female b. male)

Larval distinction and measurement of FAW

It was difficult to define characters of each larval instar thus divided into two groups i.e. mature and young larvae which make it much easier and definite to understand.

Young or immature larvae (L1 to L3)

The FAW typically has six larval instars and earlier larval instars are difficult to determine morphologically because most noctuids have the same visuals in this stage. Instead of that, young larvae of FAW were commonly in yellowish, greenish, and brownish color with a black head and white longitudinal stripes. These larvae were always found lighter in color as compared to the mature larvae. The dorsal surface spots of the larval body were not always equal in number and the mark on the head was also not visible until larvae molted into third instar. The head turned orange in color in the second instar and increased in size. A pinkish shape was commonly seen on both sides of young larvae. The bumps and hairs were more prominent on young larvae. The later bands were not visible on small caterpillars as compared to mature larvae; meanwhile pale to yellow dorsal line on center of the larval body, pale to yellow dorsal thick line, pale to yellow latero-dorsal lines started to appear on third larval instar. In the third larval instar, pinacula and setae were more visible and the upper surface (dorsal area) of the larval body started to become brownish in color and with white lateral lines (Fig.3).Young larvae were observed with similar action of semi-loopers in walking. Meanwhile, the measurement of head capsule width ranging from 0.39±0.01 mm to 0.91±0.01 and total larval length were 2.41±0.09 to 8.30±0.21 in young larval instars from L1 to L3. Early larval instars possess small black spots from which primary setae protrude; however later instars were lacking primary setae and generally smooth (Fig. 4). Meanwhile, the measurement length of head capsule ranging from 1.76 ±0.12 mm to 2.60±0.02 and the total larval length was 13.73 ± 0.53 to 33.08 ± 0.67 mm in mature larval instar from L4 to L6 (Table 2).

Table-2. Head capsule width and larval body length of *S. frugiperda* (L1-L6)

Larval instars	Head capsule width (mm)	Total larval length(mm)
L_1	0.39±0.01	2.41±0.09
L_2	0.65±0.04	4.63±0.27
L_3	0.91±0.01	8.30±0.21
L_4	1.76±0.12	13.73±0.53
L_5	2.36±0.15	19.60±0.92
L_6	2.60±0.02	33.08±0.67

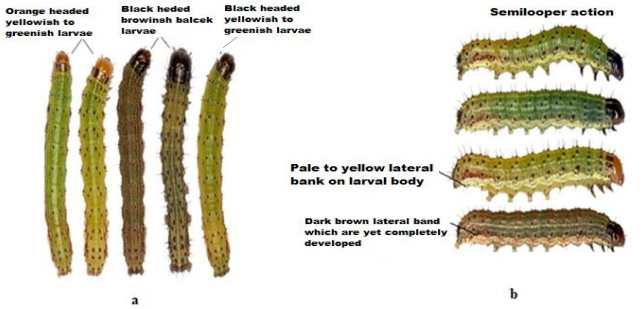


Figure-3. Young larvae of *S. frugiperda* (a. dorsal b. lateral view)

Mature larvae (L4 to L6)

The color of FAW in the late stages was mostly brownish but may include variations of light to dark brown and sometimes greenish to blackish. In case of overcrowding, the darker colors of mature larvae were noticed. The dorsal surface spots of the larval body were not always equally apparent even on

mature larvae like young larvae. The lower lateral side bands completely appeared from fourth instar and were lighter in color with a brown or black band just above the light pale to yellow thick line (in parallel). However, the general appearance of mature larvae (4th to 6th instar) was brown, blackish, or greenish in color and still the head was black, brown, and orange in color. Larger caterpillars were never observed walking with a looping action. Raised spots appeared prominently on the dorsal surface of mature larvae; they were usually dark in color and bear spines.

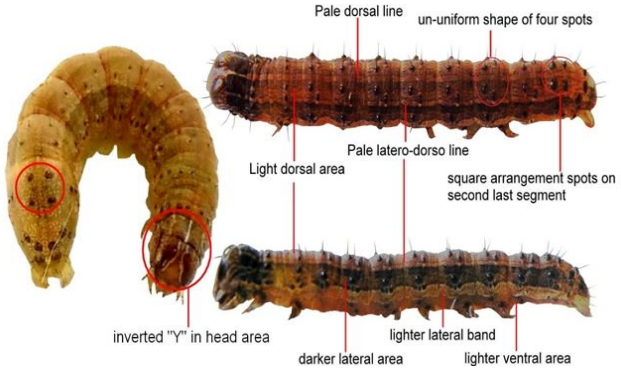


Figure-4. All larval instars of *S. frugiperda* from 1st to 6th instars

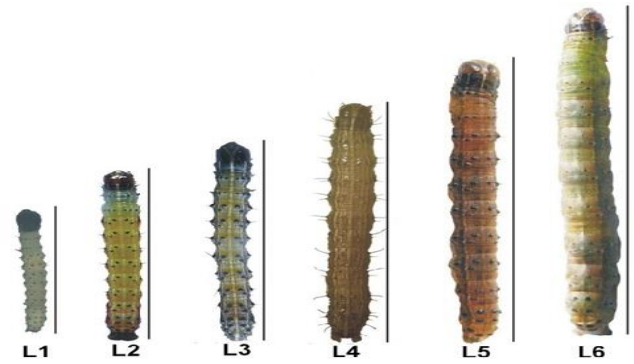


Figure-5. Most prominent characteristics of FAW in mature larva showing square dots on the 2nd last segment of body and inverse Y shape on the head

The larger caterpillars were in more characteristic marks and spots. The face of the mature larva was with an obvious mark of inverted white "Y" shape and the epidermis of the larva seemed rough or granular in texture when examined closely in 5th to 6th instars larvae but soft in touch. The best identifying feature of the FAW was a set of four large spots that formed a square on the upper surface of the second last segment of the mature larval body (Fig. 5). In addition, pale dorsal line on center of larval body, pale to yellow latero-dorsal lines, light dorsal and ventral light area, parallel dark black to brown and pale to yellow bands, less uniformed four spots on each segment completely developed and apparent on 5th and 6th larval instars of FAW.

Pupal Stage

The fully-grown larvae stopped feeding during the pre-pupal stage and started dwindling and creating cocoons. The pupa was reddish brown in color about 14.14±0.31mm in length. Two thin spikes known as cremaster at bottom (Fig. 6) were present which are common in pupa of order Lepidoptera.

Figure-6. Pupal length and cremaster

Discussion

Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) is a serious pest of maize and presently invasive in the Sub-continent. After confirmation of its first report from Karnataka city (India) by Kalleshwaraswamy et al. (2018), a big threat has generated for its neighbor countries as this pest keeps invasive behavior. Pakistan being adjacent to India had a great chance of invasion and requires special attention to report and manage this pest. In this connection, a survey of maize crop was conducted, and insect specimens were collected which later brought and reared at an entomological laboratory for proper identification. The orthodox method of insect identification is mostly carried out through its male genitalia and other morphological characters like wings (male and female). Furthermore, larval characteristics are also imperative to know as species belonging to order *Spodoptera* are complex and require close and detailed observation. Therefore, this study reports an occurrence of FAW in Pakistan particularly in few districts of southern Sindh. In dissection of male genitalia, coremata was observed with a single lobe

that confirmed the identification of FAW. According to Brambila (2009), if genitalia have only one lobe then specimen is neither *S. litura* (F.) nor *S. littoralis* (B.). Such findings suggest that single lobed coremata can be only found in very few *Spodoptera* species i.e. *S. frugiperda*, *S. eridania* (S.), *S. albula* (W.) and *S. exigua* (H.) as compared to *S. litura* and *S. littoralis* which possess two lobe coremata. Similar results regarding single lobe of coremata in male genitalia of *S. frugiperda* were previously presented by some other researchers (Kalleshwaraswamy et al., 2018). For reference, we also dissected male genitalia of *S. litura* collected from the cotton crop to make our arguments stronger regarding identification of *S. frugiperda* through male genitalia while studying the cormeta lobe. The species clearly indicated two lobes of *S. litura*, not quadrate ampula valves and costal process was most projected as compared to *S. frugiperda* (Fig.7). These findings clearly indicate that both these species are quite different and existence of invasive alien species *S. frugiperda* is obvious in the region.

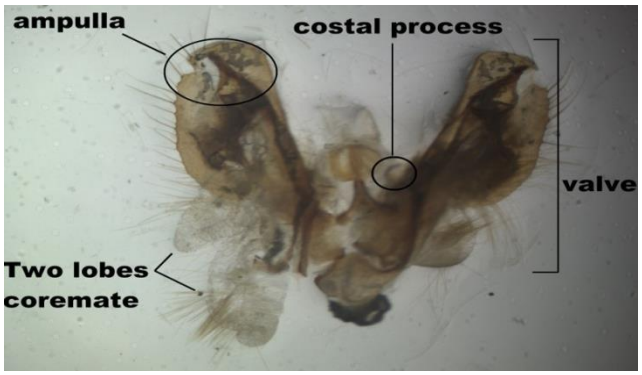


Figure-7. Referred diagram of dissected male genitalia of *S. litura* in comparison of *S. frugiperda* showing clearly two coremata lobes.

In addition, the structure of complete male genitalia of FAW in this study was the same as described in handouts on male genitalia of *S. frugiperda* (Kalleshwaraswamy et al., 2018). According to this description male genitalia were with two valves, both broad and quadrate in shape with curved ampulla and pointed at top. At the base of both valves, clavus was short with costal process narrow, straight, elongate, and inclined. Juxta was concave at base and with dorsal process. After verification of these parts, it was revealed that the collected species was *S. frugiperda*. However, male genitalia of *S. litura* was with small costal process, cucullus truncate and juxta triangular with a narrow base whereas in *S. eridania* clavus was absent, costal process was with very small bump (EPPO, 2015). Apart from male genitalia, wing patterns in adult moths were also observed of *S. frugiperda* in the present study. In both adults (male and female), wing patterns were different in forewings and such patterns were also varied from *S. litura*. The males have brown shaded forewing with clearly visible white spots in triangular shape and row of small hour-glass shape marking near apical margin. However, the hind wings of both adult moths were with silver-white marking and a fine border of golden color. These results are in line with Sidana et al. (2018) who described that forewings of male adults were light brown, oblique orbicular spots with few contrasting transverse lines. Similarly, Brambila (2013) also stated that forewings of native moths from America including *S. frugiperda* with unclear kidney shape spot, slightly in black outline and small v-shaped markings. Prasanna et al. (2018) studied the measurement of wingspan and suggested that wingspans of FAW were 32 to 40 mm in length. The male moth of *S. litura* was with a white fork made of veins in the outer median area of the wing and yellowish area at the inner median area. *S. frugiperda* can be confused with males of other species of *Spodoptera* i.e. African armyworm *S. exempta* (Walker) and *S. ornithogalli* (Guenee) (EPPO, 2015; FAO-CABI, 2019).

The morphological characters of forewings reported earlier for *S. frugiperda* are like our findings. Wing patterns in many species of genus *Spodoptera* are almost similar, that is why genitalia dissection is necessary for exact identification of required species as did in present study. In the observation of immature larvae, it was figured out that initial instars were without any identification signs. EPPO (2015) suggested that "Young larvae of *S. frugiperda* are hard to identify morphologically because early instars of several other noctuids are very similar. FAO-CABI (2019) reported that the head of the mature larvae was with obvious mark of square inverted white "Y" shape and a set of 4 square shape spots on the second last segment of mature larval body and similar signs were identified in present study but this Y shape appeared in third instar larvae and four dots also appeared on each segment of larval body with varied shape from square to less rectangle shape. Furthermore, in *S. exempta* and *S. litura* resemblance with *S.*

frugiperda in terms of color, longitudinal lateral stripes and inverted "Y" mark on the head but it lacks a set of four rectangular dark spots of the second last segment. Morphometric study of Prasanna et al. (2018) showed dissimilarity in the length of larvae whereas results were similar in terms of head capsule width.

Conclusion

This preliminary survey at southern Sindh, Pakistan showed an apprehension of the entry of one more new invasive pest belonging to genus *Spodoptera* is known as Fall Armyworm (FAW), *S. frugiperda* (J. E. Smith) in April 2019. This report regarding identification of FAW has been made based on morphological characteristics and the key previously provided by CABI bioscience and FAO.

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Contribution of Authors

Bhatti Z: Designed research methodology
Ahmed AM: Designed research methodology
Khatri I: Guided the student for identification of species through male genitalia
Rattar QA: Assisted the first author in preparation of this manuscript and advised for technical writing
Rajput S: Guided the student for identification of species through male genitalia
Tofique M: Helped the post graduate student during his research in laboratory and field
Younas H: Helped the post graduate students during his research in laboratory and field