

NEW OCCURRENCE OF THE *SPODOPTERA LITURA* (FABRICIUS)
(LEPIDOPTERA: NOCTUIDAE) INFESTATION ON COCOA IN INDIA

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ABSTRACT. *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae) is a highly polyphagous insect pest, which inflicts serious economic damage on many agricultural and horticultural crops globally. During our recent surveillance surveys, an unusual defoliation was noticed by a caterpillar pest on cocoa saplings at ICAR - Central Plantation Crops Research Institute, Regional Station, Vittal farm, Karnataka, India. Morphological identification and molecular characterization using mitochondrial cytochrome-c-oxidase (*COI*) gene confirmed the collected insect samples as *S. litura*. Hitherto, this species was only known to infest *Leucaena leucocephala* (CABI), a shade tree of cocoa plantations in Indonesia. To the best of our knowledge, this is the first record of infestation by tobacco cut worm on cocoa in India. Consequently, solarization of rooting medium has been suggested for the destruction of the quiescent pupal stage.

Additional key words: Polyphagous pest, identification, morphology, molecular characterization, management

Cocoa (*Theobroma cacao* L.: Malvaceae) is an important commercial perennial crop known for its beans to produce chocolates and is native to South America, specifically in the region of American rainforest (Ploetz 2007). It is widely cultivated in the humid tropical countries around the world (WCF 2014); however, maximum production comes from West Africa, Latin America and South East Asia (Lahive et al. 2019). In India, it is cultivated approximately in 97,000 hectares and produce around 25,800 million tonnes annually (DCCD 2022); production concentrated in Southern states of India like Karnataka, Kerala, Tamil Nadu and Andhra Pradesh (DCCD 2022, Beg et al. 2017). Meanwhile, cocoa production represents an important breadwinning activity for cultivators, entrepreneurs and traders worldwide (Sujatha et al. 2018, Apshara 2019, Voora et al. 2019).

The global demand for cocoa has increased from past 15 years, leading to an expansion of cultivated area and intensification of production (WCF 2014, Thoithimihaly et al. 2019). However, there are several production constraints such as insect pests, diseases, nutrition and climatic conditions limiting its productivity. However, insect pests are the major factor affecting cocoa production in India (Thube et al. 2019). There are more than 50 insect pests including sap feeders, defoliators, capsule and wood borers known to affect its production globally (Entwistle 1972, Mohan et al. 2022, Thube et al. 2022). As a commercial crop, cocoa planting materials were distributed to several places of the world and thus may favor the occurrence and spread of pest and disease in cocoa plantation (Ploetz 2016, Mujica Mota et al. 2019). More often, the insect

pests can adapt and feed on new host plants or closely related host species due to change in climatic conditions for survival and to meet their nutritional demand for growth and development (Anim-Kwapong & Frimpong 2008, Buxton 2020). In addition, possibility of occurrence of new insect pests on different crops is not unusual in agricultural eco-system (Lastuvka 2009). In September 2022, a sudden occurrence of defoliator pest on cocoa saplings causing moderate damage was observed in ICAR — Central Plantation Crops Research Institute (CPCRI), Regional Station, Vittal, Karnataka, India. However, the preliminary investigation on the morphology of the pest, revealed defoliation due to noctuid caterpillar, *Spodoptera litura*.

Spodoptera litura (Fab.) (Lepidoptera: Noctuidae) is a global insect pest on agricultural crops and referred by many names such as tobacco cutworm, tropical army worm and leaf caterpillar. More than 35 species of genus *Spodoptera* were recorded mainly in the tropics and subtropics (Porgue 2002). The emergence of the exotic fall army worm, *S. frugiperda* has threatened the production potential of maize in several parts of the country adds greater significance to establish the identity as well. Being a highly polyphagous, *S. litura* is able to cause significant damage to many important crops such as tobacco, cotton, pulses, oil seeds, vegetables and many more (Gao et al. 2004, Shahout et al. 2011, Ahmad et al. 2013). Till date, it has been recorded from 290 host plants belonging to 99 families due to its greater adaptability under diverse climatic conditions and long-distance migratory potential (Garad et al. 1984, Wu et al. 2004, Dhaliwal et al. 2010, Fu et al. 2015). In India, it is an important insect pest



FIG. 1. *Spodoptera litura* infestation on Cocoa. (a) larvae feeding on leaf lamina (b) devoured entire leaf leaving mid rib.

which inflicts serious damage in many agricultural crops causing economic loss from 26% to 100% (Dhir et al. 1992, Vijayalakshmi et al. 2016). In addition to its aggressive feeding potential, it can destroy young seedlings, hibernate in soil and other substrates during transportation across national and international borders. Furthermore, the favorable environmental conditions at Vittal region (Karnataka, India) might have supported its establishment on the new host plant after introduction.

Previously, its presence was documented on *Leucaena leucocephala*, which is a shade tree of cocoa plantations in Indonesia (CABI 2018). However, no other studies have reported its occurrence on cocoa. Here we report for the first time its occurrence and infestation on cocoa from India. Furthermore, this knowledge would facilitate to understand its host plant selection behaviour and implementation of suitable management strategies to limit its spread and establishment.

MATERIALS AND METHODS

Sample collection

Samples were collected on cocoa nursery in September 2022 at ICAR - CPCRI, Regional Station,

Vittal, Karnataka, India (12.7704° N, 75.1136° E). The cocoa saplings were preferentially inspected based on the presence of feeding damage such as skeletonization of leaves at younger stage and extensive defoliation in the matured stage (Fig. 1). In addition, the selected cocoa canopy trees were also visually inspected in the farm for the presence of *S. litura* life stages if any. The larvae were collected using a fine camel brush and transferred into the plastic boxes (60 x 30 cm) containing fresh cocoa leaves and brought to the laboratory for morphological and molecular studies.

Morphological identification

A detailed morphological description of suspect insect was conducted at Division of Entomology laboratory. The sampled insects (caterpillar) were studied under stereomicroscope (Leica MZ 12.5) and their images were captured with a camera mounted in a system at magnifications from $\times 20$ to $\times 62.5$ with motorized z-axis drive and inbuilt mounting software (Leica MC 170 HD). The specimens were identified based on the morphological characteristics (EPPO 2015), following which a part of insects was transferred to plastic containers (60 x 30 cm), and those reared by offering fresh cocoa leaves were allowed for pupation. A

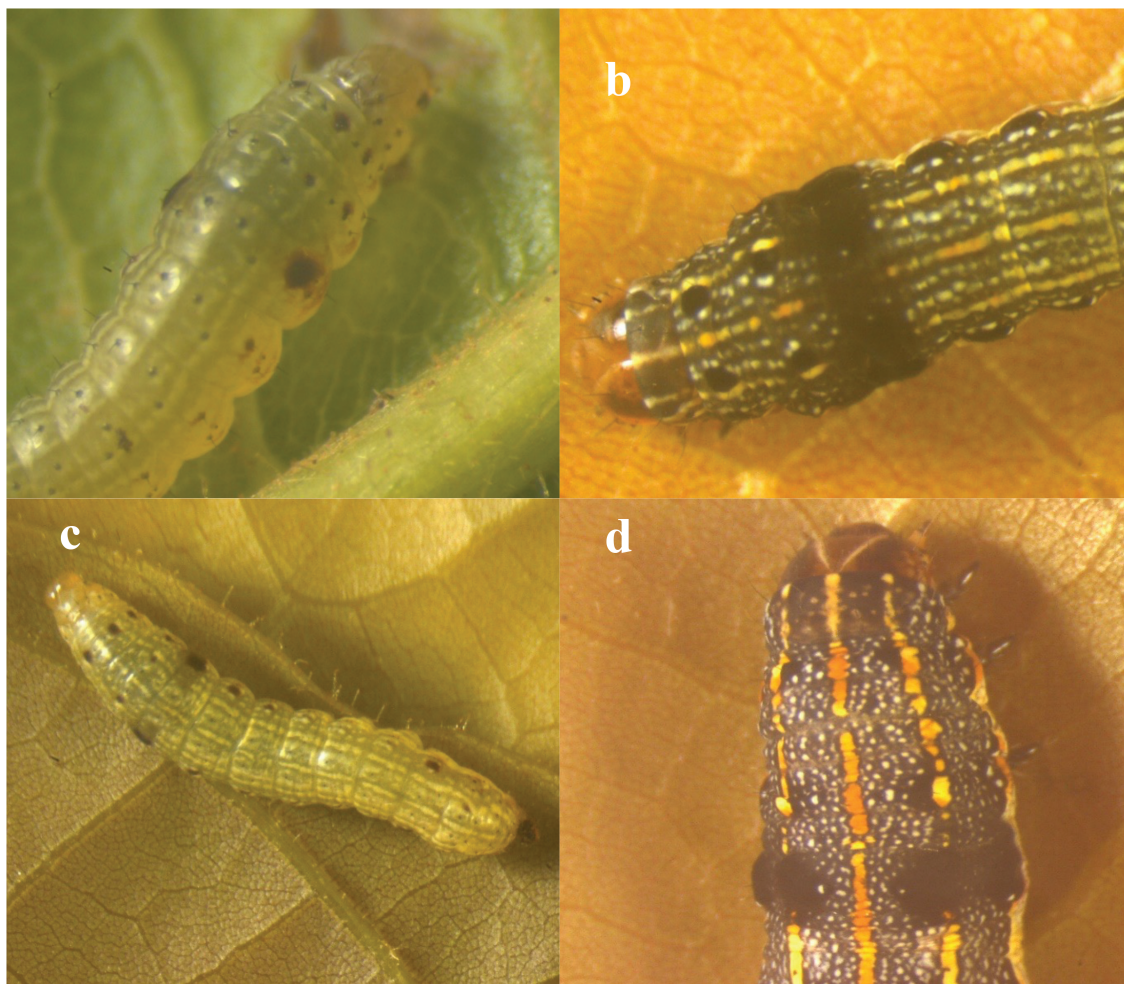


FIG. 2. *Spodoptera litura* larvae. (a) amber-brown spot on 1st abdominal segment (b) yellow dot in black dark spots of meso and meta thorax (c) yellow mid dorsal line on the abdomen (d) prominent Y shaped suture on head

subset of pupae was maintained and pupal morphological features were also recorded. Upon moth emergence, morphological identification of *S. litura* adults was carried out as described by Brambila (2013). The identified specimens of *S. litura* adults from India on cocoa were kept as a voucher specimen at ICAR-CPCRI, RS, vittal insect repository (ID VTL311).

Molecular characterization

Morphologically identified larvae and adult specimens were subjected to molecular characterization of mitochondrial cytochrome c oxidase I (*COI*) gene for species level identification. Total insect DNA was extracted from legs and antennae of adults and entire larvae using Qiagen DNeasy Blood and Tissue Kit® (QIAGEN Strasse 1, Hilden, Germany), following the manufacturer's protocols.

Polymerase Chain Reaction (PCR) was performed to amplify the partial *COI* gene using a 50 μ l reaction volume containing 5.0 μ l Taq Buffer A (Genei labs Pvt. Ltd., Bengaluru, India), 2.0 μ l each of the forward (LCO1490 '5-GGTCAACAAATCATAAAGATATTGG-3') and reverse (HCO2198 '5-TAAACTTCAGGGT GACCAAAAAATCA-3') primers (10 pmol/l) (Folmer et al. 1994), 2.0 μ l 10mM dNTP's, 1.0 μ l Taq DNA polymerase (5U/l), 2.0 μ l DNA and 36.0 μ l sterile distilled water. Thermo-cycling (Bio-Rad T100, Hercules, CA, USA) consisted of an initial denaturation at 94°C for 3 min, followed by 30 cycles of denaturation at 94°C for 20 s, annealing at 53°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 5 min. The amplified products were verified by electrophoresis on an agarose gel (1.2%) (Sambrook,

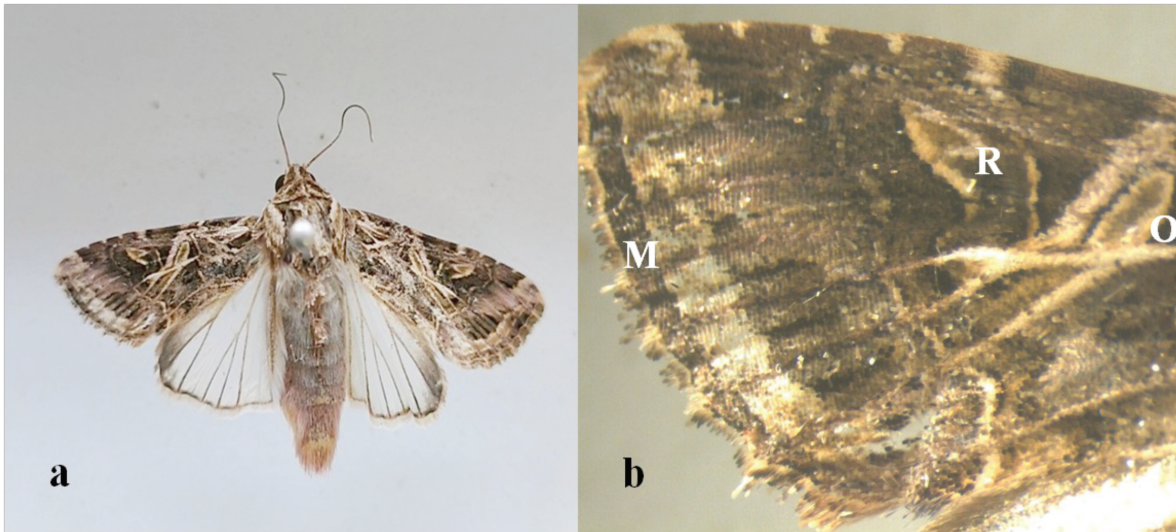


FIG. 3. (a) *Spodoptera litura* moth. (b) Forewing morphological features; presence of reniform spot (R) elongated orbicular spot with a white margin (O), a row of brown hour-glass markings on the outer margin (M).

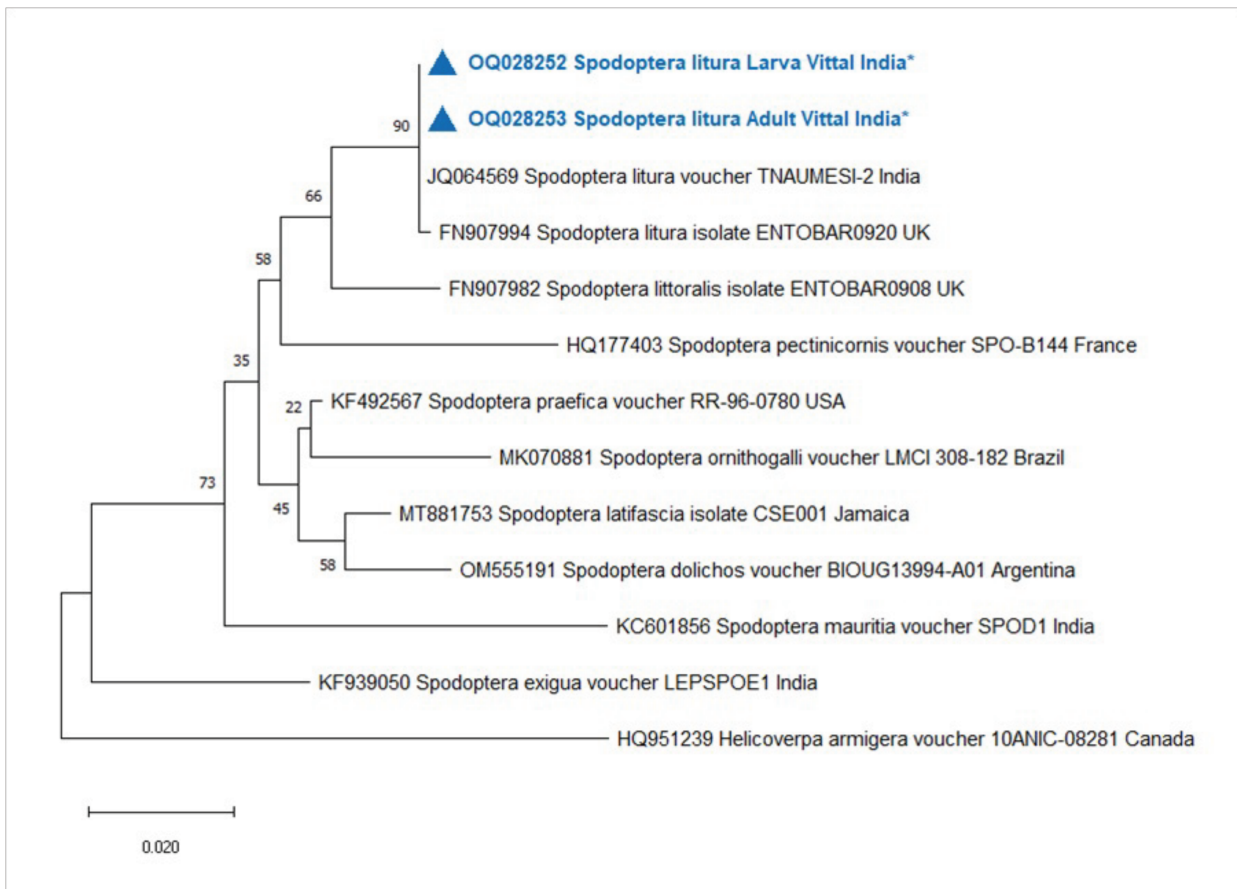


FIG. 4. Phylogenetic tree depicting relationships derived from 13 mtCOI sequences. 2 sequences from specimens collected on cocoa. 10 sequences from all the *Spodoptera* species available in the Genbank; 1 species from an outgroup species (*Helicoverpa armigera*). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap is shown next to the branches.

2001). PCR amplified products were sent for Sanger sequencing for species identification (Biokart India Pvt. Ltd., Bengaluru, India).

The sequenced results were aligned using BioEdit (Biological sequence alignment editor —Tom Hall, <http://www.mbio.ncsu.edu/BioEdit/bioedit.html>) and each sequence was BLAST searched in Barcode of Life Data (BOLD) database (<http://www.boldsystems.org/>) for a species match. The obtained sequences were deposited in NCBI database. Further, Phylogenetic analysis was performed using MEGA software (t) for both larvae and adult sequences.

RESULTS AND DISCUSSION

Field detection and morphological identification

At sampled nursery areas, *S. litura* larvae were observed feeding on young and matured cocoa leaves, 'windowing' (scraping lower surface leaving upper epidermis) in the initial stage; 'skeletonization' (eating soft lamina tissue leaving mid-rib) in later stage and causing extensive defoliation of cocoa leaves when larva at matured stage (fig. 1). More than 70% cocoa saplings were infested by *S. litura* in the experimental station during the month of September and October when the cocoa seedling production was at its peak.

Spodoptera species are highly variable in color pattern both within and between populations; thus, it requires more attention to decipher all morphological features for precise identification at species level. However, several authors provided larval description of economically important *Spodoptera* species across the world on various hosts for taxonomic identification (Dewhust 1975, Passoa 1991, Lalanne-Cassou et al. 1994, Zenker et al. 2007). The collected insect sample (larvae) were identified as *S. litura* based on the following morphological characteristics. The early instars larvae are pale green to dark green color, measuring 1–8 mm long and presence of amber-brown colored spot in the first abdominal segment (Fig. 2a). As the larvae matured, yellow to white dot appeared in the black dorsal spots on the meso and meta-thoracic segments (Fig 2b); the mid dorsal line becomes prominent and often turn to bright yellow or orange color (Fig 2c). Late instars larvae were greenish to brown, measuring 40 to 45 mm long, the head was brown to black with reticulate pattern on the lateral side and presence of prominent Y shape suture on the head/thorax shield (Fig 2d). Further, we observed a conspicuous dark patch on the dorsal side of abdominal segments (1–8) and often have a bright yellow mid dorsal line; sometimes, dark colored patches observed on the meso and meta-thoracic segments but smaller

than first abdominal segment spot. However, in adult moth forewing consists of brown colored reniform spot, outlined in white with a white margined light brown area at apex, elongated orbicular spot with a white margin, a row of brown hour-glass markings on the outer margin and light-yellowish or light-brown patches on the median area (Fig 3). Whereas in *S. frugiperda*, reniform spot is indistinct and orbicular spot is brown, oval and not as narrow as in the *S. litura*. Furthermore, we have also observed important genital features of *S. litura* including presence of two windows separated at right angle, Ampulla was slightly curved, triangular juxta with a narrow base and coremata with two lobes. Sometimes, larvae of *S. littoralis* closely resembled as *S. litura*; consequently, molecular studies are needed for reliable identification.

Molecular studies

Analysis of the partial *COI* nucleotide sequence derived from both larvae and adults were 100% identical to each other and are deposited in NCBI (GenBank OQ028252 and OQ028253). The DNA sequences of larvae and adults had 100% nucleotide homogeneity with the *COI* DNA sequences of *S. litura* (GenBank JQ064569 and FN907994) available in the BOLD database (). Further, phylogenetic analysis revealed that larvae and adult *COI* nucleotide sequences formed a separate sub clade along with *S. litura* sequences reported from other countries and different from that of *S. littoralis*, a closely species to *S. litura* (Fig. 4). The systematic studies of morphological and molecular characterization of the new insect pest on cocoa, thus confirmed as *S. litura*.

Cocoa is widely known as a 'chocolate tree'; and it provides raw materials for chocolate and pharmaceutical industries. Although cocoa production in India has been expanding for the last few years, there is a scarcity of information on the occurrence and distribution of new pest and diseases. The new epidemic outbreak of *S. litura* on cocoa could be the cause of concern on cocoa production and their allied industries due to its destructive feeding nature. Therefore, the study reported here should trigger researchers in India for systematic monitoring of this pest on cocoa in national level. Furthermore, studies on its bionomics and development of effective management strategies are crucial before it becomes endemic. Incorporation of available pest control strategies to reduce the insect load (larva) along with solarization of the planting medium is therefore mandatory to destroy the pupal stages if any present so as to arrest the pest establishment in cocoa nursery.

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