Original Research

Journal website : www.jeb.co.in ★ E-mail : editor@jeb.co.in

Journal of Environmental Biology

JEB™ p-ISSN: 0254-8704
e-ISSN: 2394-0379
CODEN: JEBIDP

DOI : http://doi.org/10.22438/jeb/42/2/MRN-1512

Occurrence of *Pyrausta panopealis* on sweet basil *Ocimum basilicum* in India

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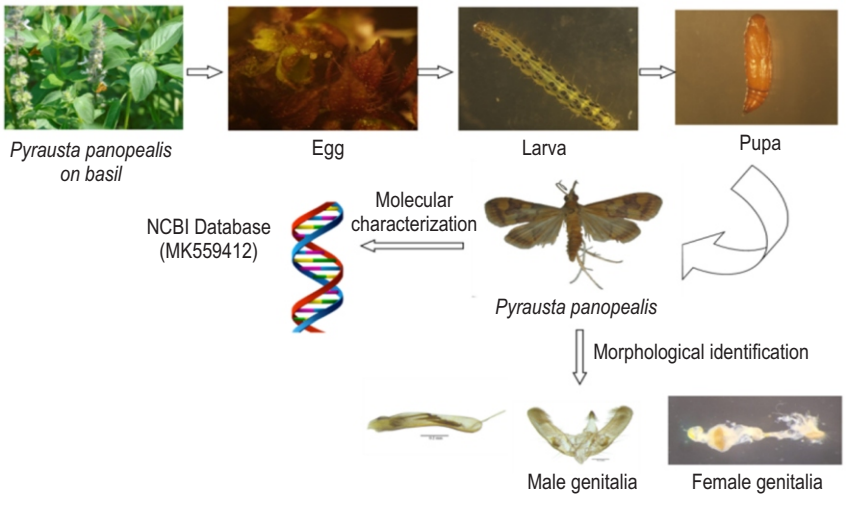
Received: 12.05.2020 *Received*: 25.08.2020 *Accepted*: 23.10.2020

Abstract

Aim: The present study aimed at reporting the occurrence of pest, *Pyrausta panopealis* on sweet basil *Ocimum basilicum*. The biological parameters and population dynamics of the pest was studied on its newly reported host plant.

Methodology: Description of *P. panopealis* was done using morphological and molecular approaches. Male and female genitalia of adults were dissected for morphological identification. DNA was isolated from insect sample (adult legs) and amplified using mtCOI primers (LCO 1490; HCO 2198). The larva of *P. panopealis* was reared on sweet basil under laboratory conditions for observing the growth parameters.



Results: Male genitalia with distinct spoon-shaped clasper; female genitalia corpus bursae with appendix bursa. The sequenced DNA product was submitted to NCBI- GenBank (Accession No: Mk559412). The mean incubation period, larval period and pupal period of the pest was 3.19 days; 13.81 days; 5.40 and 6.27 days. The total life span of male and female insects ranged from 22 to 33 days and 24 to 35 days, respectively. The fecundity of adult varied from 91 to 131 (average 111.75 eggs). The peak population of pest (11.5 larvae/ plant) was recorded during 32nd standard meteorological week, during which all the observed plants were infested by larvae of *P. panopealis*.

Interpretation: This is the first report of association between *P. panopealis* and sweet basil. The data on life cycle parameters, seasonal incidence and severity can be used for developing integrated pest management strategies to *P. panopealis* on sweet basil.

Key words: Genitalia, Perilla leaf moth, *Pyrausta panopealis*, Seasonal incidence, Sweet basil

How to cite : Shivakumara, K.T., T. Venkatesan, M.C. Keerthi, P.R. Shashank, N. Pradeeksha, A.C. Polaiah, R.N. Reddy, P.L. Saran and P. Manivel: Occurrence of *Pyrausta panopealis* on sweet basil *Ocimum basilicum* in India. *J. Environ. Biol.*, **42**, 265-270 (2021).

Introduction

Basil popularly known as "Sweet basil", (*Ocimum basilicum* L.) is used in both Ayurvedic and Unani systems of medicine. It is widely grown as an aromatic crop and used as a culinary herb or spice or condiment or as an ornamental plant (Simon *et al.,* 1990). The herb is indigenous to lower hills of Punjab and Himachal Pradesh and in India, and is cultivated over an area of 25,000 ha and accounts for around 250-300 tons of essential oil annually (Smitha *et al.,* 2014). The economical parts of sweet basil are its leaves and seeds, from which essential oil is extracted and used as flavoring agent in food, perfumery and pharmaceutical industries (Simon *et al.,* 1990). The flavor and smell of sweet basil variety is largely determined by the presence of chemical components like cinnamate, citronellol, geraniol, linalool, methyl chavicol, myrcene, pinene, ocimene and terpinol in the essential oil. The essential oil from sweet basil has antioxidants (Lee *et al.,* 2005), antimicrobial (Koba *et al.,* 2008), antifungal and insect repelling properties (Dube *et al.,* 1989). Herb and essential oil have anti-depressant, anti-microbial and anti-oxidative properties (Muralidharan and Dhananjayan, 2004).

While the demand of essential oil in the aromatic industry is increasing, there is a growing concern about improving the production and quality of sweet basil (Smitha *et al.,* 2014), hence, efforts should be made to reduce the loss due to biotic and abiotic factors (Gahukar, 2018). Sweet basil has been found to be infested by more than 30 species of insects and mites, but only a limited number of insect species have been reported to cause serious damage (Hamasaki *et al.,* 1994; Dhiman and Datta, 2013). The perilla leaf moth, *Pyrausta panopealis* Walker (Lepidoptera: Pyralidae) is distributed in South-East Asia including China, Japan and India, and also in South America (Oh *et al.,* 2010). The insect has earlier been reported to feed on Labiatae group of plants (Smedley *et al.,* 1990), however, in India the pest was observed and reported based on the light trap collection (Gurule and Nikam, 2013; Raha *et al.,* 2017). As reports on its host range, biology and incidence of the pest are meagre; the present study was carried out to investigate the pest inflicting severe foliar damage to sweet basil. Furthermore, the details of pest identification, occurrence, biology and seasonal incidence of pest on sweet basil were also investigated.

Materials and Methods

The present investigation was conducted during 2018-19 at the ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India.

Morphology based identification of *Perilla moth*: The later instar larva of perilla leaf moth were brought to the laboratory and held for the emergence of adults. For genitalia preparation, the abdomen of male and female adults was separated from the thorax and placed in a test tube containing 10% KOH solution for maceration. The abdomen was then transferred to a cavity block containing water and washed repeatedly to remove excess KOH. The genitalia were dissected and compared with earlier findings (Landry, 2015). Photographs of adults were taken using an Olympus E330-ADU1.2X mounted on an Olympus SZX16 stereo zoom microscope. Genitalia photographs were taken using a Leica DFC 425 mounted on a Leica M205C.

Molecular characterization of Perilla moth

DNA extraction: Genomic DNA was isolated from Perilla moth legs with a DNA extraction kit (QIAGEN DNeasy, Germany), following the manufacturer's protocols. Isolated genomic DNA was used for amplification of mitochondrial COXI gene with primer pair of forward primer (LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer (HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') following standard protocol (Folmer *et al.,* 1994).

Polymerase Chain Reaction (BioRad C1000™) was carried out in flat-capped 200 µl volume PCR tubes (Tarsons, Kolkata, India). A 50 µl reaction volume contained: 5 µl Taq buffer, 1 µl 10 mM dNTP mix, 1 µl (20 pmol μl^{-1}) forward primer, 1 µl (20 pmol μl^{-1}) reverse primer, 1 µl Taq DNA polymerase (1 U μl^{-1}), 5 µl DNA (50 ng μl^{-1}), and 36 µl sterile water. PCR was performed by following conditions *viz.,* 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. The amplified products were separated on 1.5 % agarose gel electrophoresis. The amplified products were sequenced by M/s Chromous Biotech Ltd., Bengaluru. Partial sequence of current study isolate was deposited in NCBI GenBank Database and compared with available sequence in NCBI database.

Biology: The larvae of perilla leaf moth were collected from the field and reared on leaves of sweet basil under controlled conditions to establish a laboratory colony. The adults were collected and released to rearing box (26×20×20 cm) provided with 10% honey solution. The eggs were collected and kept in Petri dish (15×16×2.5 cm) and observed at 3 hr interval for hatching. After hatching, the larvae were released individually in Petri dish containing fresh sweet basil leaf and the food was changed on a daily basis. The biological parameters *viz.,* incubation period, instar and total larval duration, pre-pupal period (n=30), pupal period (n=20) and also the biological characteristics of adults such as fecundity (n=10) and adult longevity (n=20) were recorded. The head capsule width of each instar larvae (n=20) was measured with ocular micrometre.

Seasonal incidence: The adult perilla moth activity was observed on basil during from 41st standard metrological weeks (SMW) to 48th SMW of 2018 and from 25th SMW to 34th SMW of 2019. Altogether, 30 randomly selected plants were taken for observing the pest incidence. The data collected were pooled and analysed. Further, the incidence was correlated with weather parameters like temperature (maximum and minimum), relative humidity (morning and evening) and rainfall that prevailed during observation.

Statistical analysis: Correlation and regression coefficients were derived using SAS 9.5 software SAS (2010). All other statistical analyses were performed with Microsoft Excel 2010.

Results and Discussion

Morphological characters of male and females of *P. panopealis* were studied. Forewings of adults were orange yellow, with broad ragged red-brown lines across them. Fore wings with an outwardly-oblique sinuous antemedial line; a sinuous post medial line highly excursed between veins 6 and 2 with a broadband of crimson suffusion inside joining the antemedial line at inner margin. A diffused crimson sub marginal band and marginal line. The hind wings were red-brown with broad splotchy deep yellow bands across them and the apical area more or less suffused with dull crimson or fuscous, narrowing to vein 2. The hind wings of females were generally darker than that of males. Male genitalia with distinct spoon-shaped clasper; corpus bursae of female genitalia with appendix bursae was noted (Fig. 1). Genitalia of male and female dissected was similar to the earlier report of Landry (2015), strongly suggesting that the species was *P. panopealis*. The COI generated sequences showed 100% similarity to *Pyrausta panopealis* (Walker, 1859) was then deposited in NCBI GenBank database and accession number was obtained (MK559412).

The growth parameters of egg, larvae, pupae, and adults were recorded and is in presented (Table 1). The adult insects were found to lay eggs singly as well as in groups on flower buds, shoots, petiole and on lower surface of the leaf. Freshly laid eggs were pale yellow in colour and had a flat base. The dark head of young larva was observed inside the shell of eggs one day prior to hatching. The incubation period ranged from 2- 4 days with an average of 3.19 ± 0.53 days. During its developmental period, the caterpillar moulted four times and had five larval instars. The total larval period ranged from 12-16 days with an average of 13.81±1.18 days (Table 1). The newly hatched larvae were active and light white in colour with dark head. The larval body was covered with a number of minute hairs. As the larva advanced to second instar, the body colour slightly changed to pale white to light yellow. The third instar larva was morphologically similar to that of second instar with a prominent light block spot on either side of the abdomen. However, the larva was light yellowish to green colour. The fourth instar larva was golden yellowish in colour. Initial two days of fifth instar larvae were yellowish-green in colour with conspicuous dark spots. However, a day before entering into pupal stage, the colour of larvae changed to brick red. The mean head capsule width of five instars larvae were 0.21, 0.33, 0.48, 0.71 and 0.98 mm, respectively. Once the larval period was completed a loose silken cocoon was constructed in the affected webbed plant parts.

The pupa was elongated and oval in shape. The freshly formed obtect pupa was yellowish and gradually attained brown colour. Total pupal duration varied from 5 to 7 days. The difference between male and female pupae could be easily seen based on

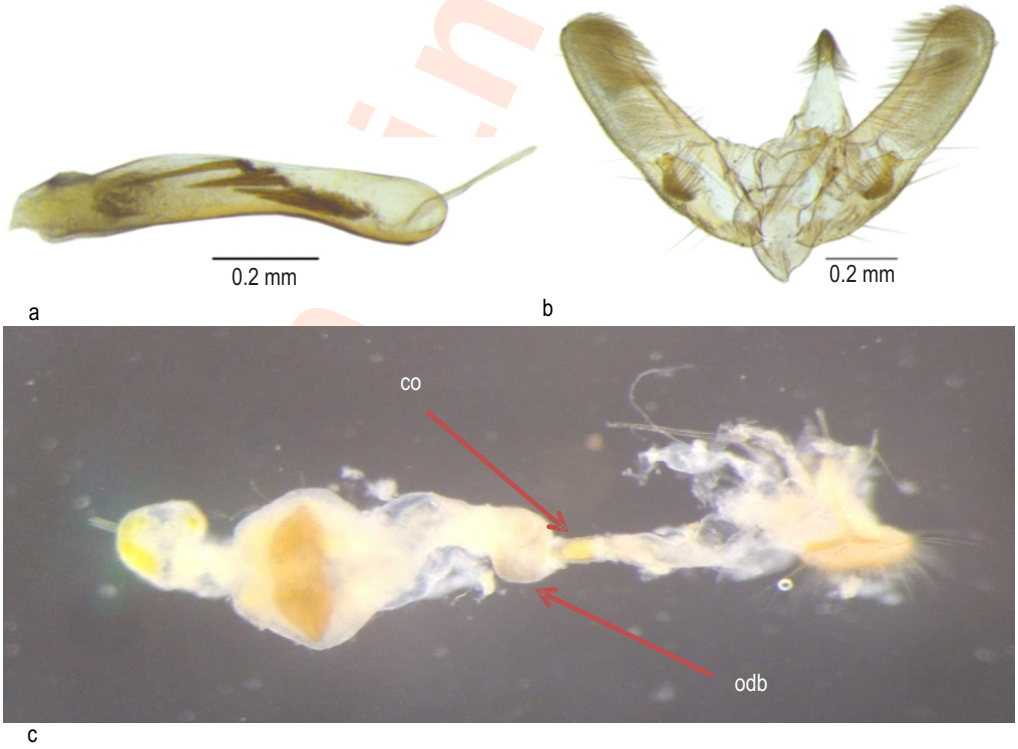


Fig. 1: Male and female genitalia of *Pyrausta panopealis*: (a) Phallus; (b) Genitalia without phallus and (c) Female genitalia; co colliculum and odb outgrowth of ductus bursae.

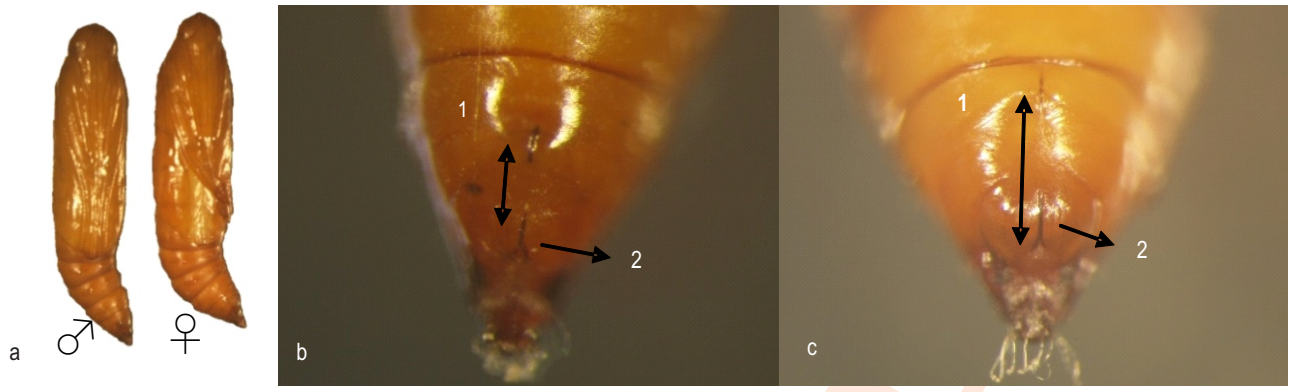


Fig. 2: (a) Male and female pupa of *Pyrausta panopealis*; (b) Male pupa with 1 genitalia, 2 anal slot and (c) Female pupa with 1genitalia, 2 anal slot.

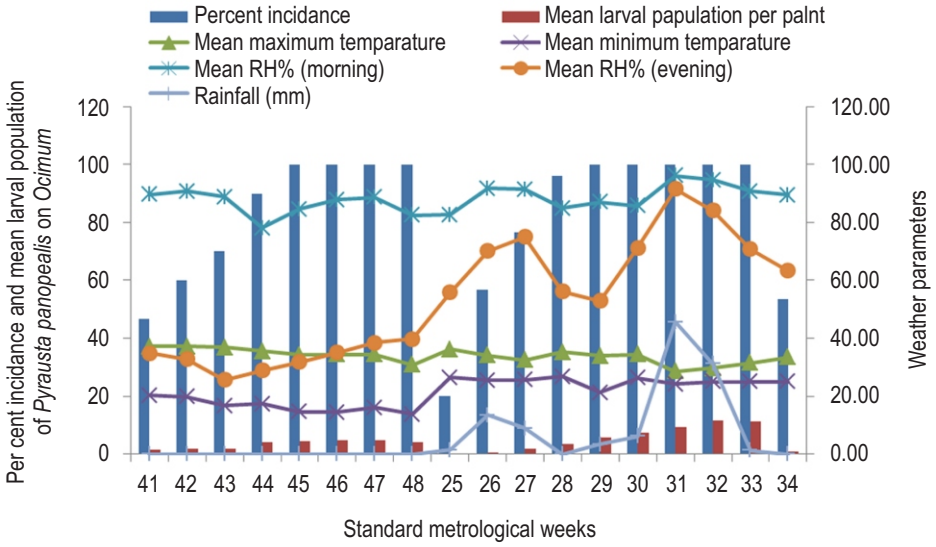


Fig. 3: Seasonal incidence of *Pyrausta panopealis* on *Ocimum basilicum*.

the size and also the distance between genital opening and anal slot. The gap from each other was more in females than males (Fig. 2). The appearance of male moth was nearly same as female, but it was smaller in size and had pointed abdomen.

The average longevity of adult male was 2.0 to 5.0 days with an average of 3.86 days. The average longevity of adult female was 3.0 to 6.0 days with an average of 4.40 days. The total life span of male and female insects ranged from 22 to 33 days (average 27.49) and 24 to 35 days (average, 28.90), respectively. The number of eggs laid by female varied from 91 to 131 with an average of 111.75 eggs. Earlier, *Hyptis capitata* Jacquin (Labiatae) was the only food plant reported against perilla leaf moth in Puerto Rico (Schaus, 1940) and was a most potential host for *P. panopealis* in new and old world tropics (Mabberley, 1987). Further, the endangered scrub balm, *D. frutescens* was found as a new larval host of *P. panopealis* in Florida (Smedley *et al.,* 1990). This was because *Hyptis* and *Dicerandra frutescens* Shinners share a minimum of five Monoterpenes (Luz *et al.,* 1984; Tanowitz *et al.,* 1984; Malan *et al.,* 1988; Eisner *et al.,* 1990) which either singly or in combination play a significant role in oviposition or phagostimulatory activity for *P. panopealis.* Recently, Cochange and Eusebio (2017) merely mentioned *Ocimum basilicum* and *Mentha viridies* as a host plant of *P. panopealis* in Peru, however, they did not study their life history parameters on the reported host. Monoterpenes are known as oviposition stimulants (Fatzinger and Merkel, 1985; Hanula *et al.,* 1985; Leather, 1987) and phagostimulants (Harborne, 1988) for many lepidopteran insects, the presence of one or more similar monoterpenes in sweet basil may result in crop damage. The Perilla leaf moth populations were observed on sweet basil from 41st standard meteorological week (SMW) to 48th SMW during

Table 1: Biological parameters of *Pyrausta panopealis* reared on *Ocimum basilicum*

Life stages	Mean ± SD	Range (days)
Incubation period	3.19 ± 0.53	2- 4
larval period	13.81± 1.18	12- 16
I instar	3.53±0.50	3- 4
II instar	2.21±0.41	2- 3
III instar	2.38±0.48	2- 3
IV instar	2.56±0.49	2- 3
V instar	3.15±0.44	3- 4
Pre pupa	1.22±0.41	1- 2
Pupa		
Male **	5.40 ±0.49	5- 6
Female**	6.27±0.44	6- 7
Adult longevity		
Male **	3.86±1.05	2- 5
Female **	4.40 ±1.07	3- 6
Fecundity *	111.75 ±14.06	91- 131
Total life span		
Male	27.49±3.67	22-33
Female	28.90±3.64	24-35

Mean of 30 observations; * Data of 10 adults; **Data of 20 pupa and 20 adults

2018 whereas population was observed between 25th SMW to 34th SMW during 2019. The incidence of pest varied from 46.66 to 100 % during Rabi season and 20 to 100% during kharif season (Fig. 3). Pooled data on percent incidence and mean larval population of *P. panopealis* on sweet basil analyzed and is presented in Fig. 3. During 2018, severity of pest was 1.56 larvae/ plant during 41st SMW and reached its peak (4.90 larvae per plant) during 47th SMW. A similar trend of population increase was noticed again during 2019, *i.e.,* 0.07 larvae/ plant during 25th SMW and 11.5 larvae/ plant during 32nd SMW. Normally, the incidence was noticed when the crop was 25-30 day old and continued till 90-95 days, when the crop was harvested the pest activity shifted to sweet basil grown in the neighboring plot. Surprisingly, during the study period there were no natural enemies observed on the pest.

Pooled data for both years revealed that the mean maximum temperature (-0.528*) and minimum temperature (-0.293) were negatively correlated with incidence of pest. On the other hand, relative humidity (morning and evening) and rainfall had positive but weak relationship on percent incidence of *P. panopealis* (r = 0.015, 0.108 and 0.216, respectively). Regression studies for both years revealed that weather factors had 48.10 percent influence over the incidence of insect pest. Similarly, the mean maximum temperature (-0.688**) had a strong negative relation with mean larval population. Whereas, evening relative humidity (0.473*) and rainfall (0.527*) were positively correlated with the mean larval population of *P. panopealis*. The regression studies for both the years revealed that weather factors had 47.90 percent influenced the mean larval population of the pest. The pest activity and damage symptoms of *P. panopealis* were recorded in accordance with the findings of Choi *et al.* (2008) who also reported peak activity during August month from central provinces of South Korea. Feeding by 4th and 5th instar larvae was higher than early instar larvae which resulted in severe yield losses. Similar results were observed with perilla plant, the larval period was taken in perilla leaf longest as 26.8 days at 20ºC and shortened as temperature went up to 30ºC as 11.3 days in Naju, Jeollanam-do province in Korea (Oh *et al.,* 2010). The results of head capsule width measurements in different larval instars are similar to the reports of Oh *et al.* (2010). Investigation of this study revealed the occurrence of *Pyrausta panopealis* on *Ocimum basilicum* for the first time in India. Identification was confirmed using morphological and molecular tools.

This pest was found to be a voracious feeder and resulted in severe yield loss on sweet basil during 2018-19. The percent incidence reached up to 100 during both the observed seasons. As scientific information pertaining to biology, seasonal incidence and population dynamics of *P. panopealis* on *O. basilicum* are meager, hence the present findings would be more useful to farmers engaged in sweet basil cultivation in understanding pest infestation status during growing period.

Acknowledgments

The authors are thankful to the Director, ICAR-DMAPR, Anand and Director, ICAR-NBAIR, Bangalore for providing necessary facilities to carry out the research.

Add-on Information

Authors' contribution: K.T. Shivakumara: Conception and design of the experiment, Data collection; **T. Venkatesan:** Data analysis and interpretation; **M.C. Keerthi:** Drafting the article; **P.R Shashank:** Data analysis and interpretation; **N. Pradeeksha:** Data collection; **A. C. Polaiah:** Data analysis and

interpretation; **R. N. Reddy:** Critical revision of the article; **P.L. Saran:** Data collection; **P. Manivel:** Critical revision of the article.

Research content: The research content is original and has not been published elsewhere

Ethical approval: Not Applicable

Conflict of interest: The authors declare that there is no conflict of interest.

Data from other sources: Not Applicable

Consent to publish: All authors agree to publish the paper in *Journal of Environmental Biology*.

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