EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
ENTOMOLOGY

A

ISSN
1687-8809

WWW.EAJBS.EG.NET

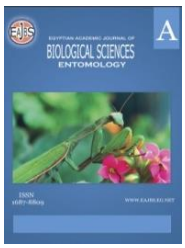
Vol. 14 No. 4 (2021)

Citation: *Egypt. Acad. J. Biolog. Sci. (A. Entomology) Vol. 14(4) pp.109-116 (2021)*
DOI: 10.21608/EAJBSA.2021.207224

Egypt. Acad. J. Biolog. Sci., 14(4):109-116(2021)

Egyptian Academic Journal of Biological Sciences
A. Entomology

ISSN 1687- 8809
http://eajbsa.journals.ekb.eg/



Sex Discrimination by Morphological Traits for Larvae and Pupae of Potato Tuber Moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae) Using Binocular Microscope

Salem Mohamed Salem[1]; Heba Yehia Mady[2] and Maha Moustafa Ahmed[2]
1- Plant Protection Research Institute, Agricultural Research Center, Egypt.
2- Zoology Department, Women's College, Ain Shams University, Egypt.
E-mail***** : salem.postdoctor@gmail.com- hepa.yehia@gmail.com- maha.moustafa@women.asu.edu.eg

ARTICLE INFO
Article History
Received:24/10/2021
Accepted:27/11/2021
Keywords:
Phthorimaea operculella, potato tuber worm, morphological characteristics, Lepidoptera.

ABSTRACT

The potato tuber worm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is one of the most destructive potato pests in the world. Sexual differentiation is sometimes required to conduct investigations on sex-specific responses, mating, pesticide resistance inheritance, and sterile, Insect-Inherited, Sterility Technique and Control Tactics. The goal of this study was to use a binocular microscope to determine distinct morphological traits of immature stages male and female larvae (L.) and pupal stage (p.). We present a straight forward approach for sexing *P. operculella* in this paper. Male larvae were distinguished by the presence of dark-colored gonads that were plainly visible through the cuticle. The sex of a male pupa can be determined using a Binocular microscope by the appearance of dark-colored gonads on the dorsal side. Furthermore, the morphology of the genitalia opening at the end of the abdominal segment distinguishes males from females.

INTRODUCTION

Because the potato tuber worm, *P. operculella* (Zeller) (Lepidoptera: Gelechiidae intimate interaction with its host, great flexibility to daily and seasonal variations, high reproductive potential, and economic damage, the potato tuber worm, *P. operculella* (Zeller) (Lepidoptera: Gelechiidae), is regarded one of the most important potato pests have the economic damage (Bacon 1960a; Foot 1974a, 1979; Briese 1986, Herman *et al*., 2005). Potato, *Solanum tuberosum* L., is important pest of solanaceous crops and weeds, including *P. operculella* (Fenemore 1988; Das and Raman 1994; Gilboa and Podoler 1995; Coll *et al*., 2000; Keller 2003).

Using a Binocular microscope, we morphologically detected the sex-related features of *P. operculella* last instars larvae and pupae in this work. Larvae are 0.5 to 0.6 inches long, white or yellow in color, with a brown head and prothorax (the insect's midsection) (Alvarez *et al*., 2005). Each segment of the thorax features tiny black points and bristles. As larvae mature, their color shifts from white to yellow to pink or green. Before pupation, the larvae feed on their host plants for up to two weeks. Male larvae were distinguished by the presence of dark-colored gonads that were plainly visible through the cuticle.

Pupae are 0.5 inches long, white, and thin. Pupae require 10 to 30 days to fully grow, depending on the climate (Raman 1980; Alvarez *et al*., 2005). Soil and debris can get into the silky cocoon woven around pupae.

The sex of a male pupa can be determined early on using a Binocular microscope by the emergence of dark-colored gonads on the dorsal side. The morphology of the genital hole at the end of the abdomen can also distinguish male from female pupa. (Loewy *et al*., 2013; Turner, 1992, 1995, and 2014). Gender specific features in *P. operculella* larval and pupal stages give valid gender information for pest management. Sex differentiation can also be utilized to forecast outbreaks of this pest in the field and to analyze population dynamics. It's also crucial to understand the distinctions between female and male insects when they're living, as this will help with sexual dimorphism-related biological and ecological studies, such as differences in female and male insect longevity (Renault and Coray 2004).

The goal of this research is to create quick and nondestructive methods for determining the gender of *P. operculella* larvae and pupae. This character was photographed and indicated on the sex of immature stages (larvae and pupae) in this study, as well as being confirmed by adult emergence. This is likely to aid researchers in determining the sex of *P. operculella* pupae, as well as other applications.

MATERIALS AND METHODS

Insect Rearing:

Dr. Ahmed Abdel-Menem Taha, senior researcher at the Central Laboratory for Organic Agriculture, generously contributed the several *P. operculella* moth stages. The *P. operculella* were raised in the lab according to the protocols outlined by (Taha and Refaat, 2021).

Binocular Microscopic Observations:

P.operculella larvae and pupae were obtained from potatoes and preserved in 70% alcohol for binocular microscopic observations. In the central laboratory of the Plant Protection Research Institute, larvae and pupae were examined and photographed to differentiate between male and female using a ToupTek Photonics FMA037, fixed Microscope Adaptor with industrial digital camera 18 MP.

RESULTS

The last (fourth) larval instar and pupae of the potato tuber worm, *P. operculella*, were studied in the lab at 26 °C, 60 % RH, and a photoperiod of 14: 10 hours (L: D). To distinguish between males and females, larvae were examined under a light microscope. Purple-red testes visible through the skin on the dorsal side of the fifth abdominal segment distinguish mature male larvae. Male and female larvae were identified using the characteristics described by (Shanower *et al*., 1993), who found that males and females of *Aproaerema modicella* (Deventer) (Lepidoptera: Gelechiidae) can be distinguished during the larval stage by the presence of pink gonads visible through the cuticle, which were photographed under a binocular.

Even with the naked eye, the gonads can be seen through the cuticle (Fig.1&2). Under a Binocular microscope, the sex of a male pupa can be determined by the presence of dark-colored gonads on the dorsal side. The rise in the cuticle's hardening process, gonads become invisible (Figs. 3&4).

Under a Binocular microscope, the sex of pupae can also be determined by the appearance of the genital area on the ventral side of the last abdominal segments.

Furthermore, the shape of the genitalia opening at the end of the abdomen distinguishes males from females. When compared to the female's oviduct aperture and bursa copulatrix, it is located more away from the proximal side of the end abdominal segment.

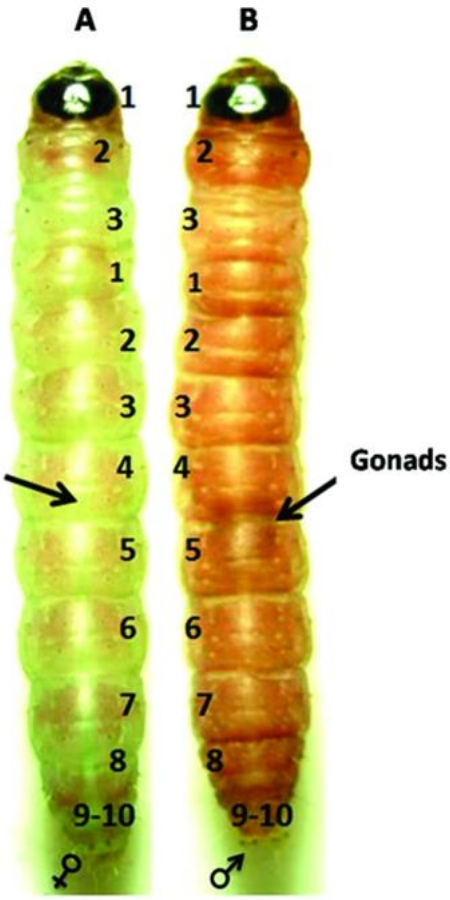


Fig. 1. Light microscopic photographs of total larval body female (**A**) and male (**B**) of *P.operculella*, from the dorsal side, dark reddish brown spot shown by arrow represents gonads (testes) in male,40X

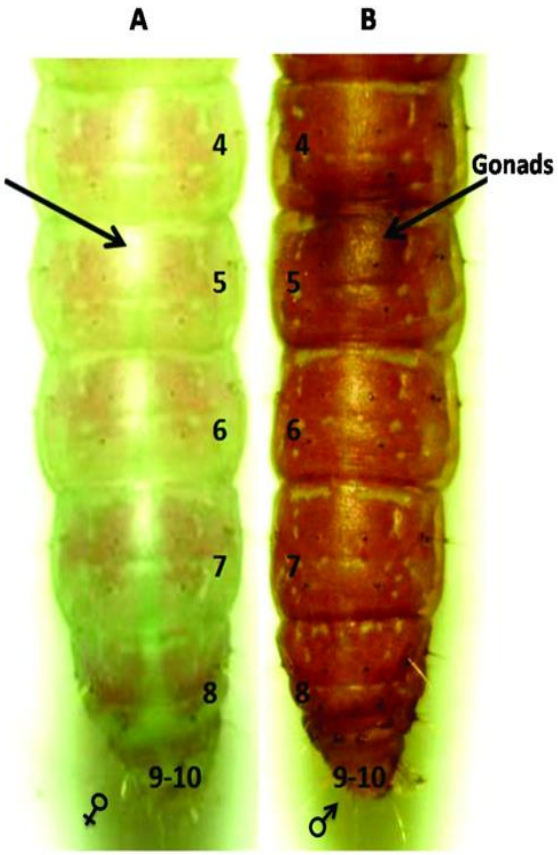


Fig. 2. Light microscopic photographs, showing enlargement the abdominal segments from 4-10 in female (**A**) and male (**B**) larvae of *P.operculella* from the dorsal side, dark reddish brown spot shown by arrow represents gonads (testes) in male, 80X

The genital aperture of the male pupa (Fig. 5), is approximately in the middle third of the last abdominal segment, whereas the genital aperture of the female pupa is approximately in the one-third of the last abdominal segment, closer to the proximal side of the segment, giving the impression that a male pupa has an additional segment before the segment on which the genital aperture and the anus occur in order to determine the specific pupal characters of the pot. The potato tuber worm had ten visible abdominal segments in the end, the tenth of which included the cremaster and had an anal entrance.

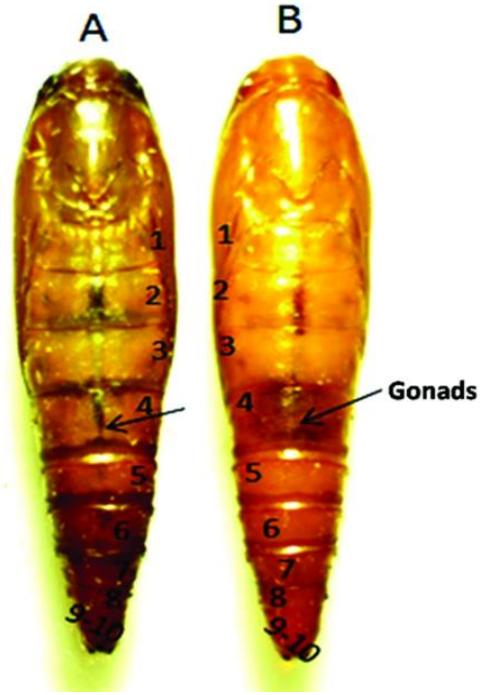


Fig. 3. Light microscopic photographsin female (**A**) and male (**B**) Pupae of *P.operculella* from the dorsal side, dark reddish brown spot shown by arrow represents gonads(testes) in male,40X

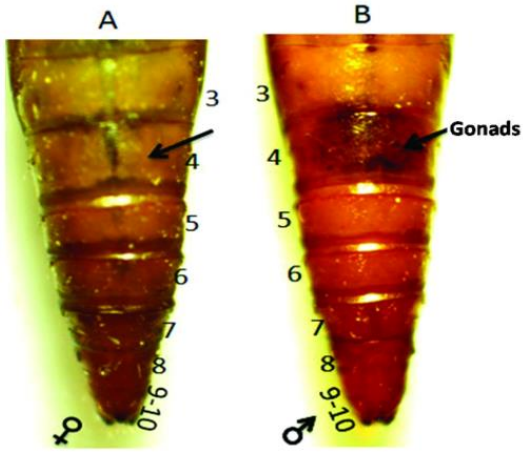


Fig. 4. Light microscopic photographs, showing enlargement the abdominal segments from 3-10in female (**A**) and male (**B**) pupae of *P.operculella* from the dorsal side, dark reddish brown spot shown by arrow represents gonads (testes) in male, 80X

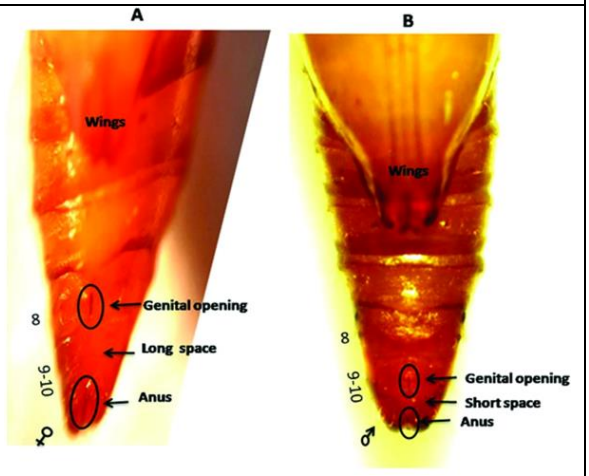


Fig. 5. Light microscopic photographs of female (**A**) and male (**B**) pupae of *P.operculella* on ventral side of abdominal segments. Posterior abdominal segments from the 4th to 10th were presented. The location of genital opening and anus for each pupa were labeled, 80X

DISCUSSION

Although there have been several papers comparing Sex discrimination by physical features of immature stages, there have been a few studies on *P. operculella* to date (larvae and pupae). The majority of studies just provided body length and width, with a focus on males, or perhaps there was no mention of *P. operculella's* sex. In general, the last segments were the most important for determining the sex of individuals

(Genc 2015; Posada *et al.*, 2011). Using a Binocular microscope, we morphologically displayed the sex-related features of *P. operculella* last instars larvae and pupae in this work. As a result, the existence of well-developed gonads of the male pupal stage on the dorsal side was first evaluated, and the identification of *P. operculella* was subsequently confirmed based on external morphological traits. There have been few papers comparing Sex discrimination by morphological features of larvae and pupae, despite the fact that there have been multiple studies on *P. operculella*. (MacLellan, 1972) established the methods for detecting the sex of live larvae in some species. Male testes (gonads) are used for sex differentiation in *P.operculella* larvae in the current study, in the same way that Chauman and Verma (1991) discovered that no sexual dimorphism is observed until the third larval stage of *P.operculella*, where initial sexual structures are visible; at the last larval stage, males are distinguished from females by having two elongated yellowish testes in the 5th and 6th abdominal segments. The presence of pink-colored gonads in the region of the sixth and seventh abdominal segments was also a distinguishing feature of male larvae of the groundnut leaf miner, *Aproaerema modicella* (Deventer), and the gonads were plainly visible through the cuticle even with the naked eye (Shanower *et al*., 1993). The fourth instar male moth larvae of *Pectinophora gossypiella*, have dark-colored testes visible to the naked eye in their fifth abdominal segment, while all other larvae lacking this feature turned into female moths (Ramya *et al*., 2019).

The similar conclusion was discovered by (Chaitra *et al*., 2021), who discovered sexual dimorphism in male and female *P. gossypiella* larvae (dark reddish-brown spot representing the testes in males). We present a reliable and rapid approach for distinguishing male and female *P. operculella* larvae without the use of specific equipment or supplies in this work. *P. operculella* sexual dimorphism is not noticeable until the fourth instars larvae have yellowish testes; a gradual change in eye pigmentation indicates distinct pupal stages (yellow, early red, middle red, late red, and black eye pupa) (Chauhan and Verma, 1991). The genital apertures that appeared as a slit were noted as sexual traits to separate male pupae, and they were visible at 9 abdominal segments from the ventral side, according to our findings.

In addition, (Posada *et al*., 2011) discovered the identical structures on the posterior abdominal segments of male and female cocoa pod borer, *Conopomorpha cramerella* pupae, with the genital entrance identified between the 8th and 9th segment in female pupae and between the 9th and 10th segment in male pupae. (Tuncer and Aker, 2017) looked at the morphology of the genital apertures of *Hyphan triacunea* pupae, the slit on the 8th segment for females and the 9th segment for males. Female pupae, on the other hand, have a single aperture on the eighth segment, while male pupae have a spherical "pit-like genitalia opening" in the middle of the ninth segment, which fits with the finding made by (Mukherjee, 1949). The major characteristics of the sex are known to be found in the posterior part of the abdomen. In the tomato leaf miner, various studies have shown that investigations of these segments revealed certain differences and similarities based on drawings that the genital aperture was in the eighth abdominal segment in females and the ninth in males (Coelho and Franca, 1987).

As a result, the findings corroborated the earlier research. Tomato leaf miner pupae, on the other hand, are quite small, and the last abdominal segments of a live pupa in some individuals are bent inwards. Because the separation between the segments is not very apparent, observing the segments in male and female pupae during laboratory research is difficult. In this case, having good photographs of living specimens with reliable sex's unique traits is critical and valuable.

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ARABIC SUMMARY

التمييز بين الجنسين من خلال الصفات المورفولوجية لليرقات وعذاري عثة درنات البطاطس باستخدام المجهر الضوئى الماسح.

[1]سالم محمد سالم ،[2]هبه يحي ماضى ،[2]مها مصطفى أحمد
1- معهد بحوث وقاية النباتات - مركز البحوث الزراعية.
2- قسم علم الحيوان - كليه البنات - جامعه عين شمس.

الهدف من هذه الدراسة هو التعرف على الخصائص المورفولوجية المميزة للمراحل غير الناضجة (ليرقات الذكور، الإناث وكذلك طور العذراء) تحت المجهرالضوئى الماسح. حيث يتناول البحث طريقة مباشرة تسمح بتحديد جنس عثة درنات البطاطس *operculella*.P عن طريق وجود الغدد التناسلية ذات اللون الداكن والتي يمكن رؤيتها بسهولة من خلال البشرة حيث انها من الصفات المميزة ليرقات الذكور، بينما يتم تمييز الجنس فى ذكور العذراء تحت المجهر الضوئى الماسح وفقًا لظهور الغدد التناسلية ذات اللون الداكن على الجانب الظهري للعذراء، ومع زيادة تصلب الكيوتيكل تصبح غير مرئية. بالإضافة إلى ذلك، يمكن التعرف بسهولة على جنس العذراء من خلال شكل فتحة الأعضاء التناسلية في نهاية البطن. ومن المتوقع أن تعطي نتائج هذه الدراسة المورفولوجية فهمًا أفضل لتصنيف هذه الحشره، ويمكن أن تكون واحدة من أهم الأمور لتطوير استراتيجية جديدة لمكافحة هذه الآفة الخطيره.