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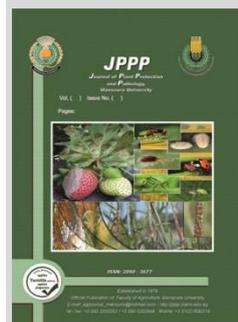
Biological Studies on Three Lepidopteran Insect Pests Tomato Crop under Laboratory Conditions

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ABSTRACT

Laboratory experiments were carried out to investigate some biological characteristics of some lepidopteran insect pests infesting tomato plants, when reared on tomato leaves at constant temperature of $27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH. These insects were *Tuta absoluta* (Meyrick), *Spodoptera littoralis* (Boisd.) and *Spodoptera exigua* (Hb). These experiments were conducted in the laboratories of Plant Protection Research Institute, Mansoura Branch. Results showed that the incubation period of the eggs stage averaged 3.0; 4.2 and 4 days for *T. absoluta*, *S. exigua* and *S. littoralis* when reared on tomato leaves respectively. Whereas, The duration of the larval stage of averaged 13.0; 15.3 and 13.5 days when reared on tomato leaves, respectively. The pupal stage lasted 3.7; 6.3 and 4.4 days for these insects respectively. The total immature development lasted 21.3; 26.7 and 22.9 days respectively. The obtained results indicated that female longevity lasted 5.9; 8.3 and 6.2 days for *T. absoluta*, *S. exigua* and *S. littoralis* respectively. The fecundity per female was 280; 480 and 870 eggs, respectively.

Keywords: Biological aspects, *T. absoluta*, *S. exigua* and *S. littoralis*.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.), is known botanically as one of the plants of the Solanaceae family and one of Egypt's and the world's primary and popular vegetable crops. In 2022/2023, Egyptian cultivation area is around 7 million feddans, with an average yield of 18.017 tons (FAO, 2023). The tomato contains 94% water, 0.9% protein, 0.2% fat, 0.5% minerals, 0.8% fiber, and 3.6% carbohydrates, as well as additional elements such as 50mg calcium, 0.12mg vitamin B-1, 27mg vitamin C, 0.4mg iron, and 356 mg carotene (BARI, 2010; Desneux *et al.*, 2010). Tomato could be infested with many insect pests, that attack all parts of the plant, including the leaves, stems, blossoms, and fruits. This crop is primarily attacked by some lepidopteran insects, namely: Tomato leaf miner, *Tuta absoluta* (Meyrick); *Spodoptera exigua* (Hb) and *Spodoptera littoralis* (Boisd). *Tuta absoluta* is one of the primary pests of tomatoes, and damage from this insect can be up to 50-80%. (Idris and Emelia 2001; Azidah and Sofian 2006; Hemmati *et al.*, 2012; Cathbertson *et al.* 2013; Kianpour *et al.* 2014; Shankar *et al.* 2014; Haque, 2015; Norma *et al.*, 2015; Ballal *et al.*, 2016; Mohamed *et al.* 2019; Zhang *et al.*, 2020; El Aimani *et al.*, 2021; Aynalem 2022; Heba *et al.*, 2024). Within a few years, *T. absoluta* has emerged as significant challenge to the sustainability and productivity of global tomato cultivation. It is now widely admitted as one of the most invasive and detrimental pests impacting tomato cultivation, particularly in countries within the Mediterranean Basin, including Egypt, Tunisia, Libya, Morocco, and Algeria, as well as in Bangladesh. Its rapid spread and destructive feeding behavior pose a serious threat to tomato yields and agricultural sustainability in these regions- (Tsai and Wang; 2001; Kim and Lee 2002, Baniamwri and Cheraghiam, 2012; Haque,

2015; Martins *et al.*, 2016 and Ramasamy 2020). The biological characteristics of *Tuta absoluta*, *Spodoptera exigua*, and *Spodoptera littoralis* have been extensively studied by researchers across various regions worldwide. (Retta and Berhe, 2015 and Alam *et al.*, 2019). The present study aimed to investigate some biological characteristics of *T. absoluta*; *Spodoptera exigua* and *S. littoralis* under laboratory conditions.

MATERIALS AND METHODS

1. Insect culture

Laboratory experiments were carried out to investigate some biological aspects of *T. absoluta*; *S. exigua* and *S. littoralis* under constant temperature of $27 \pm 2^\circ\text{C}$ and $65 \pm 5\%$ RH in the laboratories of the Plant Protection Research Institute; Mansoura Branch.

A stock colony of *S. littoralis* was established from eggs sourced from the Cotton Leafworm Research Division at the Plant Protection Research Institute, Dokki, Egypt. Prior to the experiments, larvae were reared for 30 generations in insectaries on castor bean (*Ricinus communis*) leaves. Adult moths were provided with a sucrose solution 10% concentrate for feeding. Insects were reared and maintained under controlled conditions at $27 \pm 2^\circ\text{C}$, $65 \pm 5\%$ Rh., and a 16-hour light: 8-hour dark photoperiod. A branch of oleander (*Nerium oleander*) was placed in the rearing cages to serve as an oviposition place. Egg masses were collected daily and carefully stored in 90-mL plastic cups under controlled conditions to ensure optimal incubation until hatching. (Abd-Allah and Marouf, 2015).

Spodoptera exigua larvae collected from field populations in Sekinchan, were utilized to establish a controlled laboratory culture. The larvae were reared on leaves of cabbage (*Brassica oleracea*), from the institute's

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garden in Serdang. To study the developmental stages of *S. exigua* under laboratory conditions, freshly laid eggs (within 24 hours) from laboratory-reared females were used. These females were provided with a 10% honey solution applied to aluminum foil, which had been pre-soaked in cabbage juice to stimulate oviposition and enhance egg-laying behavior.

Each batch of eggs was carefully placed in a plastic container for incubation (14 cm × 14 cm × 9 cm) with a perforated cover (6.5 cm × 6.5 cm) closed with an aluminum wire mesh to allow for ventilation. The containers were labeled with the date of egg production, and the incubation period was recorded from the date of oviposition until hatching. Upon hatching, the newly emerged larvae were individually transferred to separate plastic containers (9.5 cm top diameter × 6.5 cm bottom diameter × 12.5 cm height), each with a diameter hole 4 cm in the cover, Secured with muslin cloth to ensure proper ventilation.

The larvae were provided with either whole or sectioned fresh leaves of their respective host plants as a food source until pupation. Each larva was housed individually in a plastic container and monitored daily for ecdysis. The detached head capsule from newly molted larvae was collected and preserved in a specimen vial containing 70% ethanol to determine the number of instar stages. Following the final molting stage, vermiculite was added to the container to facilitate pupation.

Observations were conducted on a total of 83 larvae reared on cabbage and shallot, 20 larvae on long bean and lady's finger, and 40 larvae on chili. Each larva was treated as an independent replication, and its incubation period, larval development, and pupal duration were recorded.

Adults emerging from the pupae were individually housed in plastic containers (9.5 cm top diameter × 6.5 cm bottom diameter × 12.5 cm height), which had a 4 cm diameter hole in the cover covered with muslin cloth for ventilation. They were provided with a 10% honey solution as a food source. The longevity and sex of each adult were documented to assess adult lifespan and gender distribution.

The larvae were provided with either whole or sectioned fresh leaves of their respective host plants as a primary food source until they reached the pupation stage. Each larva was individually housed in a designated plastic container to prevent interaction with other individuals and to ensure accurate monitoring of its developmental progress. Daily observations were conducted to document moults. During each molting event, the detached head capsule from the newly molted larva was carefully collected and preserved in a labeled specimen vial containing 70% ethanol. This procedure was performed to accurately determine the number of instar stages the larvae underwent before pupation. Following the final molting stage, vermiculite was introduced into the container to provide a suitable substrate for pupation, ensuring optimal conditions for metamorphosis.

To assess larval development across different host plants, detailed observations were carried out using a total of 83 larvae reared on cabbage and shallot, 20 larvae on long bean and lady's finger, and 40 larvae on chili. Each individual larva was considered an independent replication to maintain the integrity of the experimental design. Throughout the study, the incubation period, duration of the larval stage, and pupation period were systematically recorded to analyze the developmental timeline of *S. exigua*.

Upon emergence, the adult moths were individually transferred to separate plastic containers measuring 9.5 cm in top diameter, 6.5 cm in bottom diameter, and 12.5 cm in height. Each container was designed with a 4 cm diameter hole in the lid, which was covered with muslin cloth to ensure proper ventilation while preventing the escape of the moths. The adults were provided with a 10% honey solution as a nutritional source to sustain their physiological functions and prolong their lifespan. The longevity of each adult was meticulously recorded, and their sex was determined and documented to evaluate gender distribution and lifespan variations within the population.

Newly emerged *T. absoluta* adults were carefully transferred to designated rearing cages designed to provide a controlled environment for oviposition, ensuring the permanent maintenance of *T. absoluta* generations during the year. The adults were housed in transparent plastic containers with a capacity of 250 mL, covered with black muslin cloth. The eggs deposited on the muslin cloth were collected and transferred to separate plastic containers. Upon hatching, the larvae were introduced onto fresh leaves of tomato, potato, and eggplant. Each larva was individually placed on a single leaf and maintained in glass Petri dishes with a diameter of 9 cm. To prevent desiccation, leaves were placed on slightly moistened filter paper spread over Petri plates. The leaves of various host plants were replaced daily on every plate. During the transfer of larvae to fresh host leaves, larval exuviae were observed to determine molting, with the entire process conducted under a stereo microscope. Pupae remained undisturbed in Petri dishes until adult emergence. Observations were recorded from 16 plates containing tomato leaves, in which the complete life cycle was monitored.

Following emergence, adults were transferred to transparent plastic containers with a 250 mL capacity, covered with dark muslin cloth. A 10% honey solution was provided for adult feeding by spreading it on the cover. The dates of some biological aspects of adults (adult longevity (female / male), female fecundity, hatching percentage, and were recorded. The collected data mean values and standard errors of the means were calculated for analysis. (Binu and Ajaya, 2019).

RESULTS AND DISCUSSION

Biological Aspects:

Effect of tomato leaves on the biological features of *T. absoluta*, *S. exigua* and *S. littoralis* was shown in Table (1) and Fig. (1). Data revealed that incubation period of *T. absoluta*, *S. exigua* and *S. littoralis*, fed on tomato lasted for 3.0, 4.0 and 4.2 days, respectively, The incubation period change compared with the other. However, larvae of *T. absoluta* respectively, resulted significantly lower incubation period compared to *S. exigua* and *S. littoralis* (Table 1; Fig. 1).

The developmental periods of the 1st instar lasted for 2.3, 2.3 and 2.0 days, for *S. littoralis*; *T. absoluta* and *S. exigua* respectively. While for the 2nd larval instar, the periods of development lasted for 2.0, 3.3 and 4.0 days, respectively. The developmental periods of 3rd larval instar lasted for 2.0, 3.8 and 3.3 days, respectively. For the 4th larval instar, the developmental periods lasted for 2.5, 3.6 and 3.0 days, respectively. For the 5th instar larvae, the average periods of development were 3.5, and 2.2 days for *S. littoralis* and *S. exigua* respectively. The developmental periods of the 6th

instar larvae lasted for 3.6, days, for *S. littoralis*. So, the total larval periods were, 15.3, 13.0 and 13.5 days when larvae were reared on tomato leaves, respectively (Table 1; Fig. 1). The obtained results demonstrated that the total larval period with *T. absslauta*, *S. exigua* did not change compared with that with *S. littoralis*. Whereas, the period with *S. littoralis* was highest than that with *T. absslauta*. The results were agreed with Esther *et al.* (2008); Wraight *et al.* (2010); Hussein *et al.* (2015); Singh *et al.* (2015); Ankita and Sangeeta, (2017).

The pre-pupal stage lasted 1.6, 1 and 0.9, days for *T. absslauta*, *S. exigua* and *S. littoralis*, respectively. The pupal

duration lasted for 3.7, 4.4 and 6.3, days, respectively (Table 1; Fig. 1). The total duration of immature stages lasted, 21.3, 22.9 and 26.7 days respectively. *Tuta absslauta* had the shortest and *S. littoralis* had the longest development.

The primary host crops of *Spodoptera littoralis* in Europe have been identified, with *Allium* spp. (onion) being among them (CABI (2022). Al-Shannaf (2011) reported that pepper is also a major host plant for *S. littoralis*. Furthermore, the incubation period, total larval duration, and overall life cycle of *S. littoralis* were found to be longer when reared on pepper compared to the castor plant.

Table 1. Developmental times of the different immature stages of main insects fed on leaves of tomato plants

Main pests	Mean developmental periods of larval instars (days) ± SE								larval duration (days) ± SE	pre-pupal duration (days) ± SE	Pupal duration (days) ± SE	immature stages, Immature development (days) ± SE
	Mean incubation period (days) ± SE	1 st larval instar	2 nd larval instar	3 rd larval instar	4 th larval instar	5 th larval instar	6 th larval instar	larval duration (days) ± SE				
<i>T. absslauta</i>	3.0±0.4c	2.3±0.2a	3.3±0.2c	3.8±0.1c	3.6±0.1b	—	—	13.0±0.1c	1.6±0.2c	3.7±0.2c	21.3±0.2a	
<i>S. exigua</i>	4.0±0.3ab	2.0±0.2a	4.0±0.3b	3.3±0.2b	3.0±0.3a	2.2±0.3b	—	13.5±0.4b	1.0±0.1b	4.4±0.3b	22.9±0.5b	
<i>S. littoralis</i>	4.2±0.1a	2.3±0.2a	2.0±0.2c	2.0±0.2b	2.5±0.2b	3.5±0.2b	3.0±0.2b	15.3±0.1a	0.9±0.2a	6.3±0.2b	26.7±0.4a	

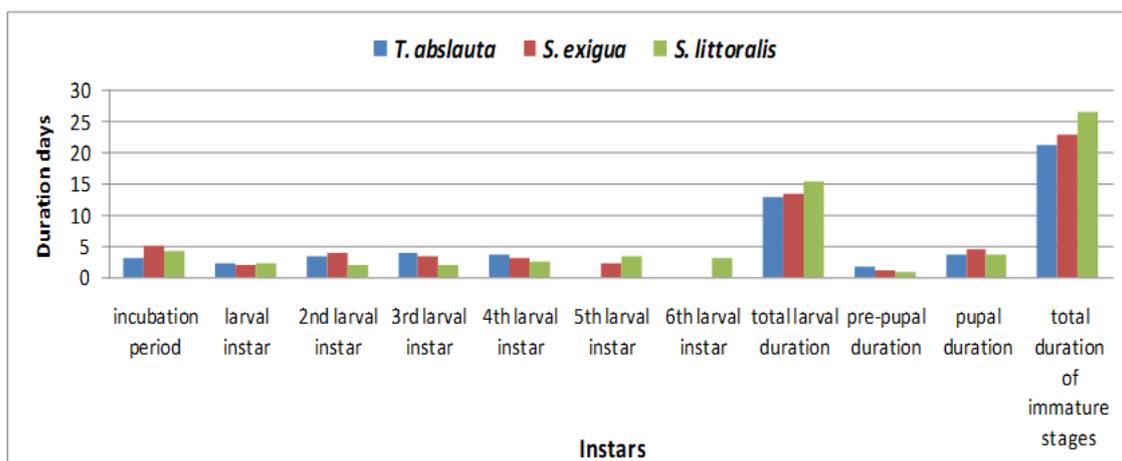


Fig. 1. Developmental times of the different immature stages of *T. absslauta*, *S. exigua* and *S. littoralis* fed on leaves of tomato plants

Data presented in Table 2 and illustrated in Fig. 2 show that the pre-oviposition period lasted 1.7, 2.2 and 1.8 days for female *T. absslauta*, *S. exigua* and *S. littoralis* which fed as larvae on tomato, leaves, respectively. These findings showed that the time needed for ovary maturation was lower with *T. absslauta* compared to *S. littoralis*. While this time increased of *S. exigua*. The obtained results exhibited that the female oviposition period lasted 3, 4.3 and 3.2 days, respectively. This means that oviposition period with *S. exigua* was longer than *T. absslauta*. The post-oviposition period lasted 1.2, 1.8 and 1.2 days respectively. These findings contrast with those of Al-Shannaf (2011), who investigated the effects of thirteen host plants on food consumption and various biological parameters of *S. littoralis*

larvae. Al-Shannaf (2011) reported that larvae fed on pepper leaves exhibited a shorter lifespan compared to those fed on castor leaves. The results of that study suggested that castor plants served as a more suitable host for *S. littoralis* larvae than pepper plants. However, similar results are discussed by Nylín and Janz (1993); Singer *et al.* (1994); Gripenberg *et al.* (2010); Chowański *et al.* (2016); Thawabteh *et al.* (2019).

Fecundity (number of deposited eggs/female) was 280, 480 and 780 eggs/female for *T. absslauta*, *S. exigua* and *S. littoralis*, respectively (Table 3; Fig. 3). These results indicate that the highest number of deposited eggs was attained in case of *S. littoralis*, followed by *S. exigua* and *T. absslauta*.

Table 2. Longevity of male and female moths of *T. absslauta*, *S. exigua* and *S. littoralis* fed on tomato leaves

Main pests	Duration ± SE (days)							
	Female moths					Male moths		
	Pre-oviposition Period	Oviposition Period	Post-oviposition period	Longevity	Generation	Life span	Longevity	Life span
<i>T. absslauta</i>	1.7 ± 0.1b	3.0 ± 0.1c	1.2 ± 0.1c	5.9 ± 0.1c	28.4 ± 0.4	32.6 ± 0.5	4.5 ± 0.4a	31.2 ± 0.5
<i>S. exigua</i>	2.2 ± 0.2a	4.3 ± 0.2b	1.8 ± 0.1b	8.3 ± 0.2b	23.5 ± 0.4	29.6 ± 0.4	6.1 ± 0.5b	27.4 ± 0.4
<i>S. littoralis</i>	1.8 ± 0.1b	3.2 ± 0.1c	1.2 ± 0.1c	6.2 ± 0.1c	24.7 ± 0.4	29.1 ± 0.4	3.9 ± 0.3c	26.8 ± 0.4

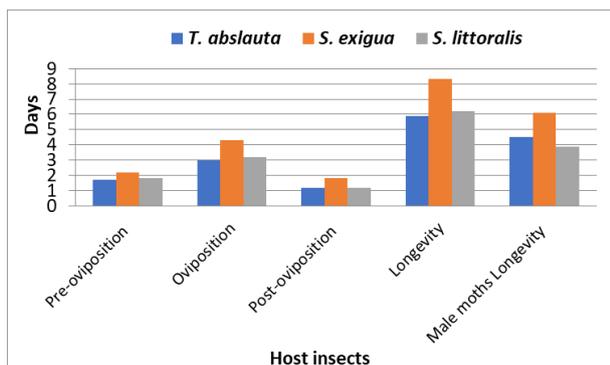


Fig. 2. Longevity of male and female moths of *T. abslautata*, *S. exigua* and *S. littoralis* fed on tomato leaves

Table 3. Fecundity, fertility, adult emergence and larval weight of *T. abslautata*, *S. exigua* and *S. littoralis* fed as neonate tomato larvae.

Main pests	Mean fecundity ± SE (no.eggs/female)	Mean fertility (% egg-hatchability)	Mean adult emergence (%)	Mean weight of full grown larvae ± SE (mg)
<i>T. abslautata</i>	280.0 ± 3.22c	40.0	23.8	300.7 ± 12.36b
<i>S. exigua</i>	480.0 ± 2.91b	66.2	40.2	300.7 ± 12.36b
<i>S. littoralis</i>	870.0 ± 3.90b	70.0	46.7	450.5 ± 15.56c

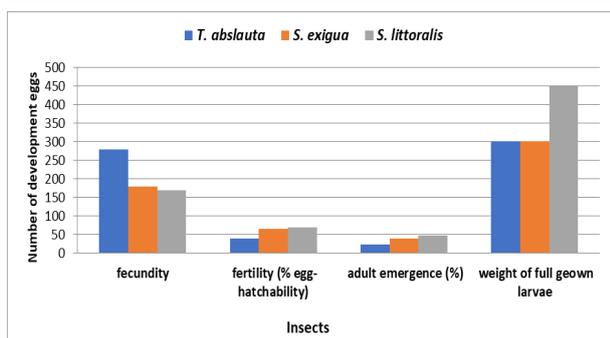


Fig. 3. Fecundity of *T. abslautata*, *S. exigua* and *S. littoralis* females fed on leaves of tomato plants

Fertility (% egg-hatchability) was species dependent with the highest egg-hatchability (70.0 %) recorded for *S. littoralis* moths, whereas the lowest egg-hatchability (40%) was *T. abslautata* moths (Table 3). In a descending order, egg-hatchability as follows: *S. littoralis*, *S. exigua* and *T. abslautata*. These findings are consistent with those of Al-Shannaf (2011), who demonstrated that female moths emerging from larvae reared on castor bean leaves laid the highest number of eggs (2,171), whereas those reared on pepper leaves laid the lowest number (790). Similar results were obtained by Diédhiou *et al.* (2021); Maharani *et al.* (2021); Al-Ayat *et al.* (2022); Altaf, *et al.* (2022); Gopalakrishnan (2022); Nandhini *et al.* (2023).

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دراسات بيولوجية على ثلاث آفات حشرية من رتبة حرشفية الأجنحة التي تهاجم محصول الطماطم تحت الظروف المعملية

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المخلص

تم إجراء تجارب معملية لدراسة بعض الخصائص البيولوجية لبعض الآفات الحشرية من رتبة حرشفية الأجنحة التي تصيب محصول الطماطم، عند تربيتها على أوراق الطماطم تحت درجة حرارة ثابتة تبلغ $27 \pm 2^\circ\text{C}$ ورطوبة نسبية $65 \pm 5\%$. وهذه الحشرات هي *Tuta absoluta* (Meyrick)، *Spodoptera littoralis* (Boisd.) و *Spodoptera exigua* (Hb.). تم إجراء هذه التجارب في مختبرات معهد بحوث وقاية النباتات، فرع المنصورة. وأظهرت النتائج أن متوسط فترة حضانة البيض لكل من *T. absoluta* و *S. exigua* و *S. littoralis* بلغت 3.0، 4.2 و 4.0 أيام على التوالي عند تربيتها على أوراق الطماطم أما مدة الطور اليرقي لهذه الحشرات فقد بلغ متوسطها 13.0، 15.3 و 13.5 يوماً عند تربيتها على أوراق الطماطم. في حين استغرقت فترة طور العذراء 3.7، 6.3 و 4.4 أيام لهذه الحشرات على التوالي. بلغت المدة الكلية للأطوار الغير كاملة 21.3، 26.7 و 22.9 يوماً على التوالي. كما أشارت النتائج إلى أن متوسط عمر الإنث كان 5.9، 8.3 و 6.2 أيام لكل من *T. absoluta* و *S. littoralis* على التوالي. أما معدل الخصوبة لكل أنثى فقد بلغ 280، 480 و 870 بيضة على التوالي. في حين أن متوسط عمر الذكر كان 4.5، 6.1 و 3.9 أيام لكل من *T. absoluta* و *S. littoralis* على التوالي.