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Biological and Sex Pheromone Studies on Tomato Leaf Miner, *Tuta absoluta*, Meyrick (Lepidoptera: Gelechiidae) at Fayoum goveRnorate, Egypt.

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ABSTRACT

Results indicated that the developmental periods of eggs, larvae, prepupal and pupal stages were 6.70, 14.75, 2.20 and 12.80 days at 20 ˚C and 65%RH, while it lasted 3.76, 8.80, 1.25 and 6.35 days at 30 ˚C and 65%RH. The longevity of female was 27.30 days, while the male longevity was 32.60 days at 21.6˚C and 67%RH. The longevity of female was 27.3 days, the male longevity was 32.6 days at 21.6˚C and 67%RH. The number of eggs/female ranged from 75 to 180 eggs. Concerning pheromone traps study, the highest peaks of male catches recorded in the first week of Dec. and in the first week of Nov. with 550 and 880 males /3traps for both two seasons, respectively. On the other hand, water pan trap was the most effective (406 males /trap) followed by modified palm weevil bucket trap (226/males/ trap). Also, the green traps caught the higher males compared with that of yellow, red and white colors but the means of all tested colors were not significantly different. Regarding the lure types, *Tuta optima*, lure was the highest efficient (252.8 males/trap), while the prepared locally lure was the lowest (39.4 males/trap) and the differences among means of the three lure types were significant. For periodicity of male catches during scootphase and photophase it found that the attraction was higher in the 1st hour of photophase than that of the 2nd hour where the trapped moths in the 1st hour represented 5.8 folds of that of the 2nd hour.

Keywords: Tomato, *Tuta absoluta*, biology, traps, sex pheromones, lures, photophase

INTRODUCTION

The tomato leaf miner, *Tuta absoluta* is a mirolepidoptera which attacks tomato crops in Mediterranean basin and European countries (Desneux*et al*., 2010). Bavaresco *et al*. (2005) in Brazil, stated that seasonal fluctuations of *T. absoluta* effect on numbers of captured males in delta traps baited with sex pheromone lure. Synthetic sex pheromone has been used for monitoring (Harizanova *et al*., 2009; Braham 2014). Sex pheromone traps efficiency could be affected by many factors.

Concerning trap designs, Salas (2004 and 2007) in Venezuela, showed that the number of captured males in water traps was higher compared with that of delta sticky traps. Habib *et al*. (2011) reported that efficiency of delta traps is more than water traps for attractiveness in open field, and the converse under glasshouse conditions. Alasady *et al*., (2011) in Iraq, mentioned that the highest catching was recorded in open water traps and the lowest was recorded in yellow delta traps The red traps were most effective in trapping moths of *T. absoluta* (Taha *et al*., 2012). Braham (2014) reported that green water pan traps was the most effective followed by red traps while the lowest effective was the white traps. Green traps captured significantly more males of potato tuber moth, *Phthorimaea operculella* (Zeller) than red and yellow traps Hashemi (2015). For trap height, Raman (1988) found that no differences between the three heights, ground levels, 0.4 or 0.8 m. Hashemi (2015) found that water pan traps placed at 0.6 m above plant canopy captured significantly the highest number of the moths in comparison to traps placed at ground level of 0.3 m.

The present work aims to determine some biological aspects of *T. absoluta*, the efficiency of trap design; height; color and lure type on males capture. Also, determine the optimum period of day for males activity and effect of weather factors on males response to sex pheromone traps.

MATERIALS AND METHODS

Stock culture:

Tomato leaf miner *T. absoluta* larval stage was collected from infested tomato field located at yousef El-Sedek district, Fayoum Governorate, Egypt. The larvae were placed in Petri dishes (5cm in diameter) and provided with fresh tomato leaves (as source of food) leaves were changed every two days until pupation. Pupae were isolated in plastic containers until emergence Emerged adults were separated in five pairs in chimney glass cages (Fig. 1A). Each cage was placed in Petri dish and covered with muslin cloth and rubber band. The cages were provided with tomato leaves placed in vials filled with water as oviposition sites and wicks of cotton in vials filled with 20 % sugar solution as food.. The cages were examined daily to change the leaves and collect the deposited eggs.The culture was maintained under laboratory conditions (25 °C± 1 and mean 65 % ±5 RH.).

Immature stages development

Incubation period of eggs

To study the incubation period of eggs, Daily separated eggs from stock culture were divided into replicates each of 50 eggs, put in plastic containers (4.5cm height and 5.0cm diameter) and incubated under three constant temperatures (20, 25 and 30 ± 1 °C) and mean 65±5 % RH. Containers were examined daily to record the number of hatched eggs until the end of experiment. Treatments were replicated three times.

Larval and pupal stages:

To study the duration of larval and pupal stages, three groups (each twenty of newly hatched larvae) were isolated individually in plastic containers with tight lids and provided with tomato fresh leaflet. The groups were incubated under the above mentioned conditions and examined daily until pupation to record molting and change the food. Also, pupae were investigated till adult emergence.

Longevity and fecundity

To determine the longevity and fecundity, last larval instars were sexually separated in two containers and provided with food till pupation. Pupae of each sex were collected separately until emergence. Ten pairs of newly emerged adults were separated individually in chimney glass cages which were placed in Petri dishes and covered with muslin cloth and rubber band. The cages were provided with tomato leaves placed in vials filled with water as oviposition sites and wicks of cotton in vials filled with 20 % sugar solution as food. Other ten virgin females separated in similar chimney cages to study the effect of mating on laying eggs. Cages investigated daily to remove the leaves and replace it with fresh ones until adult death. Removed leaves were examined with hand lens to count laid eggs and record the beginning and the end of oviposition.

Effect of some weather factors on male *T. absoluta* catches

This experiment was carried out in autumn plantation for two succissive seasons 2013 and 2014 to study the effect of daily weather factors on the efficiency of *T. absoluta* synthetic sex pheromone in capturing males. Three palm weevil bucket trap were supplied by synthitic sex pheromone lures. These traps were sited 30 m from each other and placed 25 cm above ground at aprivate farm cultivated with tomato 935 strain in mid-September. The traps were examined every three days to count and remove the captured mals until the end of crop. Climate data (maximium, minimum temperatures, daily avrage relative humidity) were obtained from meteorological department, Fayoum Governorate.

Effect of trap colors

Water pan traps with four colors (yellow, green, red and white) baited with commercial sex pheromone lures were evaluated. One trap for each color was placed on 25cm height above ground. Traps were provided with water and 10 gram of detergent to kill captured males by drowning and placed at 50 m apart. Traps were investigated every two days to count, remove captured males and rotate the traps to eliminate position effects.

Effect of trap designs

This experiment was conducted on 11th November 2015 to determine the efficiency of four trap designs; sticky delta trap, water pan trap, palm weevil bucket trap and modified palm weevil bucket trap (fig. 1B). Traps baited with synthetic sex pheromone lures were placed on 25 cm height above ground and traps were 50 m apart. The traps were examined daily for 16 days after installation to record the captured male moths and rotate them to eliminate position effects.

Effect of trap heights

For trap elevation efficiency, three heights 25, 75 and 175 cm aboveground were selected. Three water pan traps, one at each height, were baited with *T. absoluta* synthetic sex pheromone lure and suspended at the previous heights, and 50 m apart. Traps were examined after 24hr of exposure to count captured males. Treatments were replicated three timesand conducted from 28th November to 9th December.

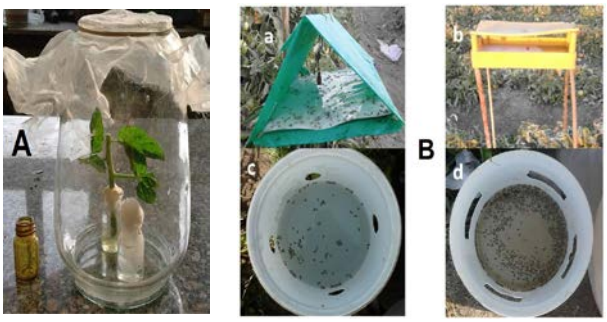

Fig. 1. A- Adult rearing chimney cage; B- traps types contains (a- sticky delta trap; b- water pan trap; c-palm weevil bucket trap; d- modified palm weevil bucket trap).

Efficiency of sex pheromone lures types

Three types of sex pheromone lures (Tuta-Optima® loaded with 0.8 mg, TurkiyedeDretil lure 0.8mg and locally prepared lure 0.5 mg obtained from plant protection institute) were tested. Three baited traps for each treatment in addition to unbaited three traps were installed on 30 cm height. These traps distributed in three rows approximately 30 m apart within the field. Traps were examined every two days to count and remove the captured male moths. After each inspection, all traps were rotated to eliminate the effect of trap position.

Statistical analysis: The simple correlation values between the number of insects and the weather factors were obtained by using SPSS 2000, version 11. Also, means subjected to one way ANOVA analysis and separated by L.S.D. test at 5% level of probability.

RESULTS AND DISCUSSION

Incubation period of eggs

Results in Table (1), indicated that the incubation period of *Tuta absoluta* eggs ranged from 3-4 days with average 3.76 days at 30 ˚C and 65% RH. Also the longest period was obtained at 20 ˚C and 65% RH where this period ranged from 6 -8 days with average of 6.70 days. The highest percentage of eggs (97.3%) was recorded at 25 ˚C and 65% RH.

Duration of larval and pupal stages

Data in Table (1), demonstrated the shortest period of larval stage ranged from 6 to11 days with average of 8.8 days at 30 ˚C and 65 % RH, while the longest period was 14.7 days at 20 ˚C and 65% RH. The duration of prepupal stage was the shortest period where it ranged from 1 to 3 days. Regarding pupal stages, the shortest period (6.35 days) was recorded at 30˚C and 65% RH, while the longest period was 12.80 days at 20˚C and 65% RH. The total immature stages ranged from 14 19 days at 20 ˚C and from 28-31 at 30 ˚C.

Longevity and fecundity of adults.

The obtained results in Table (2), Indicated that the pre-oviposition and post-oviposition periods of unmated females were longer than that of mated females.

Table 1. Effect of temperature degrees and relative humidity on development of time immature stages of *Tuta absoluta*.

Rearing conditions	Incubation period (days) &%hatch	Duration periods ±S.E (in days) Larval stage	Prepupal stage	Pupal stage	Total
20±1˚C (65±5 %RH)	6.70±0.07 (95.3)*	14.75±0.35[a] (12-17)	2.2±0.14[a] (1-3)	12.8±0.31[a] 10-15)	29.75±0.22[a] (28-31)
25±1˚C(65±5 %RH)	5.15±0.04 (97.3)	10.45±0.35[b] (8-13)	1.5±0.14[b] (1-3)	8.75±0.22[b] (7-10)	20.7±0.34[b] (18-23)
30±1˚C (65±%RH)	3.76±0.04 (91.0)	8.80±0.27[c] (6-11)	1.25±0.09[b] (1-2)	6.35±0.20[c] (5-8)	16.45±0.36[c] (14-19)

Means within each treatment followed by the same letter are not significantly at the 5% level of probability (L.S.D. test). - *the percentage of hatch.

Table 2. Longevity and fecundity of mated and unmated adult *T. absoluta* under two different room conditions.

Room conditions	Mean periods ±S.E (in days) Pre-oviposition	Oviposition	Post-oviposition	Longevity of Female	Male	No. of deposited eggs/♀	Undeposited eggs/♀
16.5˚C 77%RH	3.4±0.22[b] (2-4)	21.8±1.95[a] (12-31)	2.5±0.50[b] (1-5)	27.8±2.34 [a] (16-39)	25.9±2.50 [a] (16-39)	111.8±4.47 [a] (87-135)	4.4±1.71 [b] 0-15
21.6˚C 67%RH	2.3±0.15[b] (2-3)	20.7±1.83[a] (9-29)	4.3±0.95[b] (1-7)	27.3±2.13 [a] (19-34)	32.6±0.85 [a] (29-36)	126.2±10.51 [a] (75-180)	2.9±1.11 [b] (0-9)
Unmated 21.6˚C 67%RH	8.8±1.29[a] (4-15)	9.4±2.88[b] (1-20)	11.0±1.43[a] (5-17)	28.7±1.37 [a] (20-33)		5.1±0.82[b] (2-9)	20.3±2.78 [a] (10-33

Means within each treatment followed by the same letter are not significantly at the 5% level of probability (LSD test).

Also it found that the life span of mated females were shorter than that of unmated ones under the same condition. Also, it was found that the life span of males was longer than that of females under 21.6 ˚C and 67% RH.The number of eggs laid by mated females ranged from 75 to 180 eggs with the highest average (126.2 eggs/ female) at 21.6 and 67% RH, while the deposited eggs ranged from 2 to 9 eggs for unmated females under the same previous conditions. Statistically, there were significantly differences between means of all tested parameters for unmated females and that of mated females, while the differences between means of mated females under the different temperatures were insignificant.

Effect of some weather factors on sex pheromone trap catches

The rate of catches in the sex pheromone baited traps was very high during both study plantations compared with the chemical threshold of this pest (30 males/trap/week). In the first season, the males activity recorded sex peaks in the 3rd week of Oct., in the end of Oct., in the 2nd week of Nov., in the 3rd week of Nov., the 1st week of Dec. and in the 3rd week of Dec. the lowest peak was in the 3rd week of Nov. with 225 males/3 traps while the highest peak was in the 1stweek of Dec. with 550 males / 3 traps. The relation between the attracted males density, and maximum and minimum temperature degrees was significantly negative, while it was insignificantly positive with relative humidity. In the second season, the males trapping had seven peaks. The highest peak was in the 1st week of Nov. with 884 males / 3 traps and the lowest peak was in the first week of Dec. with 455 males / 3 traps. The effect of maximum temperature trap catches was insignificantly positive, while the effect of minimum temperature was significantly positive. The simple correlation was significantly negative between the captured males and relative humidity. Nowinszky*et al.* (2012) suggested that pheromone trap catches of *Anarsi alineatilla* Zeller and others of microlepidoptera are positively correlated with daily temperature.

Effect of trap designs

Data in Table (4), showed that trap catch affected by trap design.. The water pan trap was the most effective in capturing *T. absoluta* males(406.8 males/trap) followed by modified palm weevil bucket trap (336.9 males/trap). The trap design is one of the most important factors that affect trap catch. Water pan trap and modified palm weevil bucket trap were effectively captured and retained males. Modified palm weevil bucket trap is easily placed, examined and provided with water, as well as it's inexpensively and large capacity. Also, it was observed that the sticky trap was ineffectual in large numbers where we observed escape of moths because of trap saturation with moths or covering sticky surface with moth scales and dusts. Results agree with Valles *et al.* (1991) who stated that bucket trap was more effectively for capturing and retaining males and Houseweart *et al.* (1981) who

stated was quickly covering of sticky traps with moths and their scales reduces its effectiveness when large numbers of moths were attracted

Table (3).Three days counts of captured male moths of *T. absoluta* at yousef El-Sedek district, Fayoum Governorate, Egypt during 2013 and 2014 autumn plantations.

Date of inspection	No. of captured males	Weather factors			Date of inspection	No. of captured males	Weather factors		
		Temp. C		% RH			Temp. C		% RH
		Max.	Min.				Max.	Min.	
19/10/013	241	32.7	19.5	58	18/10/014	704	34.5	20.0	46
22/10	380	32.3	20.3	57	21/10	606	32.5	21.1	49
25/10	250	33.7	21.6	53	24/10	694	34.4	21.6	44
28/10	159	33.7	20.3	55	27/10	436	36.5	19.3	46
31/10	266	29.9	18.4	48	30/10	510	31.3	19.5	50
3/11	125	30.5	19.0	38	2/11	495	34.5	20.5	43
6/11	199	32.1	17.1	40	5/11	281	29.3	17.0	50
9/11	279	29.0	18.0	39	8/11	844	32.5	17.1	48
12/11	163	29.3	19.5	40	11/11	307	31.5	17.5	53
15/11	137	28.7	18.5	41	14/11	623	30.0	17.8	51
18/11	225	28.1	16.9	43	17/11	311	29.8	16.9	51
21/11	166	27.5	15.3	42	20/11	332	29.4	18.7	56
24/11	216	27.3	14.1	41	23/11	410	27.1	20.1	53
27/11	311	32.3	19.0	38	26/11	486	24.5	15.0	51
30/11	408	26.1	15.5	47	29/11	383	26.0	12.9	53
3/12	550	32.6	19.7	47	2/12	259	30.3	14.4	53
6/12	284	24.5	14.0	44	5/12	455	30.1	13.9	50
9/12	341	24.1	11.4	44	8/12	441	28.1	16.3	56
12/12	503	16.6	9.7	45	11/12	516	24.2	12.0	49
15/12	536	18.3	8.6	47	14/12	528	25.4	14.3	50
18/12	296	20.9	8.5	48	17/12	545	23.8	13.9	53
21/12	327	24.1	10.1	45	20/12	410	25.4	12.2	50
24/12	347	26.4	9.3	42	23/12	395	23.9	11.8	53
					26/12	298	24.3	10.8	54
					29/12	280	24.3	10.0	48
r		-0.473*	-0.434*	0.215			0.375	0.429*	-0.502*

Trap colors effect

Data obtained in Table (4), demonstrate the effect of trap color on attraction of males. The green colored traps baited with sex pheromone lures caught more males than the yellow, red and white ones,while the red colored traps were the least attractive. Effect of color factor on captured numbers of *T. absoluta* was insignificant among the four colors.

Trap heights effect

As shown in Table (4), the water pan traps baited with sex pheromone lures caught the highest number of *T. absoluta* males at 75 cm followed by 25cm height. The most effective heights (25 and 75cm) are at and just above canopy level. The differences between the means of captures males were significant between 75 and 175cm heights. This result wasstated in other Lepidoptera pests,where Herman *et al*., 2005 reported that the efficiency of both trap heights 0.3 and 1.0 m was equally in capturing *P. operculella* males with no significant differences.

Results in Table (4), indicated that the Tuta Optima lure was the most effective in catch *T. absoluta*, where the mean number of trapped males was 252.8 males per trap. The baited traps with local prepared lures were the lowest effective where the mean number of caught moths was 39.4 males / traps. Statistical analysis demonstrated that the differences between captured moths of the three lure types were significant.

Table 4. Effect of trap design, color, height and lure types on catch of *T. absoluta* under field conditions.

Parameters		Mean no. of captured males ± S.E
Trap design	Sticky delta trap	313.0±37.5^{b}
	Water pan trap	406.8±38.4^{a}
	Palm weevil bucket trap	155.6±14.0^{c}
	Modified bucket trap	336.9±31.7^{ab}
Trap color	Yellow	345.8±91.1
	Green	403.6±81.1
	Red	282.6±55.1
	white	363.0±69.4
Trap height	25cm	189.0± 26.8^{ab}
	75cm	207.9±23.0^{a}
	175cm	137.7±16.2^{b}
Lure types	TutaOptima®0.8mg	252.8±28.9^{a}
	Turkiyede Dretil lure 0.8mg	156.4±20.1^{b}
	Locally lure (0.5 mg)	39.4±4.3^{c}
	Without lure (0.0mg)	1.6±0.62^{c}

Means within each treatment followed by the same letter are not significantly at the5% level of probability

Effect of lure types

Effect of photophase and scotophase periods on male *T. absoluta* capture by sex pheromone traps

Results in Table (5), indicated that the catches of male moths started from the last hour of scotophase and increased before 30 minutes of sunrise. The attraction was higher in the 1st hour of photophase than that of the 2nd hour where the trapped moths in the 1st hour represented 5.8 folds of that of the 2ndhour. Also the highest catch was recorded with the moment of sunrise (67.1 males / trap). On the other hand, the attracted males in the first two hours of the photophase represented 6.3 times of the captured males in the scotophase and 86.3 % of the total captured males per day. Statistically there were significant differences in means of captured males between that of performed inspection at sunrise period (6.35-6.45) and the other inspections, except that of the 1st one.

Table 5. Average number of *T. absoluta* males attracted by sex pheromone traps during day hours.

Periods of inspection	captured males / trap Mean ± S.E	%
6.00 – 6.15 AM*	29.7±8.3^{c}	12.5
6.20 – 6.30 AM	44.5±9.3^{ab}	18.7
6.35 – 6.45 AM	67.1±14.4^{a}	28.2
6.50 – 7.00 AM	33.9±5.5^{bc}	14.3
7.00 – 8.00 AM	30.0±9.4^{bc}	12.6
Scotophase period	32.7±3.1^{bc}	13.8
Total / 24hr	237.8±26.4^{a}	

Means followed by the same letter are not significantly different at the 5% level of probability (L.S.D. test) - *Sunrise (6.29-6.37), sunset (4.59-4.52)

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دراسات بيولوجية وفرمونات جنسية على صانعة أنفاق أوراق الطماطم *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) في محافظة القيوم ، مصر .

ربيع جسن عوض سليمان

قسم وقاية النبات – كلية الزراعة – جامعة الفيوم

أظهرت نتائج الدراسة أن فترات نمو أطوار البيض و اليرقات و ماقبل العذراء و العذراء كانت ٦.٧٠ ، ١٤.٧٥ ، ٢,٢٠ ، ١٢,٨٠ يوم على درجة حرارة ٢٠م° و رطو بة نسبية ٦٥% . كما كانت فترة حياة الأنثى و الذكر ٢٧,٣٠ و ٣٢,٦٠ يوم على درجة حرارة ٢١.٦ و رطوبة نسبية ٦٥ %. تراوح عدد البيض الموضوع للأنثى الواحدة من ٧٥ - ١٨٠ بيضة. أما يخصوص المصائد الفرمونية في الحقل فقد سجل التعداد المصطاد ٦ قمم في الموسم الأول و ٧ قمم في الموسم الثاني و كانت أعلى القمم في الإسبوع الأول من ديسمبر و الإسبوع الأول من نوفمبر بأعداد ٥٥٠ و ٨٨٠ ذكر للمصيدة خلال الموسم الأول و الثاني على التوالي. من ناحية أخري كانت المصيدة المائية water pan trap الأكثر فاعلية (٤٠٦ ذكر للمصيدة) يليها مصيدة سوسة النخيل المعدلة (٢٢٦ ذكر /مصيدة) . أيضا وجد ان المصائد خضراء اللون جذبت ذكورا أعلى مقارنة بالمصائد الصفراءو الحمراء و البيضاء و لكن الإختلافات بين متوسطات الإصطياد كانت غير معنوية . أما بالنسبة للفرمونات فكانت كبسولة *Tuta optima*® الأكثر كفاءة (٢٥٢ ذكر /المصيدة) بينما كانت الكبسولة المعدة محليا الأقل (٣٩.٤ ذكر للمصيدة) . و قد وجد أن الإصطياد كان الأكثر في الساعة الأولى من الإضاءة photophase عن تلك الخاصة بالساعة الثانية حيث مثلت الذكور المصطادة ٥.٨ مرة من تلك المصطادة في الساعة الثانية.