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Native larval parasitoids of fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae), the recent invasive pest on maize in Egypt and its some biological aspects

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Abstract:

The fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) is the recent invasive pest species, it spreads successfully across Kom-Ombo city of Aswan Governorate, Upper Egypt in May 2019. It disrupts agriculture, particularly smallholder grain production such as maize cultivation. This study was undertaken to document two parasitoids, *Exorista sorbillans* (Wiedemann) and *Pseudogonia rufifrons* (Wiedemann) (Diptera; Tachinidae) and one parasitoid species *Microplitis* sp. (Hymenoptera: Braconidae), were detected from infected FAW larvae which, were collected from maize fields during August, September, and October. The highest parasitism rate was recorded on 1st October in two locations at Aswan by 30.77%. Moreover, some biological aspects of FAW were recorded, which an average of 156.13 ±16.57 eggs/mass were emerged after an average of 3.47 days, with hatchability of 89.18%. Larval and pupal average duration were 20.93 and 12.60 days, respectively, pre-ovipostion, oviposition and post-oviposition were 11, 5.13 and 4.93 days respectively.

Introduction

Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) outbreak was recorded for the first time in early 2016 in West and Central Africa and have currently become the new invasive species in Africa (Goergen *et al.*, 2016). The insect continued invasion and spread across African countries until it was registered in Kom-Ombo city of Aswan Governorate, Upper Egypt in May 2019. FAW is a polyphagous pest, but its greatest harm in Egypt affects maize plants. Many governments have relied on synthetic pesticides, including highly toxic ones, to control the spread of the insect, such as Ethiopia and Kenya, and the effect differs in both (Prasanna *et al.*, 2018 and Kumela *et al.*, 2019). Among the environmental alternatives that have previously been used for combating FAW is biological control, especially parasitoids **(**Sisay *et al.*, 2018 and Agboyi *et al.*, 2020). The invasive species often invade a new environment without their natural enemies, which promotes their multiplication and damage to crops. Since, the arrival of the pest to Africa, many studies have been conducted on its natural enemies, which have confirmed the presence of parasitoids of eggs and larvae (Koffi *et al.*, 2020; Castillo, 2020; Kenis *et al.*, 2019; Amadou *et al.*, 2018; Sisay *et al.*, 2018 and Agboyi *et al.*, 2020). The invasive FAW is close to several of local pests on maize and sorghum by family Noctuidae such as *Eublemma gayneri* Hübner and *Helicoverpa armigera* (Hübner), or close in terms of the genus, *Spodoptera* such as *Spodoptera littoralis* (Boisd.) and *S. exigua* (Hübner) (El-Gepaly, 2019 and Youssef, 2018), those local pests have a rich array of indigenous natural enemies, which have an opportunity to fight *S. frugiperda*. Hence, there is a need to identify the adapting natural enemies of fall armyworm in Egypt, which could be used for its IPM in the future.

Tachinidae of Diptera and Braconidae of Hymenoptera are important parasitoid families that attack a wide range of agricultural pests around the world. El-Hawagry (2018) summarized the distribution of *Exorista sorbillans* (Wiedemann) and *Pseudogonia rufifrons* (Wiedemann) around the world, African countries, and Egypt. Sharanabasappa *et al.*, (2019) recorded negligible levels of parasitism of FAW by *E. sorbillans* in South India. Gözüaçik *et al.* (2007) found *P. rufifrons* as a larval- pupal parasitoid of *Acantholeucania loreyi* (Duponchel) (Lepidoptera: Noctuidae). Also, Braconidae of Hymenoptera recorded on Lepidoptera hosts including *S. littoralis* in many countries including Egypt (Hammad *et al*., 1965; Shalaby, 1968; Ibrahim, 1974 and Abou Zeid *et al.*, 1978).

This study was undertaken to document three parasitoids of fall armyworm and record the field parasitism in Kom-Ombo, Aswan, Egypt. Also, highlight some biological aspects of FAW.

Materials and methods

1. Larval parasitoid detection:

Natural enemies of fall armyworm larvae were surveyed by collecting host larvae from maize fields in four farmer fields located around Kom-Ombo (Munihah, El-Bayyarah, Iqlit, and El-Sapeel), Aswan Governorate. Fertilizers and weed control were applied in all fields during planting and hilling. Forty FAW larvae ($20 \leq 3^{rd}$ larval instar and $20 \geq 4^{th}$ larval instar) were collected during August, September, and October 2020. Each larva was reared in a transparent plastic container tightly sealed with a silk cloth (50 ml capacity) under an average temperature of 26±2 ºC and RH. %=50±5%. Daily, the transparent container was examined from the outside to ensure the presence of parasites, data were recorded, Castor leaves were introduced daily as a feed, and jars get cleaned till parasitoid emergence or normal pupation. Dead pupae due to unknown reasons or bad handling were subtracted from totals. The percentage of parasitism was calculated according to the occurrence of parasitism (Emergence of larval parasitoids from FAW larvae), even if the parasitoid adults not occurred. Samples were preserved appropriately and transferred to identify.

2. Taxonomic Studies:

Insect specimens that were used for the taxonomic study were collected from the injured Maize plants in four locations in Kom-Ombo (Munihah, El-Bayyarah, Iqlit and El-Sapeel), Aswan, Egypt during the 2020 maize season. Material examined were; one parasitoid from one late larva at Munihah, El-Bayyarah and El-Sapeel areas on 1st Oct, three parasitoids from one late larva at Iqlit area on 1st Oct and 16 parasitoids from one early larva at Iqlit area on 2nd Sept. The specimens were killed by Ethyl acetate, and then pinned or preserved in vials or drier Eppendorf by freezing at -4Cº till used for easily dissection and study their parts for explained taxonomic characters. An illustrated key to the subfamilies, tribe, genera, and species is presented. The Identification occurs at the classification Department, Plant Protection Research Institute. Each identified species measured by an ocular micrometer after calibration attached to the dissecting microscope Olympus (Stereomicroscope) for photography of the specimens. The identified specimens were deposited in the side Collection of the Classification Department – Plant Protection Research Institute.

3. Biological aspects of fall armyworm *Spodoptera frugiperda*:

Biological aspects of FAW were carried out in Shandaweel Bio-control Lab., Bio-Control Department, Plant Protection Research Institute, Agricultural Research Center, at Shandaweel Research Station, Sohag Governorate, Egypt. (Latitude 26˚ 38¯ 05˭ N, Longitude 31˚ 39¯ 31˭ E) under an average temperature of 26±2 ºC and 50±5% RH. in October and November 2020. Fifteen egg clusters were obtained from existing colony for the study of some biological aspects of FAW. The experiment was carried out at controlled temp. (25±2 ºC) and RH.% (55±5%) room, in early October 2020. Data were recorded every day; the newly hatched larvae were transferred immediately upon hatches to avoid their predation of the remaining eggs. Each ten newly hatched larvae (For each egg cluster) were kept together in a 2-liter plastic jar covered with fine close fabric. Cutting of castor leaves were used for feed 1^{st} – 3^{rd} larval instars and replaced daily, the oldest instars were supplied with five castor leaves, so that the ten larvae were distributed between the leaves to avoid cannibalism. Daily observations were made, and larval development and duration were estimated. Pupal periods were estimated by isolated each pupa in a plastic tube (2 X7 cm) covered with a piece of cotton until the adults emerged. Some biological aspects such as, incubation period, total immature stages, life cycle, longevity, sex ratio, generation, and life span were recorded,

4. Statistical analysis:

Standard error for each biological parameter of FAW was calculated to declare data dispersal data around the means.

Parasitism ratio was calculated according to the following formula:

$$\textbf{Parasitsim}\% = \frac{\boldsymbol{No.\ of\ parasitized\ larvae * 100}}{\boldsymbol{Total\ collected\ no.-(parasitized + dead)}} \boldsymbol{x100}$$

Results and discussion

1. Parasitism ratio:

The percentage of parasitism on fall armyworm larvae was measured as soon as any of the parasitoid stages were observed during the breeding of the collected FAW larvae. Only 6 of 36 samples of parasitoid stages were developed into the adults, which will be accurately described in the next section. Data in Table (1) discuss the parasitism ratio on FAW larvae (≥3^{rd} instar and ≤ 4^{th} instar) during the last three months of maize season, 2020 in four locations at Kom-Ombo, Aswan, Egypt.

Parasitism starts to appear on 2^{nd} August sample of El-Bayyarah location on late larvae, where one pupa of the parasitoid was exited from larvae with 5.56% parasitism from pupated larvae. Parasitized larvae were continued to record at a relatively higher rate during the last three examination dates (for large larvae), and the highest parasitism rates were recorded in the last examination date in Iqlit and El- Sapeel locations by 30.77%. Only two of the small larvae that were collected showed signs of parasitism (Without the adults leaving the parasite for unknown reasons) in all sites and often in the last two examinations, with 11.76% parasitism as the highest rate in Iqlit on 2^{nd} September.

Table (1): Parasitoids and parasitism rates resulting from incubation of 40 larvae from four sites in Kom-Ombo, Aswan, Egypt in 2020 maize season.

Sample	early larvae	Following				late larvae	Following			
		Developed to Pupae	Dead	parasitized	Parasitism%		Developed to Pupae	Dead	parasitized	Parasitism%
Munihah										
1st Aug	**20**	20	0	0	0.00	**20**	18	2	0	0.00
2nd Aug	**20**	18	2	0	0.00	**20**	19	1	0	0.00
1st Sept	**20**	20	0	0	0.00	**20**	18	0	2	11.11
2nd Sept	**20**	15	4	1	6.67	**20**	18	1	1	5.56
1st Oct	**20**	14	5	1	7.14	**20**	17	0	3*	17.65
El-Bayyarah										
1st Aug	**20**	17	3	0	0	**20**	20	0	0	0.00
2nd Aug	**20**	18	2	0	0	**20**	17	2	1	5.88
1st Sept	**20**	17	2	1	5.88	**20**	15	3	2	13.33
2nd Sept	**20**	16	3	1	6.25	**20**	16	3	1	6.25
1st Oct	**20**	19	1	0	0	**20**	14	4	2**	14.29
Iqlit										
1st Aug	**20**	18	2	0	0.00	**20**	19	1	0	0.00
2nd Aug	**20**	18	2	0	0.00	**20**	18	2	0	0.00
1st Sept	**20**	18	2	0	0.00	**20**	16	2	2	12.50
2nd Sept	**20**	17	1	2	11.76	**20**	16	2	2***	12.50
1st Oct	**20**	19	1	0	0.00	**20**	13	3	4**	30.77
El- Sapeel										
1st Aug	**20**	17	3	0	0.00	**20**	17	3	0	0.00
2nd Aug	**20**	18	2	0	0.00	**20**	17	3	0	0.00
1st Sept	**20**	15	4	1	6.67	**20**	16	2	2	12.50
2nd Sept	**20**	16	3	1	6.25	**20**	15	4	1	6.67
1st Oct	**20**	17	2	1	5.88	**20**	13	3	4**	30.77

Parasitoids that completed their life cycle

*** *Exorista* (*Podotachina*) *sorbillans* (Wiedemann)** **** *Pseudogonia rufifrons* (Wiedemann)** ***** *Microplitis* sp. Foerster**

2. Taxonomical study:

Three larval parasitoids belonging to order Diptera (Two parasitoids) and Hymenoptera (One parasitoid) were detected from FAW larvae that collected from maize fields at Kom-Ombo district in the 2020 season. These parasitoids were:

2.1.*Exorista* (*Podotachina*) *sorbillans* (Wiedemann, 1830) :

Exorista (*Podotachina*) *sorbillans* **(**Wi-edemann, 1830)

Tachina sorbillans Wiedemann, 1830: 311. Lectotype male (NHMW). Canary Islands (Tenerife). **Order:** Diptera **Family:** Tachinidae **Subfamily:** Exoristinae **Tribe:** Exoristini

Distribution: AF: Cameroon, Canary Is., D.R. Congo, Kenya, Malawi, Nigeria, Sierra Leone, Uganda. OR: India, Indonesia, Nepal, Orien. China [see O'Hara and Cerretti 2016: 19], Philippines, Ryukyu Is., Sri Lanka, Taiwan, Thailand, Vietnam. Australasian: Australia, N. Australasian. PA: C. Asia (Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan), Egypt, Europe, Israel, Japan, Mongolia, and China. Egyptian localities and dates of collection are not precisely known.

The larvae (which were collected for parasitoids detection) were externally examined, two groups of parasitoid eggs Figure 1 (A and B) were observed attached to the head of one larva and another group were attached between the fourth and fifth abdominal segment of the same larva that collected from Munihah area in 1st October 2020. Parasitoid eggs hatched to maggot which immediately penetrated larval cuticle and spend 9 days before the exit of the parasitoid pre-pupae stage outside the caterpillar which turns into pupae within 30 hours. (Figure 1D). Parasitoid pupae were taken 3 days for the emergence of the adult parasitoid Figure 1 (E-J), as the parasitoids were preserved and transferred to the monitoring and Classification Research Department of the Plant Protection Research Institute through the reference insect group. Below the full taxonomic description of these species:

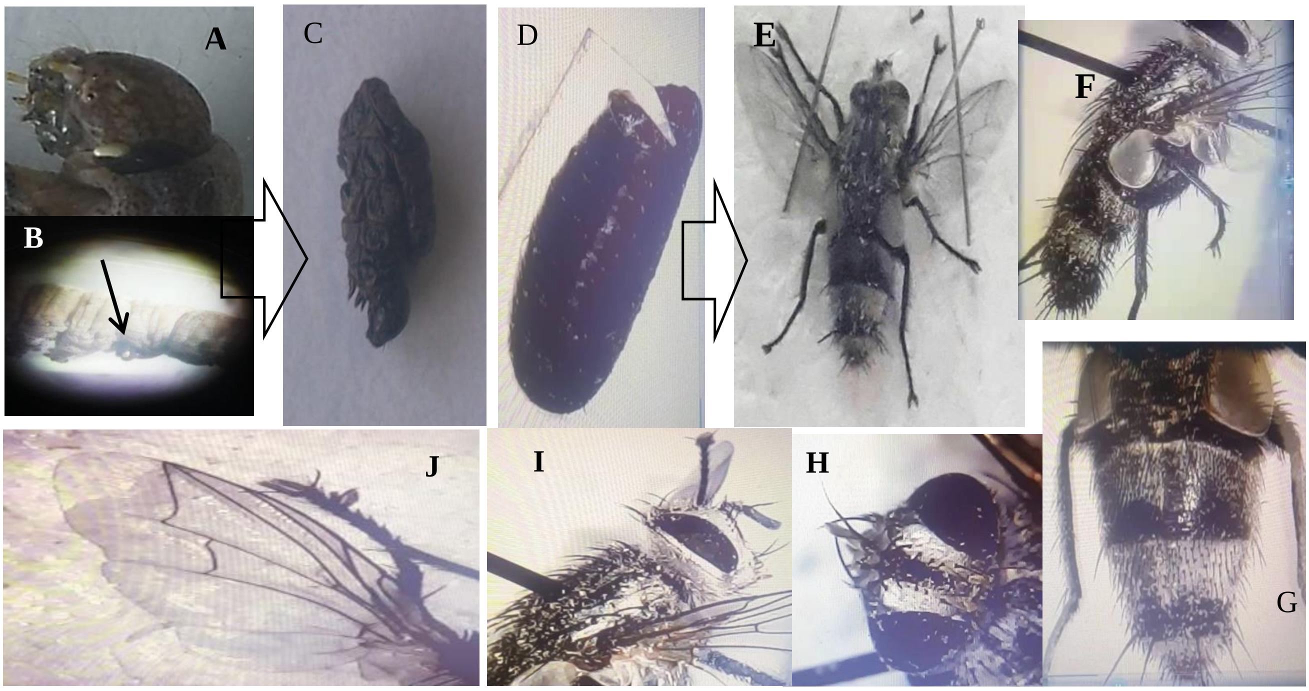

Figure (1): The parasitoid, *Exorista sorbillans* on *Spodoptera frugiperda* larva. A. An egg of parasitoid attached to head capsule. B. Eggs of parasitoid attached between the 4th and 5th abdominal segment. C. Dead larva D. Parasitoid *E. sorbillans* cocoon. E. Dorsal view of adult *E. sorbillans.* F. Lateral view of *E. sorbillans.* G. Dorsal view of *E. sorbillans* abdominal segments. H. Anterior view of *E. sorbillans* head. I. lateral view of *E. sorbillans* head. J. Wing venation.

2.2. *Pseudogonia rufifrons* (Wiedemann)

During the study, one pre-pupal stage of the parasitoid was emerged from two 5th instar of the fall armyworm larvae (Solitary behavior as usual and adult was in healthey form "Figure 2-E") at El-Bayyarah and El-Sapeel areas on 1st Oct., also three pre-pupal stages of parasitoids were emerged from one 5th instar (gregarious behavior and adult was distorted "Figure 2-H") of the fall armyworm larvae at Iqlit area on 1st Oct. Pre-pupae turn into pupae within 6 hrs. Figure 2 (C and D). Parasitoid pupae were taken three days for the emergence of the adult parasitoid (Figure 2 E-H), as the parasitoids were preserved and transferred to the monitoring and Classification Research Department of the Plant Protection Research Institute through the reference insect group. Below the full taxonomic description of this species:

Pseudogonia rufifrons **(Wiedemann)**

Tachina rufifrons Wiedemann, 1830: 318. Lectotype female (ZMUC). China.

Order: Diptera **Family:** Tachinidae

Subfamily: Exoristinae **Tribe*:*** *Exoristini*

Distribution: AF: Widespread, including Cape Verde, Nigeria, South Africa, Tanzania, U.A. Emirates, Yemen. AU: Australia, Hawaii, Melanesia, N. Australasian. OR: India, Indonesia, Malaysia, Myanmar, Orien. China (O'Hara and Cerretti 2016), Pakistan, Philippines, Ryukyu Is., Taiwan, Thailand. PA: C. Asia (Kyrgyzstan, Tajikistan, Turkmenistan, Uzbekistan), Egypt, all Europe (except British Is.), Japan, Kazakhstan, Korea, M. East (Israel), Mongolia, Pal. China (O'Hara and Cerretti 2016), Russia, and Transcaucasia. Egyptian localities: Coastal Strip, Mariout, Nuzha, Eastern Desert, Wadi Hoff, Gebel Elba, Wadi Edeib, Lower Nile Valley, Delta: Abu Rawash, Sinai, and Wadi El-Tih.

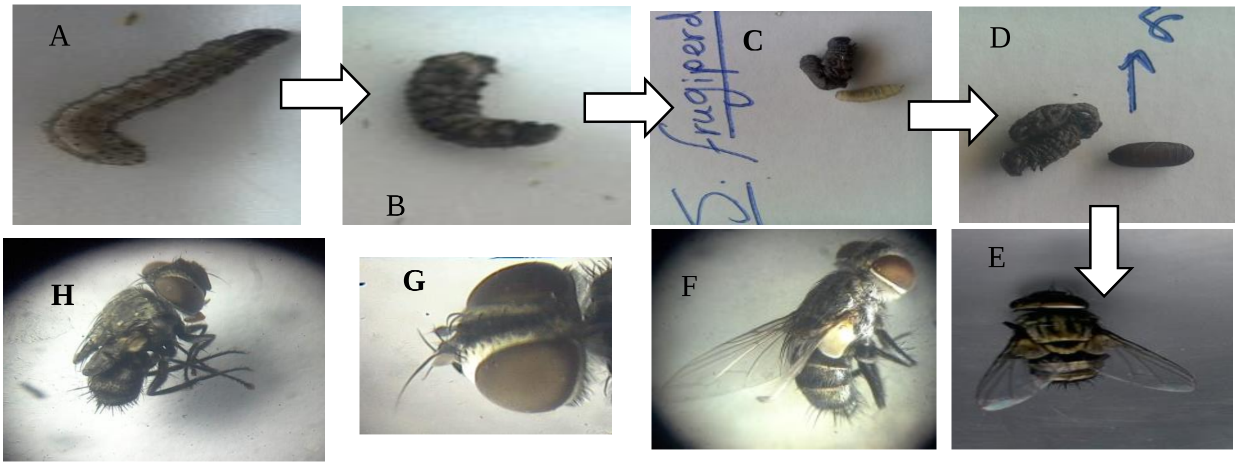

Figure (2): The parasitoid, *Pseudogonia rufifrons* on *Spodoptera frugiperda* larva. A. Infected *Spodoptera frugiperda* larva. B. Dead *Spodoptera frugiperda* larva. C. Maggot of *P. rufifrons* existing of dead *Spodoptera frugiperda* larvae. D. Pupa of *P. rufifrons*. E . Dorsal view of adult *P. rufifrons.* F. Lateral view of adult *P. rufifrons.* G. Interior view of *P. rufifrons* head. H. Dorsal view of distorted adult of *P. rufifrons.*

2.3. *Microplitis* sp.

16 pupae of parasitoid were exerted from one larva from Iqlit area on 2nd Sept 2020 (Figure 3-A), these pupae were emerged to hymenopteran parasitoids after three days of collection Figure 3 (C-F), as the parasitoids were preserved and transferred to the monitoring and Classification Research Department of the Plant Protection Research Institute through the reference

insect group. Below the full taxonomic description of this species:

***Microplitis* Foerster, 1862**

Type-species: *Microgaster obscure* Nees. Orig. design.

Order: Hymenoptera **Family:** Braconidae **Subfamily:** Microgastrinae Foerster, 1862 **Tribe*:*** Microgastrini Foerster,

Genus *Microplitis* can be recognized by a large areolet, mesopleuron without prepectal carina, roughly sculptured propodeum often with a median longitudinal carina, propodeum evenly curved in the lateral view, shape, and sculpture of first metasomal tergite, and with a weakly defined groove separating second and third tergum (Nixon, 1965; Mason, 1981; Austin and Dangerfield, 1992; 1993). Genus *Snellenius* Westwood was re-described by Mason (1981).

Distribution: *M. rufiventris* Kok. was recorded in Turkestan by Kokujev (1914) parasitizing *Spodoptera littoralis* Boisd. Also, recorded on *Heliothisarmigera* Hb. And *S. exigua by* (Thompson, 1946 and Gerling, 1971) who reported that this parasitoid was distributed in both Middle East and Europe where it attacks several noctuid species.

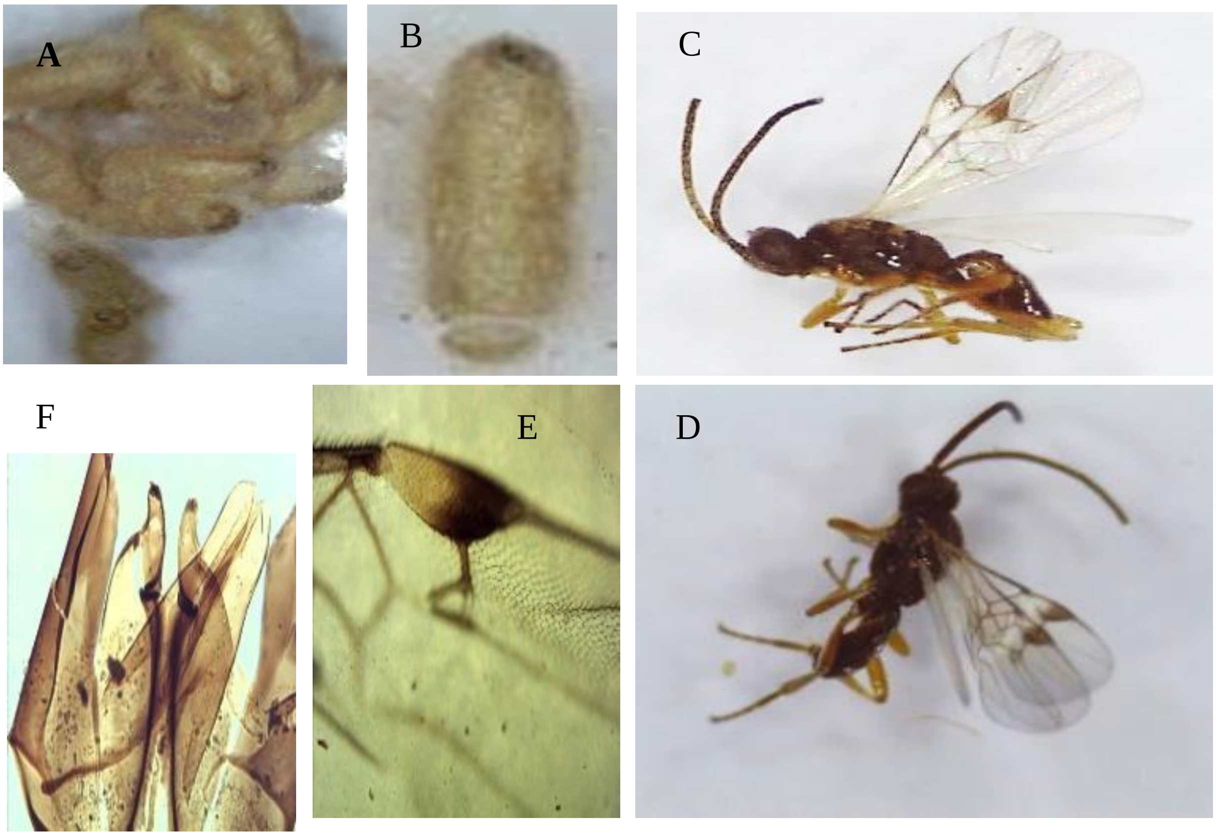

Figure (3): The parasitoid *Microplitis* sp, on *Spodoptera frugiperda* larva. A. Pupae emerged from *Spodoptera frugiperda* larva. B. An empty cocoon. C and D. Adult of *Microplitis* sp. E. Wing venation. F. Female genital system.

3. Biological aspects of *Spodoptera frugiperda* :

Data presented in Table (2) refer to some biological aspects of fifteen FAW egg masses until the death of the adult insects. No eggs were arranged between 49 – 258 eggs with an average of 156.13±16.57, these eggs were started to emerge from the 3rd day after laying to the 4th day with an average of 3.47 ±0.13 days, as the incubation period. Hatchability was ranged between 80-100% with an average of 89.18 ±1.42%. The average duration of larvae and pupae were20.93±0.68 and 12.60±0.34 days, respectively. Adults were emerged after approximately, 33.53 days from hatching (Larval and pupal durations) as total immature stages, so that the life cycle is about 37.76 days (by combining the incomplete life cycle with the incubation period of the eggs). Pre-ovipostion period reached 4.93±0.23days, bringing the average generation to 42.69 days. Oviposition and post-oviposition were recorded 11±0.31 and 5.13±0.24, respectively.

In earlier studies, Igyuve1 *et al*. (2018), Sharanabasappa, *et al*. (2018), Dahi *et al*. (2020) and Gamil (2020) reported that the incubation period was 2, 3, 3.4 and 2.29 days respectively, however, in this study the average incubation period was 3.47 ±0.13 days. In partial or full agreement with the present results, Gamil (2020**)** recorded 97.33% of eggs were hatched to larvae still 9.56 days for pupation which in turn lasted 9.56 days. Also, Dahi *et al*. (2020**)** mentioned that, larval duration was 23.7 days, pupal duration was 9.4 days while, in this study, mean hatchability, larval and pupal duration were 89.18 ±1.42% while, 20.93±0.68 and 12.60±0.34 days, respectively. The differences between studies and the present data could be attributed to the difference in the pest strains, the difference in food varieties used for larval feeding, or the different temperature and RH.% used.

The results of Tendeng *et al.* (2019) showed that the total duration of *S. frugiperda* cycle is between 22 and 28 days with an average of 25 days at 25 °C. Which gives on average 15 generations a year. Castro and Pitre (1988) have shown that *S. frugiperda* development cycle is between 28 to 38 days when the pest is fed with sorghum and 35 to 45 days when fed with corn. The FAW is a formidable invasive pest as it has a rapid development cycle that varies with temperature (Chapman *et al*., 2000 and Barros *et al*., 2010).

Table (2): Some biological aspects of *Spodoptera frugiperda* under laboratory condition at Sohag Governorate.

Egg mass	No. of eggs	Incubation period/Days	Hatchability %	No. of Larvae	Larval Period/Days	Pupal period/Days	No. of Pupae		No. of Emergence Adults		Emergency %	Sex		Male Longevity/Days	Female Longevity/Days	Pre-Ovipostion/Days	Oviposition/Days	Post-Oviposition/Days
							Healthy	malformation	Healthy	malformation and dead pupae		Male	Female					
1	125	3.5	84.00	105	20	12	89	6	86	3	96.63	36	50	15.5	22.5	4	12	6
2	141	4	85.82	121	23	14.5	107	4	102	5	95.33	45	57	13.5	23	5	13	5
3	89	4	95.51	85	19.5	15	82	3	80	2	97.56	31	49	12.5	18.5	5	9	4
4	85	3	92.94	79	21.5	13.5	79	0	73	6	92.41	33	40	13.5	20	4	11	5
5	49	3	91.84	45	23.5	14	44	1	38	6	86.36	12	26	14.5	22	6	11	5
6	150	3	100	150	19	12.5	147	3	143	4	97.28	60	83	15.5	21	4	12	5
7	236	4	90.68	214	17	10.5	210	4	208	2	99.05	95	113	14.5	23	4	12	7
8	245	3	93.06	228	20	11	222	6	217	5	97.75	97	120	12.5	20	5	10	5
9	258	3	89.53	231	20	11.5	226	5	221	5	97.79	104	117	13.5	20	6	10	4
10	186	4.5	81.72	152	24	13	150	2	148	2	98.67	63	85	14.5	20.5	5	9	6
11	165	3.5	80.00	132	23	13	128	4	126	2	98.44	60	66	15.5	21.5	4	12	6
12	235	4	84.68	199	23.5	12.5	179	7	175	4	97.77	52	123	13	21.5	6	11	4
13	147	3	85.71	126	24.5	12	121	5	118	3	97.52	50	68	13.5	22	6	11	5
14	125	3	93.60	117	20	13	115	2	112	3	97.39	52	60	14.5	22	4	12	6
15	106	3.5	88.68	94	15.5	11	91	3	88	3	96.70	34	54	14	20	6	10	4
Mean ±SE	**156.13 ±16.57**	**3.47 ±0.13**	**89.18 ±1.42**	**138.53 ±14.71**	**20.93 ±0.68**	**12.60 ±0.34**	**132.67 ±14.38**	**3.67 ±0.5**	**129 ±14.41**	**3.67 ±0.37**	96.44 **±0.83**	**54.93 ±6.81**	**74.07 ±8.09**	**14.03 ±0.26**	**21.17 ±0.34**	**4.93 ±0.23**	**11 ±0.31**	**5.13 ±0.24**

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