LABORATORY STUDIES ON *COCCOPHAGUS SCUTELLARIS* (DALMAN)(HYMENOPTERA : APHELINIDAE), PARASITOID OF THE SOFT SCALE INSECT, *PULVINARIA TENUIVALVATA* (NEWSTEAD), A PEST OF SUGAR CANE IN EGYPT

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Abstract

The aphelinid *Coccophagus scutellaris* parasitizes the coccid *Pulvinaria tenuivalvata*, one of the most serious pests of sugar cane. The different stages of the parasitoid were described and illustrated .The mean incubation period of the parasitoid egg was 2.75 days. The mean durations of the larval stages, prepupa and pupa were 16.19, 1.25 and 8.96 days, respectively. This aphelinid species is a primary endoparasitoid and attacks the pest throughout its development. It is also shown that *C.scuttelaris* prefer the second and third nymphal instars to any of the other stages host. Female laid an average of 67 eggs and lived for an average of 13.5 days.The females lived longer than males (7.3 days). The sex ratio of *C. scutellaris* recoded 1:4.6 as males : females. One or more adult parasitoids developed successfully in one host. The maximum number of adult parasitoids emerged from one individual host was ten, depending on stage of the host. Percentage of parasitism was 95% in 2nd instar nymph of *P. tenuivalvata*. It is concluded that *C. scutellaris* can be considered as a potential biological control agent for this insect.

INTRODUCTION

The aphelinids represent one of the most interesting groups among the parasitic Hymenoptera. Most aphelinids are primary parasites of Sternorhynchus Homoptera (Aphidoidea, Aleyrodoides and Coccoides); a few are known to develop on other hosts (eggs of Orthoptera and Lepidoptera; pupae of Diptera). Their bioecology shows some peculiarities that demonstrate the most advanced strategies for reproduction, biocenotic interdependence, and competition. Moreover, several species are important regulators of pest populations and maybe successfully used in biological and integrated control (Viggiani 1981; Civantos and Sanchez 1993; Velimirovic 1994).

Coccophagus is cosmopolitan genus in the family aphelinidae (Viggiani 1994). Many of its species, which are frequently encountered parasitoids of soft scales, have been used successfully in biological control programs in various parts of the world (Ken-

nett *et al.* 1966; Prakasal *et al.* 1987; Moglan and Moglan 1997; Viggiani 1999).

In the present study, the parasitoid *Coccophagus scutellaris* (Dalman) was recorded for the first time in Egypt on the red – striped soft scale insect *Pulvinaria tenuvalvata* (Newstead) (Hemiptera: Coccidae) infesting the sugar cane at Luxor (Quena Governorate) and Atfieh (Giza Governorate) districts. Specimens of the parasitoid were sent to Dr. Gregory Zolnerowich , Department of Entomology Kansas State University who identified it as *C. scutellaris* (Dalman). This parasitoid was found to be an endo-parasitoid on *P. tenuvalvata*. In the present paper, the description and duration of the different immature stages of this parasitoid and some biological characteristics were studied.

MATERIALS AND METHODS

Host rearing: A stock culture of the soft scale insect *P. tenuivalvata* was collected from Luxor (Quena Governorate) and Atfieh (Giza Governorate) districts. The soft scale was reared on sugar cane seedlings kept under the laboratory conditions.

Rearing of the parasitoid: Rearing unit, which was used to maintain the parasitoids, consists of a transparent plastic cylinder cage measured 17 cm in diameter and 50 cm height. The cylinder was placed over the previous infested sugar cane seedlings. The upper opening of the cage was covered with muslin cloth kept in position by a rubber band. Droplets of honey were scattered on the inner surface of the cage as a source of food for the adult parasitoids. The seedlings inside the cages were exposed to adequate number of the adult parasitoids, which removed after 24 hr.

Biological experiments: To study the durations of the immature stages of the parasitoid *C. scutellaris*, adequate pairs of newly emerged adults were introduced into a cage ,which has some sugar cane seedlings infested with the host. The cage was provided with honey droplets on the inner surface and left for 24 hr, then the exposed parasitized scale insects were daily replaced with others new till death of the parasitoid adults. Regardless of the sex, the scales were examined daily, by dissecting under a stereomicroscope to record the durations of the different stages of the parasitoid, till emergence of the parasitoid. The different stages were also measured and drawn by using a squared glass lens at microscope of different lens powers.

Host preference and number of individuals per one host: To determine the preferred stage of the soft scale insect for *C. scutellaris* and the number of parasitoids developing in a single host under laboratory conditions, the following stages were

offered simultaneously; a) 2nd nymphal instar, b) 3rd nymphal instar, c) Newly developed adult females, d) Full grown adult females. The stages were obtained by infesting sugar cane seedlings with newly hatched nymphs of the soft scale on successive days. Heavily infested seedlings harboring all the abovementioned stages of the scale were placed under transparent plastic cylinder cage (17x90 cm) and covered with a piece of organza cloth fixed with a rubber band. A large number of parasitoids was introduced into the cage, which was provided with droplets of honey on the organza cloth. These stages of the soft scale insect were exposed to the parasitoids for 24 hr; then each seedling was put into a separate glass jar, which was then closed with fine organsy mesh. Daily checks were made for emergence of parasitoid. The number of emerged parasitoids and the number of different host stages with emergence holes, from which they emerged, were recorded

Sex ratio: Eight sugar cane seedlings of 30 cm height, harbored different stages of the soft scale insect, each was put under transparent plastic cylinder cage (90 x 17cm) and five pairs of newly emerged parasitoid adults were placed into each cage. The cage was covered with a piece of organza cloth fixed with a rubber band. Each cage was supplied with honey droplets and left for 24 hr. The cage was opened, the adult parasitoids were gathered out and the seedling contained parasitized scales was left until emergence of parasitoids. The emerged parasitoids were classified into females and males.

Number of eggs / female parasitoid: A pair of newly emerged adults was introduced into a plastic cylinder as described before. Five replicates from this unit were used and left until death of the adults. The parasitized scales were dissected under a stereomicroscope to count number of eggs deposted by one female.

RESULTS AND DISCUSSION

Egg: The freshly deposited egg is typically hymenopterine in shape. It is translucent white, elongate – ovate, slightly bent toward one side with one end slightly broader than the other. The chorion is smooth and translucent. The length ranged between 0.20 and 0.25 mm with an average of 0.24 ±0.016 mm. The width [at the widest part] ranged between 0.050 and 0.061mm with an average of 0.055 ± 0.007 mm, Table 1and Figs. 1&2.

Larva: *C.. scutellaris* has three instars; tend to be homogenous in shape and structure, Fig.1, the dimensions of these instars are given in Table 1.

First instar:

As shown in Table 1 and Fig 1, the newly hatched larva is of caudate hymenopterous form, it has translucent white color changed to opaque white as a result of feeding on the host. The body has 13 segments without any spiracles and the last segment is very much attenuated to constitutes the tail. The body measure averaged about 0.39 mm in length and about 0.079 mm in width. The head-capsule measured about 0.046 and 0.065 mm in length and in width, respectively. Mandibles are well developed and each measures 0.0098 mm in length and 0.0065 mm in width. *C. scutellaris* male is a secondary endoparasitoid of *P. tenuivalvata* scale . The first instar is a teleaform .

Second instar: The second instar larva has an elongate body, with a long tail.. It is opaque-white, with yellow stomach contents. The larva, upon exposure, usually assumes a bent position more or less like the letter C. There are 13 distinct body segments without any spiracles, as in the first instar. It shows reduction of the mandibles. These results are in agreement with Viggiani (1984) who indicated that in endophagous species of aphelinids the second instar larva shows reduction of the mandibles. Average dimensions of body of the second instar larva are as 0.78 mm in length and 0.27 mm in width. The average length of the head capsule is 0.130 ± 0.022 and the average width is 0.115 ± 0.0002 mm, Table 1 and Fig. 1.

Third instar: It is rather similar to the subsequent instar .It is opaque white, with yellow stomach contents occupying most of the body cavity and body becomes curved and notably swollen. The head is distinct, slightly retractable and is followed by 13 body segments. The tracheal system is well developed. There are nine visible pairs of spiracles in the larva on segments two through ten .The spiracles are heavy sclerotized and are situated in the dorsoventral area. The body of the third instar larva measures about 1.59 mm in length and 0.57 mm in width. At its greatest thickness with a head capsule of about 0.239 mm long and 0.210 mm wide .The mandibles are larger in size and more pigmented than the first instar ones. Each mandible has a broad rounded base and a taper to sharp end .It is about 0.0385 mm in length and 0.0277 mm in width, Table 1 and Fig. 1.

Pre-pupal stage: As soon as the miconium is discharged out of the body, most of the larval characteristics are lost and a prepupa is formed .The meconial substance is ejected in small clumps and consists of dark –brown granules in a viscous dark fluid. Two days after voiding, the fluid has dried away and the meconium now is represented

Table 1. Dimensions (in mm) of *C. scutellaris* stages on *P. tenuivalvata* under laboratory conditions.

Stage	Body		Head capsule		Mandible	
	length	width	length	width	length	width
Egg	(0.20 - 0.25)	(0.050 - 0.061)				
	0.24 ± 0.016	0.055 ± 0.007				
1st instar	(0.26 - 0.42)	(0.070 - 0.082)	(0.042 - 0.049)	(0.061- 0.070)	(0.0095 - 0.014)	(0.0063 - 0.0069)
larva	0.39 ± 0.032	0.079 ± 0.009	0.046 ± 0.005	0.065 ± 0.004	0.0098 ± 0.0002	0.0065 ± 0.0001
2nd instar	(0.50 - 0.85)	(0.19- 0.31)	(0.110 - 0.145)	(0.011- 0.119)		
larva	0.78 ± 0.045	0.27 ± 0.053	0.130 ± 0.022	0.115 ± 0.0002		
3rd instar	(0.95- 1.75)	(0.32 - 0.610)	(0.23 - 0.25)	(0.20 - 0.24)	(0.0356 - 0.0405)	(0.0259 - 0.0292)
larva	1.59 ± 0.290	0.570 ± 0.050	0.239 ± 0.0082	0.210 ± 0.01	0.0385 ± 0.0004	0.0277 ± 0.0007
Prepupa	(1.11 - 1.37)	(0.570 - 1.400)	(0.220 - 0.550)	(0.350 - 0.560)		
	1.25 ± 0.19	0.710 ± 0.230	0.360 ± 0.1000	0.450 ± 0.070		
Pupa	(1.24 - 1.45)	(0.44- 0.65)	(0.218- 0.365)	(0.305- 0.522)		
	1.33 ± 0.065	0.51 ± 0.14	0.260 ± 0.0178	0.320 ± 0.447		
Adult	(0.870 - 1.088)	(1.750- 1.894)	(0.174- 0.260)	(0.261- 0.435)		
	0.979 ± 0.052	1.861 ± 0.150	0.218 ± 0.0470	0.304 ± 0.0178		

Table 2. Durations of *C. scutellaris* stages on *P.tenuivalvata* under laboratory conditions.

Stage	Duration (day)		
	Minimum	Maximum	Average + S.E
Egg	2.5	3	2.75 ± 0.12
1st larval instar	4	7	6.00 ± 0.88
2nd larval instar	4	7	5.86 ± 0.85
3rd larval instar	3	5	4.33 ± 0.29
Prepupa	1	1.5	1.25 ± 0.09
Pupa	7	10	8.96 ± 0.73
Total	21.5	33.5	29.15 ± 2.60

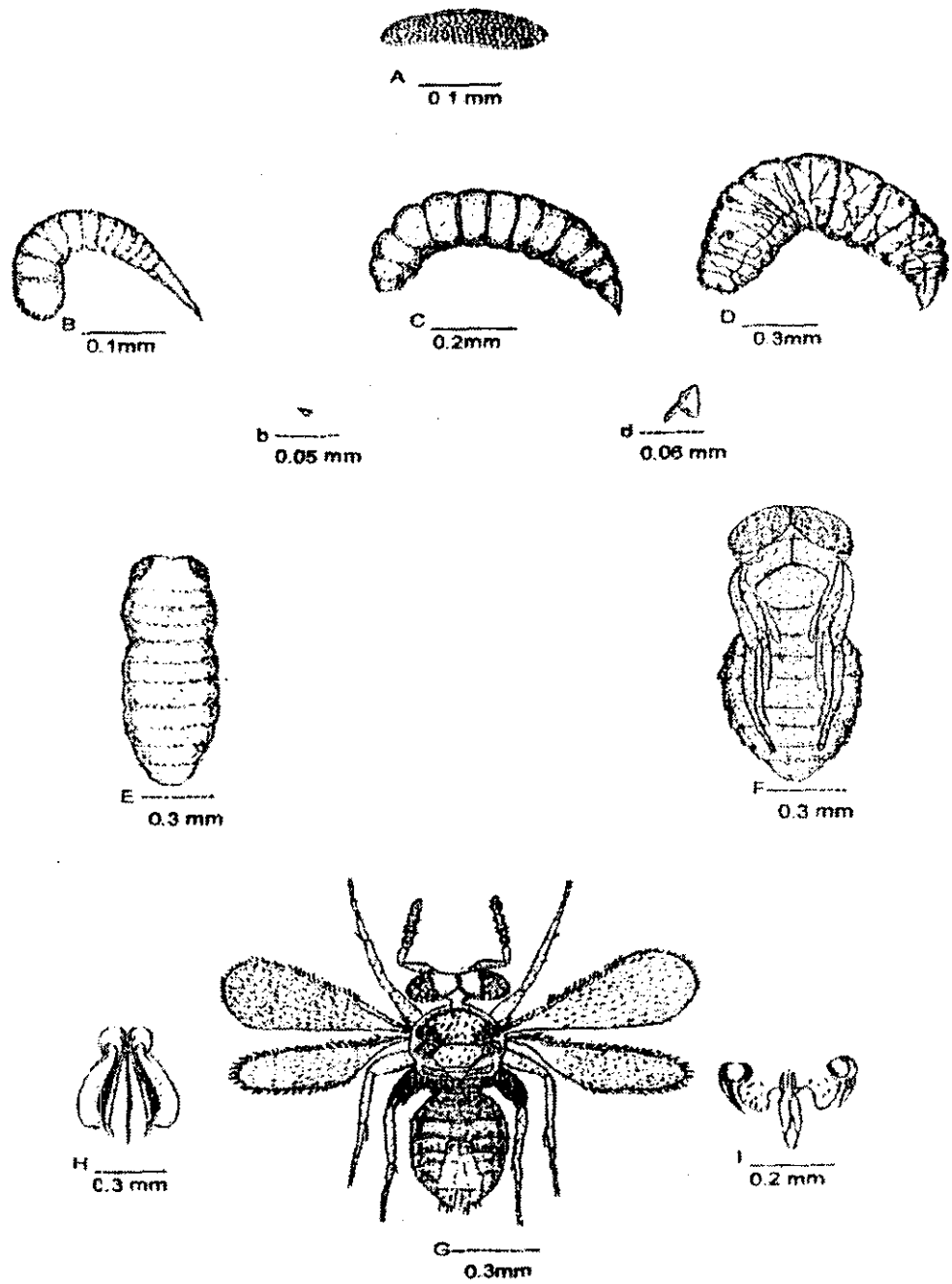

Fig. 1. Different developmental stages of *Coccophagus scutellaris*, male genitalia and ovipositor

A. Egg B. First instar larva (b) Mandible of 1st larval instar C. Second instar larva D. Third instar larva (d) Mandible of 3rd larval instar E. Pre - pupa F.Pupa G.Adult H. Male genitalia I. Ovipositor

by three or more hard dark – brown pellets lying on each side of the pupa against the lateral scale integument. The body length and width of this stage averaged 1.25 and 0.71 mm, respectively. The head-capsule averaged 0.36 mm long and 0.45mm wide, Table 1 and Fig. 1.

Pupa: This stage is also formed inside the host. It is about 1.33 mm in length and 0.51 mm in width. Its color is opaque white and the compound eyes appear red after that turned gradually to black. The head capsule measured 0.218 mm in length and 0.304 mm in width, Table 1 and Figs. 1&2.

During the course of these events, the scale contents turn completely opaque white. The integument is at first colorless and transparent and the parasitoid is visible inside. Then, it turns a light brown color. Toward the end of the larval stage, the host integument turns from light brown to progressively deeper hues of brown, but remains transparent to some degree.

Adult: Average dimensions of the body are 0.979 ± 0.052 mm (0.870 – 1.088) in length and 1.861± 0.150 mm (1.750 – 1.894) wing span, with a head capsule of about 0.218 ± 0.0470 mm in length and 0.304 ± 0.0178 mm in width.

Female: Its general color is black with an apical portion of scutellum more or less extensively yellow. The legs are yellow except middle and hind coxae and hind femora, which are black. Scape of usual shape, slightly fusiform, about four times as long as wide. Pedicel a trifle more than one and one-half times as long as wide and about two –thirds as long as first funicle joint . First funicle joint longest, slightly more than twice as long as wide; second and third each successively slightly shorter and almost imperceptibly wider so that the third is slightly less than one and one-half times as long as wide. First club joint slightly the longest and widest; slightly longer than wide and not quite as long as the preceding funicle joint. Second and third club joints each progressively shorter and narrower. Fore - wings faintly and uniformly infumated. The marginal vein is clearly longer than the sub-marginal one; postmarginal produced about as for distal as stigmal. Wings finely and densely ciliated. Marginal fringe short. Ovipositor not exerted, Table 1 and Figs. 1&2.

Adult, when ready to emerge, pierces the scale integument with her mandibles and increases the size of this hole until it is large enough for the body to pass through.

Male: Similar to the female except that the scutellum is entirely black and genitalia . Figures.1&2 show a male genitalia type characterized by a phallobase shape provided

with narrow digiti without claspers. Adageus with bacilliform spondemes. Josnosh (1976) pointed out the key significance of male genitalia structure for the elucidation of generic relations and suprageneric groups- subfamilies. Also, Viggiani and Bttaglia (1984) indicated that a male genitalia in the aphelinidae is important in the classification.

Duration of *C. scutellaris* immature stages: Regardless of the sex, the durations of various stages of *C. scutellaris* reared on *P. tenuivalvata* are presented in Table 2. Data showed that the incubation period of the egg averaged 2.75 ± 0.12 days. The first instar larva lasted 4 - 7 with an average of 6 ± 0.88 days. The second instar larva lasted 4 – 7 with an average of 5.86 ± 0.85 days. The duration of the third instar was 3-5 with an average of 4.33 ± 0.29 days. Total larval period lasted 16.19 days. The pre-pupal and pupal stages lasted 1.25 ± 0.09 (1-1.5) and 8.96 ± 0.73 (7-10) days, respectively. The total developmental period of this parasitoid, from the egg to adult emergence, averaged 29.15 ±2.59 days with a minimum of 21.5 and a maximum of 33.5 days.

Fecundity, sex ratio and adult longevity: As shown in Table 3. number of eggs / female ranged between 32 and 90 with an average of 67±5.76 eggs. The results indicate that female emergence occurs at a high rate, relative to that of males. The sex ratio of *C. scutellaris* recorded 1: 4.6 as males: females. Female adults lived longer (13.5 days) than male ones (7.3 days).

Number of parasitoid individuals/one host: Data obtained in Table 4 and Fig.2 indicated that 90% of the parasitised 2nd nymphal instar scale contained only one parasitoid, while 5% contained 2 individuals .

Also, 32% of the parasitised 3rd nymphal instar scales contained only one parasitoid, while 36, 23 and 2% contained 2, 4 and 6 individuals, respectively.

As for the newly developed host female, 12% of them contained one parasitoid while 16, 19, 9,5 and 3% harboured 2,4,6,8 and 10 parasitoids, respectively.

Also 10,11,15,7,4 and 3% the full-grown female contained 1, 2,4,6,8 and 10 parasitoids, respectively.

The presented data indicate that, one or more adult parasitoids developed successfully in one host. The maximum number of adult parasite emerged from one individual host in this study was ten, depending on stage of the host.

These results show that the female parasite attack the host scale after the scale ceased the crawler activity and settled down. It parasitized the second and third nymphal instars, the newly adult females and the full grown adults. From all the aforementioned data, it is clear that the 2nd nymphal instar is the most favorable stage for the parasitoid, the percentage of parasitism reached 95% and it is reduced in the older 3rd nymphal instar, newly developed female and full-grown female (93, 64 and 50 %, respectively).

It is to be concluded that *C. scutellaris* can be considered as a potential biological control agent for *P. tenuivalvata*. Confirmative results were obtained by Ceballos and Hernandez (1988) and Murphy (1991) where *Coccophagus* sp. And other parasitoids were used as bioregulator for scale insects.

Table 3. Fecundity, sex ratio and adult longevity under laboratory conditions.

No. of eggs / female	67±5.70 (32 – 90)
Total no. of males	12±1.92 (9 – 15)
Total no. of females	55±2.81 (50 –69)
Sex ratio (Male : female)	(1 :4.6)
% of female	82.1
Female longevity (in days)	13.5±1.39 (5–15)
Male longevity in days	7.3±1.25 (2– 10)

Table 4. Number of *C. scutellaris* emerged from one host of various stages of *P. tenuivalvata.*

No. of parasitoids emerged / individual host	% parasitism in:			
	Nymph		Adult	
	2^{nd} instar	3^{rd} instar	New emerged	Full-grown
1	90	32	12	10
2	5	36	16	11
4	0	23	19	15
6	0	2	9	7
8	0	0	5	4
10	0	0	3	3
Total	95	93	64	50

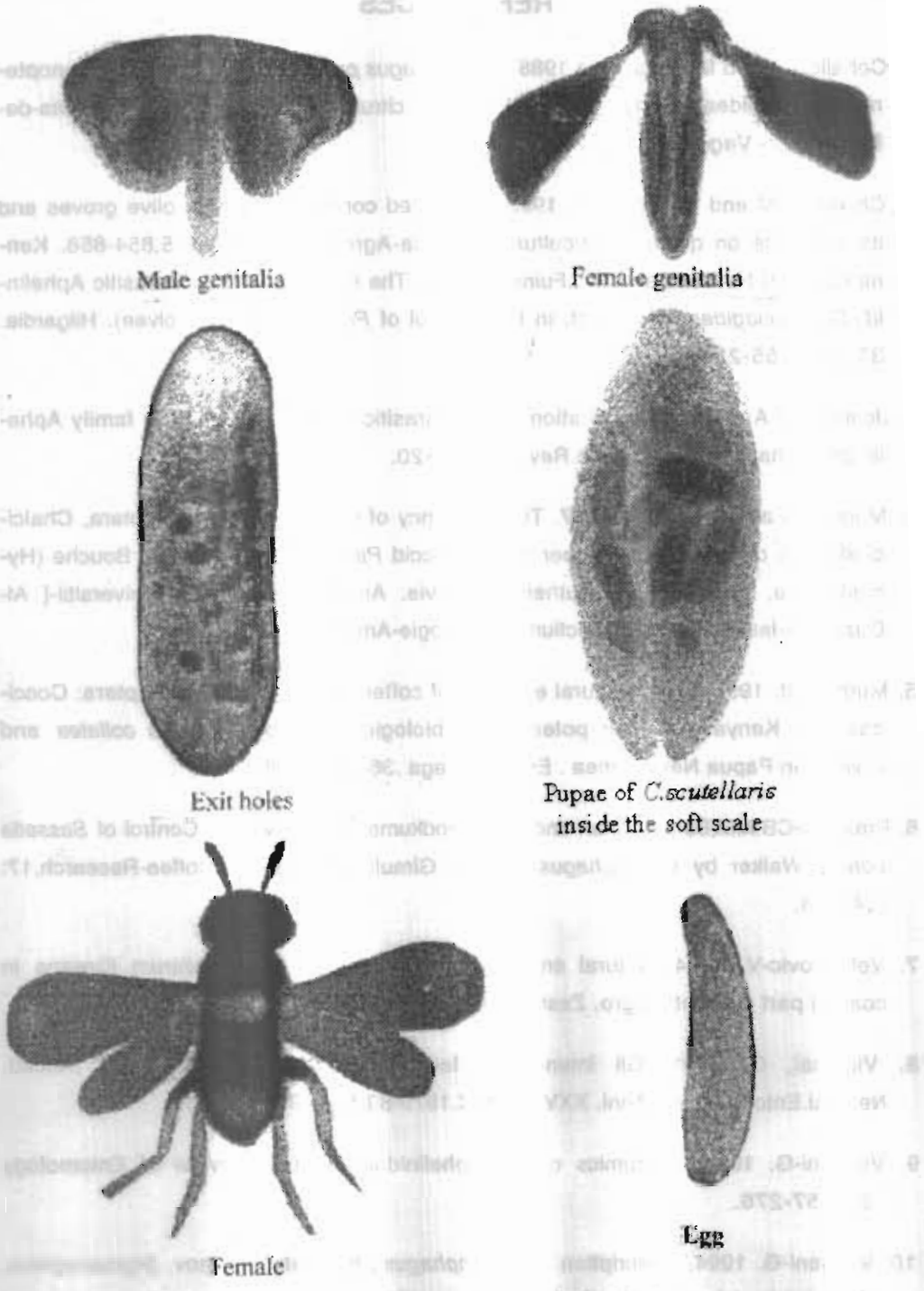

Fig. 2. *C. scutellaris* stages

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دراسات معملية على طفيل *COCCOPHAGUS SCUTELLARIS* من عائلة افيلينيدى على الحشرة القشرية الرخوة *TENUIVALVATA PULVINARIA* التي تصيب محصول القصب في مصر

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ظهرت الحشرة القشرية الرخوة حديثا على محصول القصب مسببه له خسارة كبيرة ونتيجة لحدوث الإصابة في وقت تكون فيه النباتات متشابكة ومزدحمة مما يصعب معه مكافحة الحشرة بالمبيدات لذا كان من الضروري التفكير فى وسيلة مكافحة أخرى وقد ثبت من الدراسة وجود طفيل داخلي *C. scutellaris* يهاجم الحشرة خلال مراحل نموها المختلفة وقد تم دراسة الصفات المورفولوجية للأطوار المختلفة للحشرة مدعمة بالرسومات التوضيحية .أتضح من الدراسة البيولوجية للطفيل أن متوسط فترة حضانة بيض الطفيل ٢,٧٥ يوم وكان متوسط عمر الطور اليرقي وطور ما قبل العذراء وطور العذراء هو ١٦,١٩ و ١,٢٥ و٨,٩٦ يوما، على التوالي. ويتضح من الدراسة تفضيل الطفيل للعمر الحورى الثاني والثالث للعائل كان العدد الكلى للبيض الذي تضعه الأنثى الواحدة ٦٧ بيضه خلال فترة حياتها وهى ١٣,٥ يوم وعمر الإناث أطول من عمر الذكور(٧,٣ يوم) وكان متوسط النسبة الجنسية هو ١ ذكر : ٤,٦ أنثى. يخرج من العائل الواحد طفيل أو أكثر حيث أمكن حصر أعداد تتراوح ما بين طفيل إلى عشرة طفيليات وذلك تبعا لعمر العائل.

وبلغت أعلى نسبة تطفل ٩٥٪ على العمر الحورى الثانى للحشرة القشرية ولهذا يمكن استخدام هذا الطفيل في برنامج المكافحة لهذه الحشرة الخطيرة .