

Potential of the parasitoid species, *Aganaspis daci* (Weld) (Hymenoptera: Eucoilidae) against the peach fruit fly, *Bactrocera zonata* (Saund.) (Diptera: Tephritidae)

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ABSTRACT

The peach fruit fly (PFF), *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) is one of the serious tephritid insect pests attacking tropical and subtropical fruits. In Egypt, the pest is present and widespread over most of the Egyptian governorates causing serious problems to many fruit crops. The present study aimed to shed light on some morphological and biological characteristics of different stages of the exotic parasitoid species, *Aganaspis daci* Weld. (Hymenoptera: Eucoilidae) under laboratory conditions, as well an evaluation of a field release trial of the parasitoid. Biological studies included; durations of different immature stages, fecundity, longevity, survival rate and sex ratio. All studies were carried out under the laboratory conditions of $25\pm 2^{\circ}\text{C}$, 54-65% R.H. and 14:10 L: D photoperiod. Total developmental period of the parasitoid was 23.3 days. Fecundity of *A. daci* averaged 39.57 eggs/ female (32-55 eggs). Sex ratio (female: male) was 1: 1. Fed female and male longevity attained 18.8 and 17.3 days, respectively. A successful field release trial of the parasitoid *A. daci* was recorded at North Sinai Governorate, Egypt. The first recovery was about a month post releasing date, as percentage of parasitism attained 9.7%. Further data, particularly on its potential release is still needed.

Keywords: Morphology, Biology, Characteristics, *Bactrocera zonata*, Parasitoids, *Aganaspis daci*, Release.

INTRODUCTON

Fruit crops are subject to attack by serious insect pest species (polyphagous and/or monophagous). Fruit flies are among the agricultural pests that have great economic importance. They include about 4000 species. Out of which 1200 species belong to family Tephritidae. Most of these species are polyphagous and about 40% of them attack several fruits, while the rest attacks the flowers, stems, leaves and roots. Most of the fruit flies belong to 5 genera. Genus *Bactrocera* is the greatest one which contains about 500 described species. Many of the fruit flies are prevail in Egypt, causing losses for most of the fruit crops because their larvae feed inside the fruits, and damage them, in addition to the growth of fungi and infestation of secondary pests (dry fruits beetles), which cause more damage and reducing the quality of fruits and the non-suitability of them for consumption and marketing.

The peach fruit fly (PFF) *Bactrocera zonata* (Saund.) is an invasive species, native to Southeast Asia. It was first recognized as a new pest of guava and mango in 1998 in the northern region of Egypt (El-Menshawy *et al.* 1999). It is now a serious pest of fruits and some vegetables replacing the Mediterranean fruit fly (Medfly) *Ceratitidis capitata* (Wied.) in most of the Egyptian governorates. PFF is one of the most dangerous species at present, because it is phytophagous and infests most of fruit crops in Egypt, causing losses of about 375 million dollars annually (FAO/STAT). PFF attacks a wide range of host plants including

peach, guava, mango, date palm, apples, bitter gourd, okra, pomegranate, papaya, common fig, quince, sweet and bitter orange and possibly melons and water melons, in addition to numerous ornamentals. Apparently there is a considerable scope for the increase of the host range of PFF as it colonizes new environments.

The widespread of the pest in recent years, in most of the orchards all over the country, was attributed to its wide distribution, being polyphagous, most of its life cycle is spent inside the fruits, it attacks fruits near ripening, as well as the difficulty of differentiation between the symptoms of its damage and that caused by the immature stages of the Medfly (Action plan 2000).

Parasitoids are of potential effective for controlling fruit flies. The native parasitoids regulating the populations of fruit flies in Egypt, as well as those of African origin seem to be not compatible with *Bactrocera* species of the Asian origin. African parasitoids that attack fruit flies do not have the co-evolutionary history with the new host *B. zonata*.

The pest problem of *B. zonata* in Egypt is a classic example of an invasive species that is accidentally moved from Asia to the African continent without its specific natural enemies. Therefore, the fly increased without check and became a serious pest. The release of new candidate parasitoids from the native region of this pest may be one of the control methods to reduce its populations to manageable levels. Thus, the introduction of Asian parasitoids from the native region of the pest to be established for help in suppressing the fly populations in Egypt appears to be a logic approach.

Through an Egyptian-American collaborative project, "Non-toxic control of Peach Fruit Fly in Egypt", carried out during the period 2008-2011, the exotic parasitoid species *Aganaspis daci* (Weld) (Hymenoptera: Eucolidae) was imported from Asia through USDA, to provide an additional mortality against PFF. The native area of this species is South-East Asia. The parasitoid was imported from USDA, Hawaii to be tested in Egypt. *A. daci* is a larval-pupal parasitoid of several species of genus *Dacus* (Diptera: Tephritidae) in Southeast Asia and Australia (Weld, 1951 and Clancy *et al.*, 1952). The parasitoid species was reared successfully for several generations on *B. zonata* larvae under laboratory and quarantine conditions in Egypt. The parasitoid was also introduced to Hawaii and successfully reared on *C. capitata* and *Bactrocera dorsalis* (Hendel) (Clausen *et al.*, 1965). Recently, it has been reared on the Medfly and released against that pest in France and Israel (Papadopoulos and Katsoyannos, 2003). Generally, there are insufficient data on the life cycle of *A. daci* and its biological control potential.

In an effort to evaluate the potential of this parasitoid species on *B. zonata* in Egypt, the present study focused on studying morphological and biological characteristics of *A. daci* under laboratory conditions, as well a preliminary trial to release and evaluate it under field conditions.

MATERIALS AND METHODS

1.

2. Rearing of the parasitoid *A. daci*

The parasitoid species, *A. daci* is an exotic species, introduced from USDA-ARS, Honolulu, HAWAII, USA through the Egyptian–American collaborative project, “Non-toxic control against Peach Fruit Fly in Egypt” to the quarantine laboratory of the Agricultural Research Center (ARC), Giza, Egypt.

A laboratory stock culture of *B. zonata* was established according to the technique described by Marwa *et al.* (2012).

The parasitoid was reared for more than 80 generations on *B. zonata* under the laboratory conditions of $26\pm 2^{\circ}\text{C}$, $60\pm 5\%$ RH and photoperiod 14:10 light and dark. Second and third larval instars (3-7 d old) were exposed to the parasitoid in ovipositional units. Parasitization units were introduced to the cages (35 x30 x 30 cm) and provided with undiluted bee honey and water, as a source of food.

The parasitoid was allowed to parasitize *B. zonata* larvae in two different types of parasitization units according to the host age. Small host larvae, 2-3 days old, were provided with larval diet in plastic trays. After parasitization, these units were transferred to transparent jars and covered for maturation. Large larvae, 4-7 days old, were exposed in modified Petri-dishes, tightly fitting with organdy lids. After exposure of larvae to the parasitoid for 24-48 h, pupae were collected into a plastic jar containing a thin sand layer in its bottom and covered with tightly fitting organdy or clothes lid until adult emergence. The jars were maintained under the laboratory conditions of ($25\pm 2^{\circ}\text{C}$, $60\pm 5\%$ RH and a photoperiod of 14:10 light and dark) (Marwa, *et al.* 2011).

2. Morphological and biological studies of *A. daci*

2.1. Morphology of *A. daci* immature stages

In order to measure and determine each stage of the parasitoid, the host larvae were exposed to the parasitoid in Petri dishes (15 cm diameter) containing 300 host larvae of 2nd instar of *B. zonata* in their artificial diet, with 10 mated females of the parasitoid and covered with organdy. Exposure period was 24 hr, then the parasitoid females were removed and transferred to rearing cages and the larvae were left in the medium to complete development. The larvae were dissected every 12 hr after parasitization, until the 3rd instar parasitoid larvae were formed, and then every 24 hr until the emergence of adult parasitoids.

Parasitized hosts were dissected in drops of the physiological saline and observed under a stereomicroscope. A semi-permanent mounting of the egg, 1st and 2nd instars in Hoyer's media was used for photography. Large specimens of 3rd, 4th instars and pupae were placed after measuring in 70% alcohol and glycerin. The measurements were taken using a micrometer lens (1 cm, with small scale = 50 μ).

2.2. Biology of *A. daci*

2.2.1. Life cycle

A study to describe the immature stages of *A. daci*, to observe the parasitoid behavior in the host and parasitoid immature stages development from oviposition in the host till adult emergence under the laboratory conditions was undertaken.

Second instar larvae of *B. zonata*, 3 day old, were used to study the life cycle and development of immature stages of *A. daci*. The host larvae were exposed once for 24 hours to a large number of mated *A. daci* females for oviposition. Small host larvae were presented to the parasitoid in a small thin layer of larval diet in a Petri dish (9 cm diameter), contained 100 host larvae exposed to 3 parasitoid females for 6 hr. This number of host larvae and time of exposure was used to avoid superparasitism as reported by Nunez-Bueno (1982). The exposure was carried out for several times to get large numbers of parasitized hosts to use them in the dissection also, as replicates. Obtained data were recorded.

In order to observe the parasitoid's development, parasitized hosts were dissected every 24 hr. using drops of physiological saline and were examined under a microscope or a binocular. The parasitoid's egg, 1st and 2nd instars larvae were observed on the same microscopic slide using a phase microscope provided with an (1 cm) micrometer slide (with small scale =50 μ). Older parasitoids larvae (3rd and 4th instars), pre-pupa and pupa were observed using a binocular due to their relatively larger sizes.

2.2.2. Fecundity and determination of Arrhenotoky

Newly emerged parasitoid adults were left for mating. Thereafter, each couple was confined in a plastic jar and provided with 25 newly 3rd instar larvae of *B. zonata*. Each jar was provided with drops of bee honey and cotton moistened with water for adults feeding. The newly 3rd instar larvae were replaced daily with new ones until the death of the adult parasitoid female. From the daily exposed host larvae in relation to the number of emerged parasitoid adults from them, the pre-oviposition, oviposition and post oviposition periods, as well as fecundity were determined. Also, adult longevity, sex ratio and survival rate were also recorded.

For the determination of the Arrhenotoky, 30 virgin females, without males were placed in a small jar provided with a source of water and drops of honey. The females were supplied with large number of 3rd instar larvae of *B. zonata* in an oviposition unit. This unit was provided daily with a new one until the death of the adult parasitoid females.

2.2.3. Survival and Sex ratio

Survival and sex ratio of the parasitoid were estimated. Mated females of the parasitoid were provided daily by 100 larvae on artificial diet in small transparent jars until their death. Exposed larvae were collected daily and allowed to complete their development on the larval artificial diet until pupation and the parasitoids were allowed to complete their life cycle and pupae were kept in small jars until adults' emergence.

2.2.4. Longevity

Newly emerged adults of the parasitoid were placed individually into small glass vials and divided into two groups (30 pairs of males and females each) and left to complete their longevities. The 1st group was left unfed, while the 2nd was fed on small drops of undiluted honey. The longevity of each sex was determined.

2.3. Release trial

A preliminary trial to release and evaluate the potential of *A. daci* under field conditions was carried out on guava trees, located at El-Arish district, North Sinai Governorate, Egypt in September, 2010. El-Arish was the most suitable location for the trial, as it is recognized as the place, which has the two economic fruit flies; *C. capitata* and *B. zonata* together in competition and in relatively high numbers on the same host fruits. Twenty guava trees located in the center

of the orchard were randomly chosen to release the parasitoid. Out of the 20 trees, 10 were chosen for sampling. The soil beneath the 10 trees was cleared from the weeds and any other residues. A transparent plastic cover (2x3 m) was placed under each tree, covered with a thin layer of previously washed sand (4-5 cm) to receive the fallen fruits and to be used as substrate for larval pupation. Release rate was 50 parasitoid adults/ tree (sex ratio 1:1). A total of 1000 adults (500 males and 500 females) were released. Sampling was done as follows:

- a- A pre-releasing sample was taken, 24 h before release of the parasitoid,
- b- Twice a week, post releasing date, for collecting the fallen fruits,
- c- Once a week for collecting the formed puparia located in the sand layer under the trees, and
- d- Sampling continued for 6 weeks until no more parasitoids were recovered.

Statistical analysis:

All statistical analyses were carried out using Microsoft Excel 2010.

RESULTS AND DISCUSSION

1- Morphological and biological studies on *Aganaspis daci*

1.1. Morphological Studies of *A. daci*

A. daci displays hypermetamorphosis, a phenomenon common to all Cynipids. Characteristics of different *A. daci* stages are illustrated in figures (1- 16).

1.1.1. Egg stage

Egg of *A. daci* is monoembryonic, uniformly of the stalked type, with the stalk, situated at the anterior end (Fig. 1). This stalk is less than the length of the egg body. The chorion is thin and transparent without surface ornamentation. Egg measurement averaged (150-250 μ) in width and (300-450 μ) in length. Eggs hatched always after (3-4) days from deposition on the host larva. After 72 hr from egg deposition, the structure of the 1st instar larvae can be easily distinguished. When ready to hatch, the larva produces spinning and strong movements of the head and caudal end and breaks the chorion (Fig. 2).

This result is in agreement with the stated data of parasitic members of the superfamily that there are uniformly of the stalked types, with the stalk, which is situated at the anterior end, ranging in length from less than that of the main body, as in *Charips* sp., to several times its length. In *F. anthomyiarum*, it is elongated and somewhat constricted in the middle and has a stalk of about equal length. In *E. keilini*, the stalk is twice the length of the egg body, and in *Ibalia leucospoides* it is about four times as long. Also, in agreement with the obtained results, Nunez-Bueno (1982) reported that the egg of *A. daci* was stalked, monoembryonic and surrounded with an elastic chorion. The egg measurement averaged $286 \pm 0.01 - 760 \pm 16.73 \mu$ in length. Similar to these data, Sergio (1994) reported that *A. pelleranoi* produces a stalked egg, typical of Eucoilidae. The newly laid egg averaged 320 – 560 μ in length and 60 – 100 μ in width.

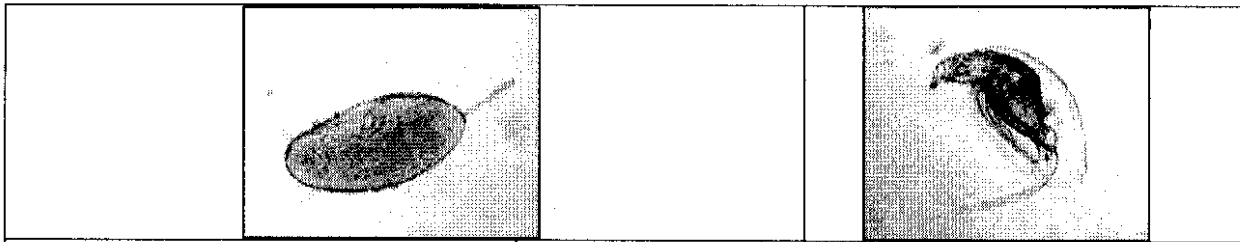


Fig. (1): Egg of *A. daci* in *B. zonata* 3rd instar larvae (stalked type).

Fig. (2): Egg of *A. daci* near hatching.

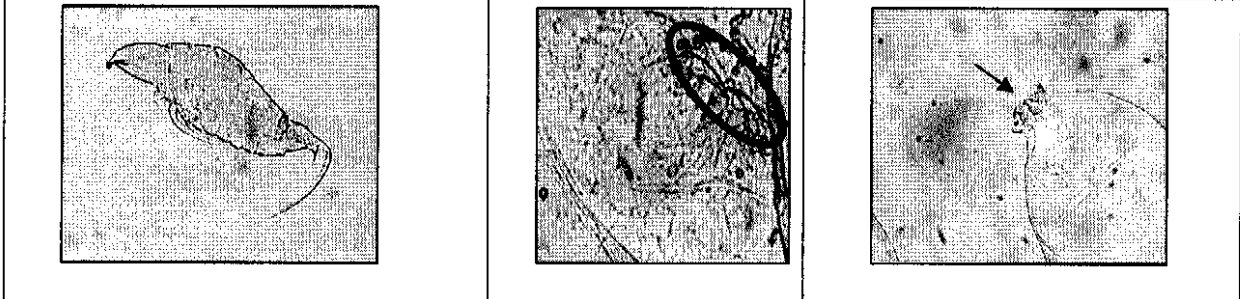


Fig. (3): First instar larva of *A. daci*

Fig. (4): Mandibles of *A. daci* 1st instar larva



Fig. (5): a) Thoracic appendage, b) Ventral process, and c) Tail seta.

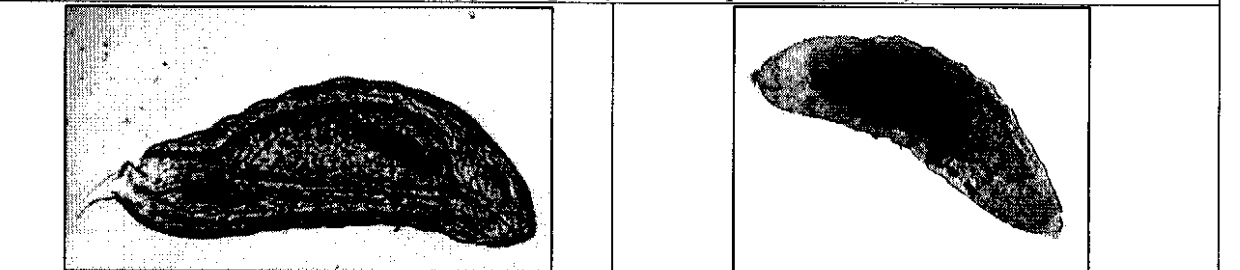


Fig. (6): Earlier and older 2nd instar larvae of *A. daci*

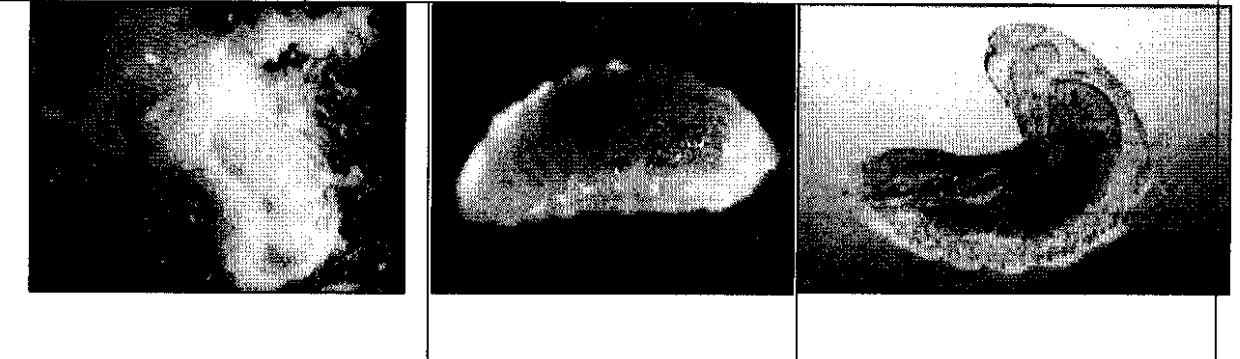


Fig. (7): Endoparasitic 3rd instar larva, white globular fat particles in thorax and abdomen (right) and Ectoparasitic 3rd instar larva (left).

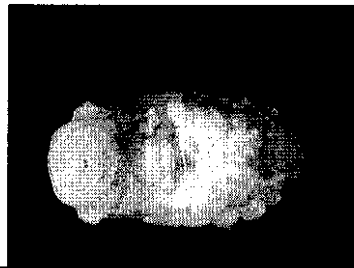
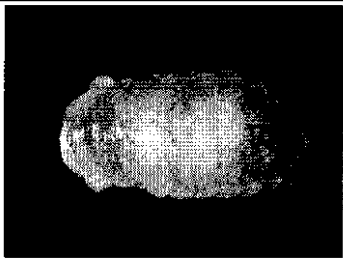


Fig. (8): Ventral and dorsal view of 4th instar larva of *A. daci*

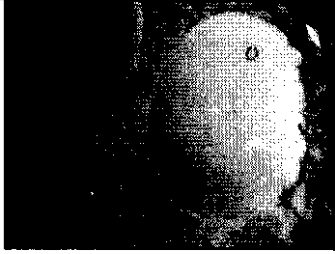


Fig. (10): Pre-pupa and meconium of *A. daci*

Fig. (9): Fourth instar larva of *A. daci* inside the puparium of the host

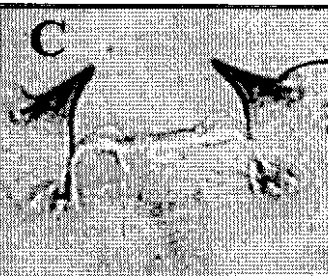
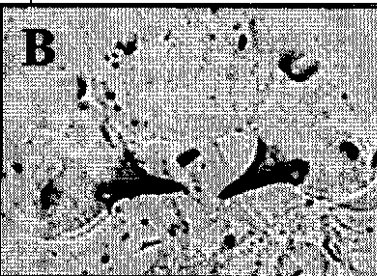


Fig. (11): Mandibles of A) 2nd instar, B) 3rd instar and C) 4th instar larva of *A. daci*

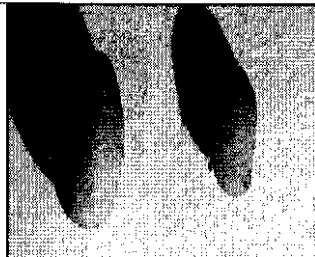


Fig. (14): Emergence hole of *A. daci*

Fig.(13): Normal and parasitized pupae

Fig. (12): Early male pupa of *A. daci*



Fig. (16): Female of *A. daci* (short antenna).

Fig. (15): Male of *A. daci* (long antenna).

1.1.2. Larval stage

Larva of *A. daci* passed through four larval instars described as follows:

1.1.2.1 First instar larva (L1)

The 1st instar larva appeared on the 4th day post oviposition, when the host puparium was formed. It is eucoiliform type, translucent, subcylindrical in shape, with an elongate distinct head (Fig. 3) and measured (650 μ) in length and (250 μ) in width. It has a pair of mandibles (Fig. 4). Each thoracic segment has a pair of long slender ventral fleshy appendages. Caudal segment is with a long tail and short ventral process. The tail bearing several small setae apically and the basal end of caudal segment with scale like ornamentation (Fig. 5). Nunez-Bueno (1982) stated that the position of 1st instar, usually adopted in mounted specimens who made it impossible to determine the presence and characteristics of the mandibles. Furthermore, Clausen *et al.* (1965) stated that the 1st instar larva of *A. daci* does not possess discernible mandibles.

1.1.2.2. Second instar larva (L2)

The 2nd instar larva was observed on the 7th day post oviposition (Fig. 6). It measured 3970 μ (3500 – 4350 μ) and 697 μ (550- 850 μ) in length and width, respectively. It is a modified eucoiliform; cylindrical in shape; whitish yellow; laterally depressed with differentiated head. Caudal segment is with a small tail.

1.1.2.3. Third instar larva (L3)

The 3rd instar larva appeared about 9th day post oviposition. It is more typically hymenopteriform larvae. Characterized with the “C” shape; yellowish with several white globular fat particles in thorax and abdomen; cylindrical in shape with a distinct head and gut easily discernible (Fig. 7). It measured 1750 – 2000 μ in length, and 600 -750 μ in width. Mandibles are sclerotized and easy to recognize under light microscope. Newly emerged 3rd instar larva was found feeding inside the host (endoparasitic behavior) as the 1st and 2nd instars did, while the old 3rd instar larva (by the end of the 3rd instar duration), it made a hole in the host tissues, partially emerged from the host pupa and then lied between the host puparium and host body to feed externally on the host puparium (ectoparasitic behavior), with the head directed towards the host's head (Fig. 7).

1.1.2.4. Fourth instar larva (L3)

The 4th instar larva is hymenopteriform; observed at 12-13 days post oviposition. Whitish yellow with many small white globular fat particles in thorax and abdomen; cylindrical in shape and head relatively large, sub-circular in front view (Fig. 8, 9 and 10). Mandibles are sclerotized and easy to recognize. It measured (3100 – 3750 μ) in length and (1500 – 1650 μ) in width (Fig. 11).

Available literatures on *A. daci* suggest different views. Clausen *et al.*, (1965) mentioned three larval instars for the same parasitic species. Obtained results agree with the finding of

Nunez-Bueno (1982), who depended in her study on the mandibles measurement of each instar. Also, the presence of four larval instars was reported in the family Eucolidae, as supposed of *Eucoila trichospila* (Sychevskaya 1974) and *Leptopilina boulandi* (Kopelman and Chabora 1978).

1.1.3. Pupal stage

The start of the pupal stage was demarcated by the development of the ommatidia of the compound eye under the 4th larval instar cuticle, followed by the constriction of the body separating the thorax from the abdomen, and differentiation of antenna and thoracic legs. The newly formed pupa is white and become dark later (Fig. 12). There was morphological differentiation between male and female. Parasitized pupa was usually smaller in size than normal one (Fig. 13). It appeared in sixteen days post oviposition in the puparium of the host and lasted from 12- 15 days until adult emergence. Emergence hole is usually at the upper broad part of the puparium (Fig. 14).

1.1.4. Adult stage

Adult of *A. daci* is black; mandibles, legs and ventral abdomen reddish. Head smooth, almost bare; from above slightly broader than thorax (Fig. 15 and 16). The female antennae with 13-segmented, moniliform and with a 9-segmented club. Wing slightly smoky. Veins brown, abdomen compressed, longer than high, and ovipositor straight at tip. The male differs by having 15-segmented antennae, tapering to tip from the enlarged and bent third which is longer than 1 plus 2 and twice as long as 4.

1.2. Biological studies on *A. daci*

1.2.1. Life cycle

1.2.1.1. Durations of immature stages

Durations of various immature stages of *A. daci* were estimated under the laboratory conditions of $26\pm 2^{\circ}\text{C}$, $60\pm 5\%$ RH and photoperiod 14:10 light and dark.

1.2.1.1.1. Incubation period of egg stage

Female of *A. daci* deposited a single egg in the body cavity of the host larva (Fig. 17) and one parasitoid adult emerged from each host puparium. Incubation period of the eggs ranged between 2-3 days, with an average of 2.7 ± 0.15 days. The chorion is broken through interiorly by the mandibles and thoracic processes, and posteriorly by movements of the tail.

The present results agree with those of Nunez-Bueno (1982) who reported that the eclosion of the first 30% of the eggs of *A. daci* on the 2nd instar larva of *A. suspense* was observed at 73 h. after parasitization and was completed in the following 12 h. The embryonic larva produced strong movements of the head and caudal end and broke the chorion which was retained around the body for a few hours and later was found in the hemocoel.

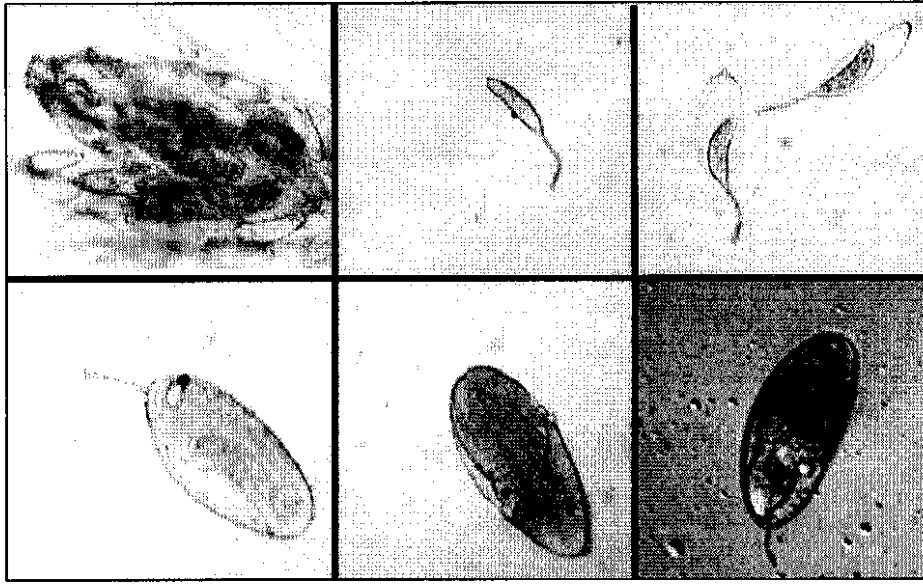


Fig. (17): Development of *A. daci* eggs in *B. zonata* 2nd instar host larvae; (a) dissected ovary in newly emerged female, (b) egg in ovary before mating, (c) eggs in ovary after mating, (d) eggs in the host after Oviposition, (e) beginning of the formation of 1st instar larvae, and (f) easily differentiation of the head and body area inside the egg.

1.2.1.1.2. Developmental period of larval stage

The four larval instars occupied 8-16 days. After hatching, the 1st instar larva actively fed on the internal tissues of the host for 2-3 days. The 2nd instar larva fed inside the host pupa for 1-2 days, then developed to 3rd instar larva, which lasted 1- 3 days. In this stage, the transformation from endoparasitic to ectoparasitic behavior was observed. The 4th instar larva fed on the remains of the host tissues for 3-5 days. By the end of 16th day from parasitism, the parasitoid had totally consumed the host and the larval meconia were released in the posterior end of the host puparium. The voidance of the meconium was considered as the end of larval development. The pre-pupa lasted from 1-3 days.

1.2.1.1.3. Duration of pupal stage

As shown in table (1), the duration of the pupal stage ranged between 10-12 days and averaged 11.2 days. When the parasitoid was ready to emerge, it removed the pupal skin, using its mandibles and legs, and the active adult started opening an irregular emergence hole in the anterior part of the puparium.

1.2.1.1.4. Total Developmental period

The total developmental period of *A. daci* from oviposition in the host larva to emergence of adult varied from 26 - 28 days in males and from 28 - 30 days in females. Thus, males tended to emerge somewhat earlier than females. The time of development of a

parasitoid is influenced by the host instar, parasitoid species, age and nutritional suitability of the host and by environmental conditions (Salt 1941 and Vinson 1980).

Table (1): Some biological data of *A. daci* when reared under the laboratory conditions of $25 \pm 1^\circ\text{C}$ and 54%-60 R.H.

Ranges	Means (days)	Developmental time (Duration)
2 – 3	2.7 ± 0.15	Egg
2 – 3	2.5 ± 0.16	Larva 1 st instar
1 – 2	1.3 ± 0.15	2 nd instar
1 – 3	1.8 ± 0.2	3 rd instar
3 – 5	4.2 ± 0.24	4 th instar
1 – 3	1.6 ± 0.52	Pre-pupa
10 – 12	11.2 ± 0.29	Pupa
20- 31	25.3 ± 1.3	Total mean period of immature stages

Information on the rate of development of *A. daci* in other hosts is scanty. Ahmad *et al.*, (1972) reported 21 to 28 days for development rate of *A. daci*, when reared in *D. zonatus* at $24 \pm 3^\circ\text{C}$. Pralavorio (1980) reported 23 days for the same species, when reared in 2nd instar larvae of *C. capitata* at 25°C and 80% RH. Nunez-Bueno (1982) who studied the development of *A. daci* on the 2nd instar of *A. suspense*, reported that not all parasitoids from the same period of egg deposition developed and emerged as adults at the same time. Most of the males emerged at 26-27 days of age, and the majority of females did so at 28 days at $27.5 \pm 2^\circ\text{C}$, 50 – 70% RH. In disagreement with the obtained results, Saiqa *et al.*, (2010) reported that the duration of the whole life cycles of male and female of *A. daci* on *B. zonata* larva were 16-18 days and 18-20 days, respectively. These days were distributed as 10-12 days for egg to adult's development period; 4-5 days of life of male *A. daci*; 8-9 days of female *A. daci*.

1.2.2. Longevity

Adults provided by water and small drops of honey lived longer compared to those unfed individuals. Female and male longevities were 18.8 ± 0.1527 and 17.3 ± 0.21 days for the fed individuals, respectively and 4.2 ± 0.35 and 3.5 ± 0.54 for unfed ones at $25 \pm 1^\circ\text{C}$. Nunez-Bueno (1982) studied longevity of *A. daci*, when reared in 1- 6 days old *A. suspense* and recorded 8.27 and 11.78 days, and 6.57 and 16.80 days for male and female, respectively on *A. daci* larvae of 1 and 6 days old. Saiqa *et al.*, (2010) mentioned that the longevity of male *A. daci* was 5-6 days after the period of mating, while longevity of female was 8-9 days.

1.2.3. Survival rate

Data revealed that the average percentage of adult emergence was 44.7 % at $25 \pm 1^\circ\text{C}$ and 54-60% R.H. In agreement with this result, Nunez-Bueno (1982) reported 47.58, 44.18 and 47.11% for *A. daci* survival rate on 1, 2 and 3 day-age of *A. suspensa* larva, and 19.58% for 6 days-age larvae.

1.2.4. Sex ratio

Sex ratio (female: male) was 1: 1 under the laboratory conditions of $25 \pm 1^\circ\text{C}$ and 54%-60 R.H. Clausen (1939), Salt (1934) and Vinson (1980) mentioned that differences in sex ratio are related to the host's condition, sex, size, superparasitism and environmental conditions, or the age of the parasitoid itself.

1.2.5. Fecundity

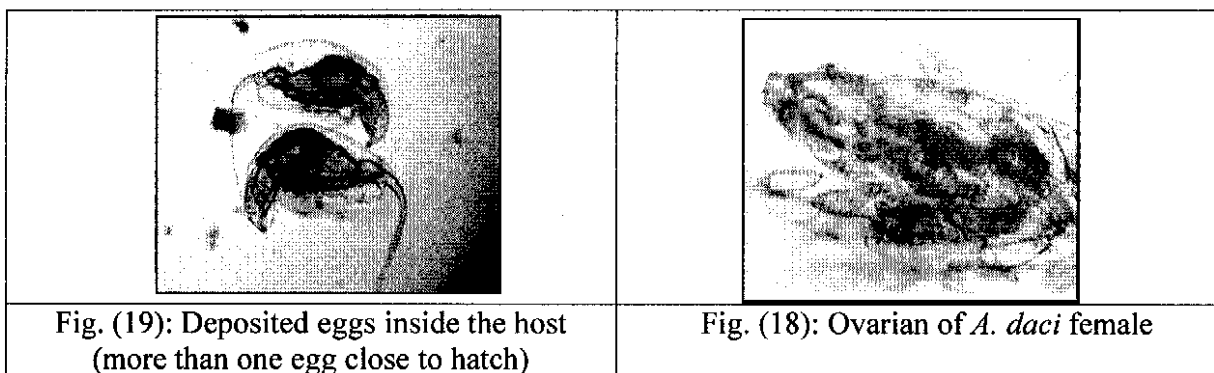
Examination of the reproductive system of gravid females revealed the presence of hundred eggs (Fig. 18), that might indicate a relatively high capacity. Each ovarian had near 80 eggs. The total number of eggs/ female averaged 39.57 ± 1.3 (32 -55 eggs). It was observed that large sized pupae of *B. zonata* produced larger adults of *A. daci*. Non- mated females showed an ability to lay infertile eggs which result in male only. Nunez-Bueno (1982) reported that total numbers of mature eggs in nonovipositing female of *A. daci* were ranged from 239.44 – 273.69 eggs, when reared on 1 and 6 day-old *A. suspensa* larvae.

2.2.6. Mating behavior of *Aganaspis daci*

Females emerge about 2 days after males. Immediately males walked to females with folded wings (vibrating wings) over the body. Males approaching females and mounted it with their dorsal side up, placing their head over the females head. As soon as male was situated on the female's thorax, male's antennae were extended forward, raised and lowered alternately with fanning wings, while the female's antennae were held straight upward and remained motionless. Subsequently, the male quickly gained contact with female's genitalia. After successful copulation females start to move, while males stop and begin to clean their antennae and body.

1.2.7. Superparasitism

Females of *A. daci* could discriminate between parasitized and unparasitized hosts, but when the parasitoid females were provided with small numbers of hosts, the phenomenon of superparasitism occurred (Fig. 19).



This result agrees with Nunez-Bueno (1982) who reported that at high parasitoid: host ratios, reduced number of eggs laid resulted from the capacity of parasitoids to restrain oviposition, as well as from interference from other females. The number of parasitoid eggs per host increased with increasing parasitoid density, but the yield of parasitoid progeny decreased as a result of competition between supernumeraries and/ or effect of host defense reaction.

2- Evaluation of *A. daci* under natural conditions

The success of rearing the parasitoid *A. daci* on larvae of *B. zonata* for tens of generations under laboratory conditions encourages the next step towards carrying out some pilot field releases to evaluate the efficacy of the parasitoid against the pest, as well as against *C. capitata* under Egyptian natural conditions. Obtained results showed that:

- *C. capitata* represented only 4.8% of the tephritid fly population emerged from the collected samples at the releasing site and the larval parasitoid species, *Pystellia concolor* was the one recovered only from *C. capitata* puparia. It was not recorded from *B. zonata*.
- Some unidentified hyperparasitoid spp. were recovered from both flies puparia.
- The trial showed a successful establishment of the *A. daci* as a new exotic parasitoid species against *B. zonata* in Egypt.
- The first field recovery record, post releasing *A. daci*, was about a month from releasing date (24 September). This is the same developmental period of *A. daci* under laboratory conditions (average temp. and RH at El-Arish district in October were 28°C and 65-70% RH).
- Percentages of parasitism attained 9.7 % on October 25th. % Parasitism looks relatively low but this was the first recovery record of *A. daci* in the releasing site. Further studies are needed to monitor and evaluate its potential in suppressing the fly population.

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