Chapter

Lepidopter Parasitoidea

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Abstract

Parasitoids have a long history of pest management, specially for control of economical important lepidopteran pests, such as Noctuidae, Tortricidae, and Pyralidae. The two major parasitoids super families Trichogrammatidae and Braconidae in relation to biocontrol of lepidopteron pests are important. In this chapter: (i) the mass production of the moth egg parasitoid, *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera, Trichogrammatidae), which has been known to occur in Iran and attack many important hosts Lepidoptera, such as the chickpea pod borer, *Helicoverpa armigera* (Hübner, 1808), Carob moth, *Ectomyelois ceratoniae* (Zeller, 1839), and the codling moth, *Cydia pomonella* (Linnaeus, 1758) in the region and (ii) biology and parasitism behavior of *Bracon* (*Glabrobracon*) *variator* Nees, 1811, as larval ectoparasitoid of *Cydia johanssoni* Aarvik and Karsholt, 1993 (Lepidoptera: Tortricidae), a seed feeder of *Anagyris foetida* Linnaeus, 1758 (Fabaceae) at forest habitats in Western Iran, are discussed. Host development was arrested immediately upon parasitism. The dissected capsules show each adult female lay three eggs close to host larvae inside seed case. Video films and photographs of the behavior as research documents were recorded. Both aestivation and hibernation of the parasitoid occur in the parasitization rate on *C. johanssoni* averaged 18.77% ± 3.80, during second generation of the parasitoid wasp, *B.* (*G.*) *variator*.

Keywords: biological agents, Chalcidoidea, ectoparasitoid, Ichneumonoidea, Iran

1. Introduction

Many Lepidoptera larvae are economically important to agriculture and forestry ecosystems. For example, Noctuidae, Pyralidae and Tortricidae. Many Hymenoptera parasitoids, such as Trichogrammatidae and Braconidae are an important potential bio-control agent for control of moth pests. Recently, attention has been focused on this strategy due to increased consumer concern with pesticide residues in food products and a wide-ranging negative impact of chemical insecticides to the environment. Thus, using parasitoid Hymenoptera can be a safe and viable method of crop protection.

2. Hymenoptera parasitoid

Hymenoptera is the third largest and perhaps the most beneficial to humans of all insect orders. It has around 320,000 species, mostly more than 75% (240,000) are parasitoids (in 12 super families). Parasitoid wasps are highly diverse and specialized to attack a particular host life stage (egg, larvae, pupae and adults) of most arthropods, mainly insects. Major species richness of parasitoid wasps is in Ichneumonoidea 100,000 (which include Braconidae 40,000 and Ichneumonidae 60,000 known species) and Chalcidoidea 22,000 known species; Most species of Chalcidoidea are tiny, <3 mm in length; as a result, they can be difficult to collect and study. Detailed study an estimated more than 500,000 species in existence belong to the Chalcidoidea; within this superfamily, Trichogrammatidae are the smallest, ~0.2 mm in length, which includes 83 genera and 839 known species. Some *Trichogramma* species and strains have a wide host range of insect eggs, while others have strong preference for the eggs of a particular moth species and many successful biological control programs have involved the introduction of highly specific parasitoids [1–4]. The two major parasitoids groups, Trichogrammatidae and Braconidae in relation to biocontrol of lepidopteron pests are important. This chapter is concerned mass production of the egg parasitoid, *T. brassicae* Bezdenko, 1968 (Trichogrammatidae); addition refer to biology and parasitism behavior of *B.* (*G.*) *variator* Nees, 1812 (Braconidae), in relation to lepidopteran pests.

3. Trichogrammatidae

Most prominent species of *Trichogramma* are mostly amenable for insectarium mass production (**Figures 1, 2** and **6**) on factitious hosts like the grain moth, *Sitotroga cerealella* (Olivier, 1789), which it selves is mass produced on factory scales and is being used for biological control of noxious Lepidopterous pests of crops worldwide (**Figures 1, 2** and **6**). *Trichogramma* adults are typically free-living and the females are responsible for finding host insects for their progeny.

3.1 *Trichogramma* biology

The development of all *Trichogramma* spp. is very similar. The eggs hatch in about 24 h and the parasite larvae develop through three instars. These are followed by a prepupa, when the adult characters form, and a pupa. At the beginning of the third larval instars, the host egg turns black due to the deposition of black granules at the inner surface of the chorion, an invaluable diagnostic character for parasitized eggs. After about 5 days, the adult wasps emerge from the pupae and escape the bollworm egg by chewing a circular hole in the egg shell (**Figure 2**). *Trichogramma* overwinter as immature forms in host eggs. This short life cycle allows multi generations per year, and rapid population increase [5]. Hence, early season releases

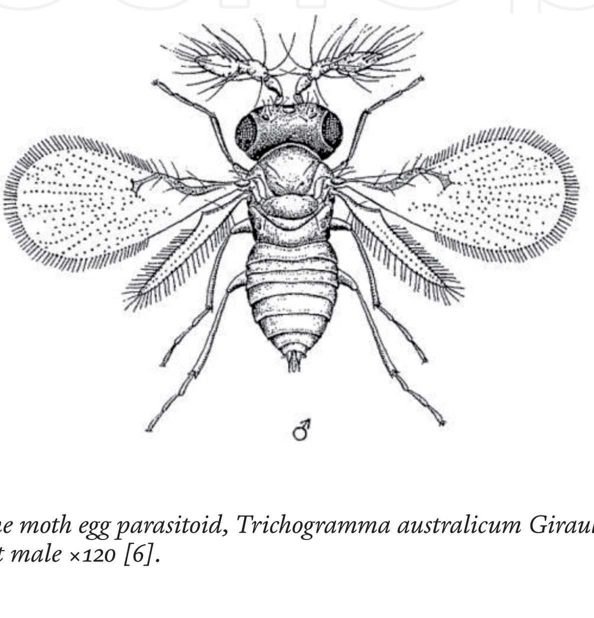

Figure 1.
General view drawing of the moth egg parasitoid, Trichogramma australicum Girault, 1912 (Hymenoptera: Trichogrammatidae), adult male ×120 [6].

produce large populations positioned to fight pest invasions. Unparasitized eggs remain light until black at hatching stage (**Figure 3**). For example, the development time for *T. australicum* Girault, 1912 (Hymenoptera: Trichogrammatidae) on the rice moth, *Corcyra cephalonica* (Stainton, 1866) (Lepidoptera: Galleriidae) at 28 ± 2 from egg to adult are completed in 14 days [5, 6]. The early stages of developed eggs (**Figure 3B**) are more suitable for parasite development. Older bollworm eggs, especially those in which the head capsule of the larva is visible, are not usually parasitized and if they are, parasite survival is much lower (**Figure 3C**) [7].

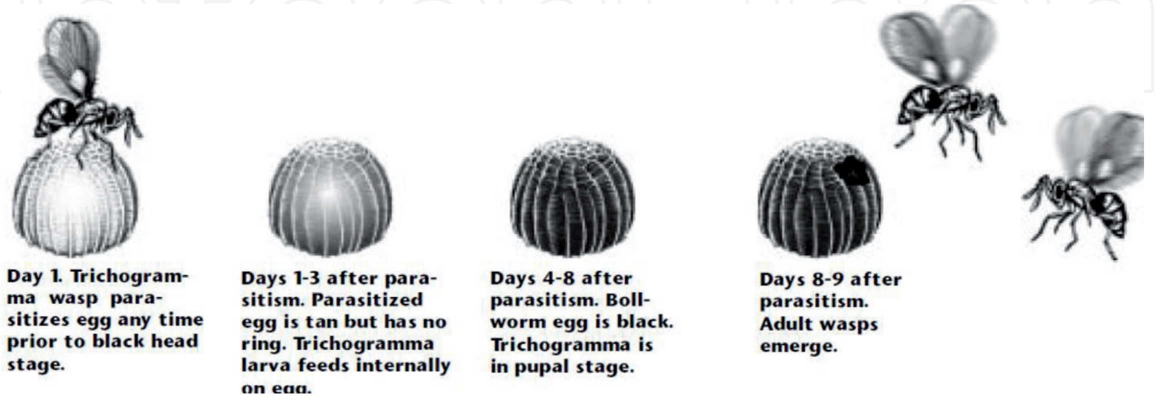

Figure 2.
Life cycle of the moth egg parasitoid, Trichogramma developing on the bollworm egg (Helicoverpa armigera) (Hübner, 1808) [5] (moth egg diameter 0.4–0.6 mm).

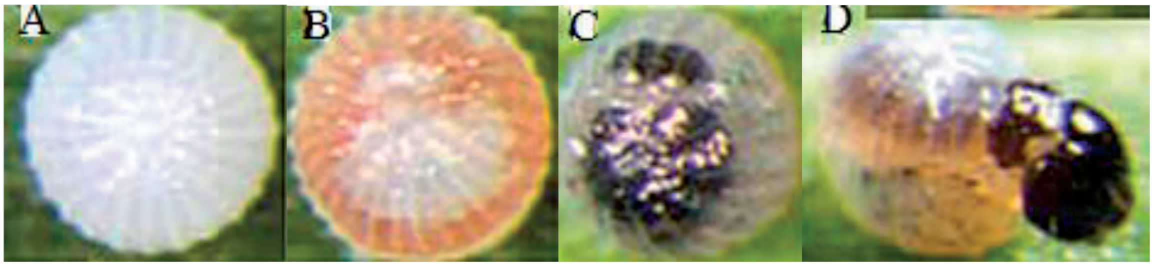

Figure 3.
Light micrographs of development of the pod borer eggs (unparasitized), Helicoverpa armigera (Hübner, 1808) showing the embryonic developmental sequences: (A) newly laid egg (yellowish-white); (B) 2 days old egg (tan egg); (C) egg before hatching or black head stage (larva visible) and (D) hatching stage (larva) (moth egg diameter 0.4–0.6 mm). Original.

Figure 4.
Images general view of the chickpea pod borer, Helicoverpa armigera (Hübner, 1808) (Lepidoptera: Noctuidae): (Right) 5th larval stage off the chickpea and (Left) The adult stage (Lepidoptera: Noctuidae); (adult wingspan 3.5–4 cm; 1.5–1.9 cm long and the mature larva or last instars was about 2.8 ± 0.05 mm long and 0.27 ± 0.08 cm wide). Original.

Figure 5.
Images general view of the pomegranate calyx worm, Ectomyelois ceratoniae (Zeller, 1839) (Lepidoptera: Pyralidae) and infested pomegranate fruit: Right to left. The adult stage of E. ceratoniae; 5th larval stage of E. ceratoniae off the pomegranate; infested split pomegranate fruit and applied cardboard capsule for infested orchards; (adult wingspan 1.6–2.5 cm, 1.5–1.8 cm long and the mature larva or last instars was about 1.8 ± 0.07 cm long and 0.75 ± 0.05 cm wide). Original.

Recently, mass rearing techniques have been developed for several parasitoids of insects pests, including, *Trichogramma* (Chalcidoidea: Trichogrammatidae). Currently, several laboratories are actively engaged in producing *Trichogramma* on local scale. These biological agents, parasitoids, are mass produced and released for controlling variety of insect pests including, the chickpea pod borer, *H. armigera* (Hübner, 1808) and the Pomegranate calyx worm, *E. ceratoniae* (Zeller, 1839), which maintain populations below economic levels [8, 9]. The chickpea pod borer, *H. armigera* (Hübner, 1808), (**Figure 4**) produces 52.5% losses [10] and more than 60% losses in the chickpea grower area every year [11]. It attacks on other Legume variety of economically important crops.

The Carob moth, *E. ceratoniae*, which known "Pomegranate calyx worm" (**Figure 5**). It is a pest that attack on variety of fruit crops such as Pomegranate, *Punica granatum* Linnaeus, 1880; Apples*, Malus domestica* Borkh, 1803; date palm, *Phoenix dactylifera* Linnaeus, 1753; Cultivated pistachio*, Pistacia vera* Linnaeus, 1753 [12, 13]. Split pomegranate fruit are more prone to infestation by larva as penetration into the fruit occurs more readily. The adult female deposits its eggs in already split fruits or on the skin or calyx of pomegranate fruit. It is widely distributed and occurs in Iran and close countries [12].

4. *Trichogramma brassicae* Bezdenko, 1968 (Hymenoptera: Trichogrammatidae)

The moth egg parasitoid, *T. brassicae* Bezdenko, 1968 is reared in private or government owned insectaries and released annually in the agricultural crops and orchards in Iran [14]. For example, the development time for *T. brassicae* Bezdenko, 1968, on the grain moth, *Sitotroga cerealella* Olivier, 1789 (Lepidoptera: Galleriidae) at 28 ± 2°C from egg to adult is completed in 14 days [14].

T. brassicae Bezdenko, 1968, pupae can be programmed to enter an overwintering condition of arrested development called diapauses. Once in diapauses, wasp pupae can be stored for up to 9 months so that the large demand for *Trichogramma* during the summer can be met [14, 15].

Cardboard capsules containing host eggs with developing *Trichogramma* are applied to release of *Trichogramma* in the chickpea farms or pomegranate orchards (**Figure 5**).

Released *Trichogramma* are at different developmental stages so that adults emerge from the capsules over several days. This increases the time interval between applications.

Two releases each at a rate of 460,000 pupae per hectare are made beginning at the first moth flight as determined by light traps. Chickpea pod borer, eggs hatch after about 5–6 days and the egg-laying period continues for 3–6 weeks. In-field reproduction of released parasites is believed to be important in providing residual control of eggs deposited after the second release. Field evaluations in Germany have shown releases result in a 70–93% reduction in corn borer larvae relative to untreated fields [15].

In western parts of Iran, releases of *Trichogramma* are a parts of integrated pest management in controlling the chickpea pod borer, *H. armigera* (Hübner, 1808) and the Carob moth, *E. ceratoniae* (Zeller, 1839).

Parasitoid attributes include: The ability to parasitize and develop in the target host egg, the species' preference for the target host egg, total egg mortality caused by parasitism, adult feeding, fecundity, development rate, sex ratio, and longevity releases [16, 17]. These characters are important in mass-rearing programs and then field releases.

5. Parasitism behavior of *Trichogramma*

Trichogramma spp. are most famous biocontrol agents and widely distributed in the world. *Trichogramma* drills a hole through the egg-shell and inserts two to three eggs into eggs of 200 pest moth species, including *Helicoverpa* spp., *Chilo* spp. the pink bollworm (*Pectinophora gossypiella* Saunders, 1844 and etc.) and preventing neonate larvae from hatching out and devouring crops. These parasitoids wasps are so small, <1 mm long; moth egg size, and hence how many of their own eggs to lay, is calculated by timing walks across moth egg surfaces. *Trichogramma* larvae eat out the insides of pest eggs, pupate, and cut an exit hole in moth eggshells for winged adults to squeeze through. Males emerge first, wait for females, and immediately mate.

6. Materials and methods: Mass production technique

Native parasitoid has proved to be one of the most potent egg parasitoid for so many important caterpillar pests. It is reared on factitious hosts such as, the flour moth, *Ephestia kuehniella* Zeller, 1879 (Lep.: Pyralidae), the grain moth, *S. cerealella* (Olivier, 1789), (Lep.: Gelechiidae) and other stored grain pests. The eggs of above hosts are utilized in mass rearing of this parasitoid [18]. *Sitotroga* eggs, test tubes, egg cards, refrigerator, UV chamber (fluorescent tube light, 15 W/Luminal flow), honey solution 20%, gam arabic, camel hair brush, Glass and adults of the grain moth, *S. cerealella* (Olivier, 1789), (Lep.: Gelechiidae). Following rearing procedure are involved in mass production of *T. brassicae* Bezdenko, 1968 (**Figure 6**).

Providing the Grain moth eggs: (i) For prevention from hatching of the eggs during and after parasitization of eggs, they should be exposed to UV rays (15 watt UV tubes) for 45 min at a distance of 5 cm.

Parasitoid species name, date of release of parasitoid for parasitization, expected date of emergence and Institute name and name of technical person should be given on the each egg sheet.

Processed egg sheet is than placed the plastic box. Introduce 6 days old duly parasitized egg cards to adult females of *Trichogramma* for 24 h. The parasitized and unparasitized eggs in each of box/containers should be in the ratio of 1:6 (parasitoid:host) to have optimum parasitization. Close the container properly for preventing escape of parasitoids. The parasitoids emerged in the container will parasitize the unparasitized eggs of the grain moth, *S. cerealella* (Olivier, 1789).

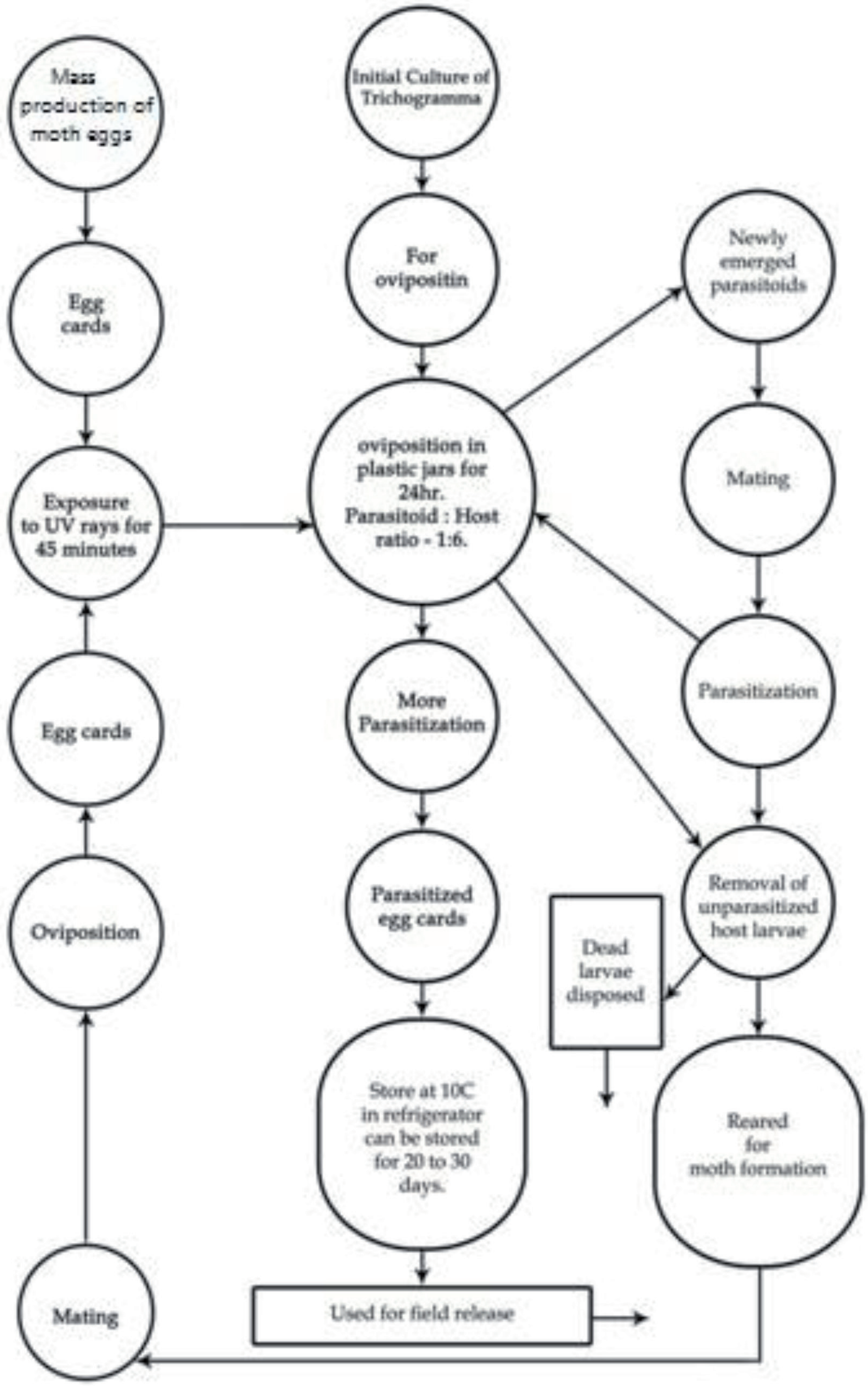

Figure 6.
Mass rearing procedure schematic: conventional production of egg parasitoid, Trichogramma brassicae, and its laboratory host, the eggs of grain moth, Sitotroga cerealella (Olivier, 1789).

Approaching the time of emergence of parasitoid, the egg color becomes blackish. Mating of sexes is essential for obtaining sex ratio, male:female, 1:1. Thus, mating is occurred in container.

Unparasitized eggs get hatched, into larvae (of host), such larvae should be removed from the container with the help of camel hair brush. The host larvae may destroy the eggs hence they should be removed from the container. The parasitoid complete its life cycle with 7–8 days.

After 4 days of parasitization, parasitized eggs of the grain moth, *S. cerealella* (Olivier, 1789), start changing their color from creamy white to blackish due to accumulation of urate granules. Such egg sheets are removed and stored in refrigerator at 10°C temperature for about 20–30 days for our convenience, duskiness point of view. The egg sheets thus preserved can be marked to farmers.

7. Method of calculation of parasitism rate and observation of oviposition behavior of *Bracon* (*Glabrobracon*) *variator* Nees, 1812, (Hymenoptera: Braconidae)

In order to determine parasitism rate of the ectoparasitoid, *B.* (*G.*) *variator* Nees, during first third of June, at least 100 infested seed pods (the stinking bean trefoil, *Anagyris foetida* Linnaeus, 1758 (Fabaceae)) by larva of *Cydia johanssoni* (Lepidoptera: Tortricidae) were collected daily and the number of parasitized larvae was counted and parasitism rate was calculated. The parasitoid was visible on the outside of the caterpillars.

In order to observe parasitism behavior, several live female wasps were collected along with pods containing unparasitized larvae and transported to laboratory. One adult female wasp was released into a petri dish with an infested pod and oviposition observed.

A digital camera, model Canon, PowerShot S3 IS and a digital Sony EXwave HAD camera connected to a stereomicroscope, model Blue Light, were used to take video films and photographs of the wasp behavior.

Immediately after oviposition, the seed capsules were dissected, under a stereomicroscope to observe the number of parasitoid eggs laid.

8. Braconidae

The Braconidae constitute one of the most species-rich hymenopterous families, with 48 subfamilies, more than 1050 genera and about 17,600 species described worldwide. The family has two major lineages: (a) the cyclostome braconids, most of which are idiobiont ectoparasitoids of concealed Lepidoptera and Coleoptera larvae although many are koinobiont endoparasitoids of Diptera and Hemiptera and (b) the noncyclostome braconids which are all endoparasitoids and are generally koinobionts, typically attacking the early instars of their hosts [19, 20].

Taxonomic significance of the genus *Bracon* Fabricius, 1804: The width of the hypoclypeal depression may be important at subgenera level; presence or absence of occipital carina and shape of first metasomal tergite (t_1) (flattened or not flattened lateral parts) [21]. *Bracon* genus is measured as a paraphyletic or also a polyphyletic group, mainly of small and middle-sized species [22] divided over several subgenera [23, 24]. For example, *Bracon* fauna of Iran include five subgenera: *Bracon* (*Bracon*) kozak Telenga, 1936; *B.* (*Glabrobracon*) Fahringer 1927; *B.* (*Habrobracon*) Ashmead, 1895; *B.* (*Asiabracon*) Tobias 1957 and *B.* (*Orthobracon*) Fahringer, 1927 [25]. Braconids also vary greatly in their biology [26–28].

Different species of parasitoids attack different life stages of the pest. For example, *B.* (*G.*) *variator* Nees, 1812, are larval ectoparasitoid and prefers 3rd–5th instars larvae and each adult female laid three eggs close to host larvae (**Figure 7**), inside seed case [32]. Most parasitoids behaviors are so identical and specialized that they can attack only a particular host life stage i.e., they attack eggs (eggs parasitoids), larvae (larval parasitoids), Pupae (pupal parasitoids), or adult (adult's parasitoids) [1]. There are considerable variations in insect parasitoids parasitism. These may be

Figure 7.
Image general view of the larval ectoparasitoid wasp, adult female, Bracon (Glabrobracon) variator Nees, 1812 (Hymenoptera: Braconidae). Original.

Figure 8.
Images general view of the larval ectoparasitoid wasp, Bracon (Glabrobracon) variator Nees, 1812, (Hymenoptera: Braconidae) and its host larva, Cydia johanssoni Aarvik & Karsholt, 1993 (Lepidoptera: Tortricidae): (A) female, oviposition on infested pod of stinking bean trefoil by pyralid seed feeder larva; (B) parasitoid's eggs ((B. (G.) variator)) laid close to the head of host larva (C. johanssoni); (C) developed parasitoid larvae inside the seed and (D) parasitoid larvae ((B. (G.) variator)) feeding upon Pyralid seed feeder larva, Cydia spp. [31].

idiobiont, whose hosts stop development, when they are parasitized. Idiobionts are either ectoparasitoids that kill their hosts or endoparasitoids that attack immobile host stages such as eggs or pupae. Koinobionts, allow the hosts to continue their development until the parasitoid's offspring matures. Most koinobionts are endoparasitoids of larval stages of insects, although a few are ectoparasitic [27]. Some female parasitoids also use the ovipositor to puncture a host and then feed on the body fluids before selecting other hosts for oviposition, thus causing two different types of mortality in the caterpillar pest population. In some cases, for example, *B.* (*G.*) *variator* Nees, 1812, the egg is laid externally on the body of the host and the larvae also feed externally. This parasitoid wasp is a highly polyphagous gregarious ectoparasitoid that attacks the larvae of a wide range of insects, such as Lepidoptera (**Figure 8**). For example, see [28], *B. (G.) variator* Nees, 1812, paralyzes the larvae of *Hadena bicruris* (Hufnagel, 1766) (Lepidoptera: Noctuidae) before depositing on average 3 eggs on 3rd-5th larval instars [29, 30]. Like *C. johanssoni* Aarvik & Karsholt, 1993, *H. bicruris* (Hufnagel, 1766) is a seed specialist, feeding on *Silene latifolia* Poir. 1789 (Caryophyllaceae) seeds. Lepidoptera larvae, attacks the young seeds before they are shed by the parent plant [32–34]. The larvae of the beech moth, *Cydia fagiglandana* (Zeller, 1841), feed inside the nuts of European beech, *Fagus sylvatica* Linnaeus, 1753 (Fabaceae) causing high seed mortality in South Sweden beech forests [35, 36]. Other economically important species are the pea moth, *C. nigricana* (Fabricius, 1794), attacking legume crops; the spruce seed moth, *C. strobilella* Linnaeus, 1758, attacking spruce seed, *Picea* spp.; *C. latiferreana* Walsingham, 1879, attacking fruits oak and the hickory shuckworm moth, *C. caryana* Fitch, 1756, an important pest of pecan [37].

First generation adult parasitoids of *B.* (*G.*) *variator* Nees, 1812, appeared in the first ten days of May, with the second generation appearing about a fortnight later, but adult wasps were most abundant, and its percentage parasitism were highest among larvae, in early June (**Table 2**). Adult activity was greatest during the hottest part of the day (12.00–14.00 hours). The parasitoid population was greatest on pods infested with *C. johanssoni* Aarvik and Karsholt, 1993, larvae, early June. Oviposition began after a lengthy search period. The female wasp first inspected the drilling into the pod (**Figure 8A**), using her antennae by tapping the pod. She then moved so that her mesothoracic legs straddled the drilling position, lifted her abdomen and inserted her ovipositor almost vertically into the pod (recorded Video film). Almost always each caterpillar had three eggs laid on it (**Figure 8B**); rarely, it was noted that more than 3 eggs were laid up to a maximum of six.

The oviposition is not an easy task. The tip of the ovipositor almost always gets stuck to tiny irregularities of the pod surface. When she loses her balance, re-starts oviposition from the beginning. Since the exact point of drilling is crucial and must be recalculated for accuracy. The eggs were white and bacilli-form, with a diameter approximately equal to the tip of a lab needle (**Figure 8B**). Eggs were oviposited directly onto the host larvae. The incubation period is 18 hours at 33°C and relative humidity of 14%. Upon hatching, the parasitoid larva penetrated the caterpillar's cuticle and fed on the body hemolymph for 4–5 days. Upon seed maturation, the parasitoid maturated larva secreted a webbed cocoon within the seed capsule and then pupated. Adults of second generation leave the seed pod in last ten days of May via a hole made by adult. Adults lived as free-living adults for up to 3–4 days until they mated and oviposited. When the larvae of *C. johanssoni* were paralyzed, their movement became reduced. As a result of feeding by the parasitoid larvae, the moth larva was weakened and eventually is reduced to the head capsule and body cuticle.

The free living adult wasps fed on resin produced when opens the first larval stage of *C. johanssoni* Aarvik and Karsholt, 1993, in the pod. The parasitoid aestivation and hibernation was as a pupa in a cocoon inside infested seeds. There are two overlapping generations during a year in natural conditions because of the diapause the parasitoid larvae go into at the end of the second generation. Additionally, parasitism activity of *B.* (*G.*) *variator* Nees, 1812, was also observed on an unknown seed feeder (Lepidoptera: Pyralidae). This was the only other host record for *B.* (*G.*) *variator* Nees, 1812. The unknown pyralid larva was green in color with large body, larger than *C. johanssoni* Aarvik and Karsholt, 1993, larva and had a very low population in the study area (**Figure 8D**).

The parasitization rate on *C. johanssoni* averaged 18.77% ± 3.80, during second generation of *B.* (*G.*) *variator* Nees, 1812 (**Tables 1** and **2**) [31].

General distribution of this wasp includes: China, Central Asia, Mongolia, Siberia, Russia, Crimea, Iran, Turkey and European country [26].

Stages	Egg	1st–3rd larval instars	Prepupa	Pupa	Adult longevity	Total	Release period	Sex ratio M/F
Day/s	1	4	1	2	7	14	1	1: 1

M = male and F = female.

Table 1.
The life stages longevity, release period and sex ratio of Trichogramma brassicae Bezdenko, 1968, reared on the grain moth eggs, Sitotroga cerealella Olivier, 1789 (Lepidoptera: Galleriidae) at 28 ± 2°C.

Date	Total number of *C. johanssoni* larvae	Number of parasitized larvae of *C. johanssoni* by *B. variator*	Parasitism rate (%)
1 June 2014	120	9	7.5
2 June 2014	105	7.5	7.14
3 June 2014	120	9	7.5
4 June 2014	120	7	5.83
5 June 2014	135	22	16.29
6 June 2014	110	20	18.18
7 June 2014	130	29	22.3
8 June 2014	110	38	34.54
9 June 2014	100	33	33
10 June 2014	110	39	35.45
Mean	116	21.35	18.77 ± 3.80

Table 2.
Total number, number of parasitized larvae and the percentage parasitism of Cydia larvae (Cydia johanssoni Aarvik and Karsholt, 1993 (Lepidoptera: Tortricidae) by the second generation of Bracon (Glabrobracon) variator *Nees, 1812 (Hymenoptera: Braconidae), Iran, 2014.*

9. Discussion

Trichogramma brassicae Bezdenko, 1968 (Hymenoptera, Trichogrammatidae) has been the object of great interest regarding its mass rearing and is used as a biocontrol agent against many moth pests, in studied area. This study also confirmed that *B.* (*G.*) *variator* is a gregarious idioboint ectoparasitoid with 2 generation in a year, attacks third-fifth instars *C. johanssoni* Aarvik and Karsholt, 1993, caterpillars. Almost always 3 parasitoid eggs were oviposited on each host larva within the pod, which had been previously paralyzed. These observations agree with those of Elzinga [29]. However, Elzinga has not mention about number of eggs and location of pupation.

Because of proper parasitism rate of *B.* (*G.*) *variator* Nees, 1812, this parasitoid plays important role in decrease population of *C. johanssoni* Aarvik and Karsholt, 1993, larvae and it is the most important natural enemy of *C. johanssoni* Aarvik and Karsholt, 1993, in Iran, therefore with conservation of this wasp would take an important step toward reduction of damage of pest moth and development of Stinking bean trefoil shrub in the west of Iran.

Parasitoid sex ratio: In the field condition, female population of *B.* (*G.*) *variator* Nees, 1812, was much more than male and male population was rare.

B. (*G.*) *variator* Nees, 1812, has been recorded from most parts of Iran, in East Azerbaijan [38], South Iran [25], North Central Iran [39] and from western part of Iran [31]. *B.* (*G.*) *variator* Nees, 1812, is already known as a larval parasitoid of the lychnis, *Hadena bicruris*, (Hufnagel, 1766), which is the most important of *Silene* *latifolia* Poir. 1789 (Caryophyllaceae). This ectoparasitoid attacks 3rd-5th instars moth larvae and stops host development immediately by paralyzing the caterpillars. *B.* (*G.*) *variator* Nees, 1812, is a gregarious parasitoid, which means that several parasitoid larvae attack a given caterpillar and produces clutches that are predominantly single-sex, mainly female (each individual parasitoid lays predominantly three sexed eggs). Females inject paralyzing venom into the host before oviposition. On hatching, the parasitoid larvae perforate the cuticle and imbibe nutrients from the paralyzed or dead host. The lychnis, *Hadena bicruris*, (Hufnagel, 1766) hibernates as a pupa in a cocoon [29]. The adult wasp *B.* (*G.*) *variator* Nees, 1812, feeds on resin of infested pods in the studied forest habitat, western Iran. *B.* (*G.*) *variator* Nees, 1812, is a widespread species, known from: China, Middle Asia, Mongolia, Siberia, Russia, Crimea, Iran, Turkey and Europeans countries [40].

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