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Effectiveness of potential biological control agent *Bracon hebetor* (Say) (Hymenoptera: Braconidae) on *Cydalima perspectalis* (Walker) (Lepidoptera: Crambidae)

Cydalima perspectalis (Walker) (Lepidoptera: Crambidae) üzerinde potansiyel biyolojik mücadele etmeni Bracon hebetor (Say) (Hymenoptera: Braconidae)'un etkinliği

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ABSTRACT

Cydalima perspectalis (Walker, 1859) (Lepidoptera: Crambidae) is the main pest of boxwood fields in many countries. It has caused significant damage especially since 6-7 years in Türkiye. Chemical control is definitely not recommended against this pest because boxwoods are found in forest areas, parks and gardens. In this study, the potential use of the idibiont larval parasitoid *Bracon hebetor* (Say, 1836) (Hymenoptera: Braconidae) against the box tree moth was evaluated within the scope of biological control. Laboratory experiments were carried out with early and late instar larvae of *C. perspectalis* at 25±1°C, 60-70% R.H. and 16:8 (light: dark) conditions. The number of paralyzed and parasitized larvae, parasitism rate, number of eggs laid per female, rate of larvae and pupae were determined. *Bracon hebetor*, which has the ability to paralyze the host larvae, showed an insecticidal effect against the pest *C. perspectalis*. It was found that the number of paralyzed larvae (10.33±1.15) in early stage (3rd instar) larvae was higher than in late stage (5th instar) larvae (4.67±0.57). On the other hand, it was determined that the number of parasitized larvae (14.68±1.50) and eggs laid (7.50±0.32) was higher in late stage (5th instar) larvae. Parasitism rates were found to be 33% and 73% in early and late instar larvae, respectively. This detailed biological data has been obtained to for the first time. *Bracon hebetor* venom showed a high toxic effect on *Cydalima* larvae. The results of the study showed that the parasitoid may be recommended as an alternative to chemical control against the box tree moth.

Key Words: Biological control, *Bracon hebetor*, *Cydalima perspectalis*

ÖZ
Cydalima perspectalis (Walker, 1859) (Lepidoptera: Crambidae) birçok ülkede şimşirlerin ana zararlısı konumundadır. Türkiye'de son 6-7 yıldır önemli zarara neden olmaktadır. Bu zararlıya karşı şimşirlerin özellikle ormanlarda, park ve bahçelerde bulunması nedeniyle kimyasal mücadele önerilmemektedir. Bu çalışmada, biyolojik mücadele kapsamında idiobiont larva parazitoiti *Bracon hebetor* (Say, 1836) (Hymenoptera: Braconidae)'un şimşir güvesine karşı potansiyel kullanımı değerlendirilmiştir. Laboratuvar çalışmaları genç ve olgun dönem *C. perspectalis* larvaları ile 25±1°C, % 60-70R.H. ve 16:8 (aydınlık:karanlık) koşullarda gerçekleştirilmiştir. Paralize olan ve parazitlenen larva sayıları, parazitleme oranı, dişi başına bırakılan yumurta sayısı ile larva ve pupa oranları belirlenmiştir. *B. hebetor* paraliz etme yeteneğine sahip olup bu sayede zararlı *C. perspectalis*'e karşı insektisit etkisi göstermektedir. Üçüncü dönemde paralize olan larva sayısı (10.33±1.15) beşinci döneme (4.67±0.57) göre daha yüksek bulunmuştur. Parazitlenen larva (14.68±1.50)

ve bırakılan yumurta sayısı (7.50±0.32) beşinci dönem larvalarda daha yüksektir. Parazitleme oranı erken ve geç dönem larvalarda sırasıyla %33 ve %73 olarak bulunmuştur. Sözkonusu biyolojik veriler ilk defa elde edilmiştir. *Bracon hebetor*'un venomu *Cydalima* larvaları üzerine yüksek derecede toksik etki yapmıştır. Çalışma sonuçları, şimşir güvesine karşı kullanılacak kimyasal mücadeleye alternatif olarak bu parazitoitin kullanılabileceğini göstermektedir.

Anahtar Kelimeler: Biyolojik mücadele, *Bracon hebetor*, *Cydalima perspectalis*

Introduction

Cydalima perspectalis (Walker, 1859) (Lepidoptera: Crambidae), which is known as box tree moth, is an exotic species. It is a primary pest that causes significant damage to Buxus spp (boxwood), which has a wide distribution in Türkiye (Öztürk et al., 2016; Toper Kaygın and Taşdeler, 2019). The first detection of this East Asian origin species in Europe was recorded Billen (2007) in Germany. Later, it spread rapidly in the most Europe country. The first report of *C. perspectalis* in Türkiye was recorded by Hızal (2012) in parks and gardens in Istanbul.

The larvae of the box tree moth can feed on several species of boxwood. It often causes the complete death of a plant. In Switzerland and Caucasus, it has been reported that an important area of forest has been lost due to the intense damage of *C. perspectalis* on *B. sempervirens* (John and Schumacher, 2013; Kenis et al., 2013; Gninenko et al., 2014). A similar damage was seen in Artvin between 2016 and 2017 in Türkiye (Göktürk, 2017). Although the number of generations of *C. perspectalis*, which is a polyvoltine species, depends on the temperature, it can complete 4-5 generations per year, but the number of generations is limited to 2-3 in Europe (Van der Straten and Muus, 2010; Leuthardt et al., 2010). In Türkiye, similarly Salioğlu (2020) reported that, *C. perspectalis* could complete 3 generations in Bartın, and 2 generations in Artvin depending on temperature. It spends the winter as a 2nd or 3rd instar larva between two leaves attached by nets. The first adult flights are observed in late May and early June, and moth adults can fly between 5-10 km (Nacambo et al., 2014, Van der Straten and Muus, 2010). Two morphological forms are dominant in adults. One of them is white colored butterflies and the other is butterflies in melanic form (Strachinis et al., 2015). Both forms of adults were also found in Türkiye (Salioğlu, 2020). It has been reported that the larval developmental period is between 28 and 85 days, the pupal development period is between 10-51 days, and generation is completed between 30-40 days under optimal conditions (López and Eizaguirre, 2019).

Biological control agents have an important role in the control of the pest *C. perspectalis*. Particularly, parasitoids belonging to the order Hymenoptera, (Braconidae) (Ichneumonidae) (Trichogrammatidae) (Herz, 2013; Wan et al., 2014) (Chalcididae), (Encyrtidae) (Brua, 2013; Wan et al., 2014). Göttig and Herz (2016) tested the effectiveness of different *Trichogramma* species on *Cydalima* eggs. This pest also has parasitoids belonging to the Tachinidae (Diptera) (Farahani et al., 2018; Martini et al., 2019; Bird et al., 2020).

The number of natural predators of *Cydalima* larvae is quite low, because those feed on boxwood leaves which have high alkaloids content (Leuthardt et al., 2013; Brua, 2013; Martin et al., 2018). The mono-basic alkaloids are metabolized while bi-basic alkaloids are retained in the larvae. Among the existing predators, *Vespa velutina* (Lepeletier, 1836) (Hymenoptera: Vespidae) is the most common species in Europe (Leuthardt and Baur, 2013; Brua, 2013; Wan et al., 2014; Feás and Charles, 2019). Egg predators are also extremely important for *C. perspectalis* because the pest eggs do not contain toxins. Therefore, the use of predatory species *Chrysopa carnea* (Stephens, 1836) (Neuroptera: Chrysopidae), *Harmonia axyridis* (Pallas, 1773) (Coleoptera: Coccinellidae) and *Orius majusculus* (Reuter, 1879) (Heteroptera: Anthocoridae) can suppress population of the pest (Herz, 2013). In the present study, the effectiveness of parasitoid *B. hebetor* on *C. perspectalis* larvae was tested under laboratory conditions for the first time in Türkiye within the scope of biological control.

Material and method

Rearing of Cydalima perspectalis

In this study, the box tree moth was reared in the laboratory for the first time in Türkiye. Box tree moth larvae were obtained from Kastamonu General Directorate of Forestry, in Türkiye. The larvae of the box tree moth at different instars were taken to the laboratory at 25±1°C, 60-70% relative humidity and 16:8 (light: dark) conditions and transferred to rearing box (19 x 24 x 7 cm) in groups of 20/each and fed with boxwood tree leaves. The larvae were checked daily and pupae were separated and placed in large rearing boxes (30 x 35 x 55 cm) where the adults emerged. Bunches of clean fresh boxwood were placed in large rearing boxes. The boxwoods have been used in the large rearing boxes to enable the butterfly adult females for mating and also, to lay their eggs on the leaves after mating. The leaves of boxwoods were also checked daily to determine eggs of *Cydalima.* The boxwoods with *C. perspectalis* eggs were separated and transferred to the rearing boxes. Figure (1) showed some photos about the rearing method.

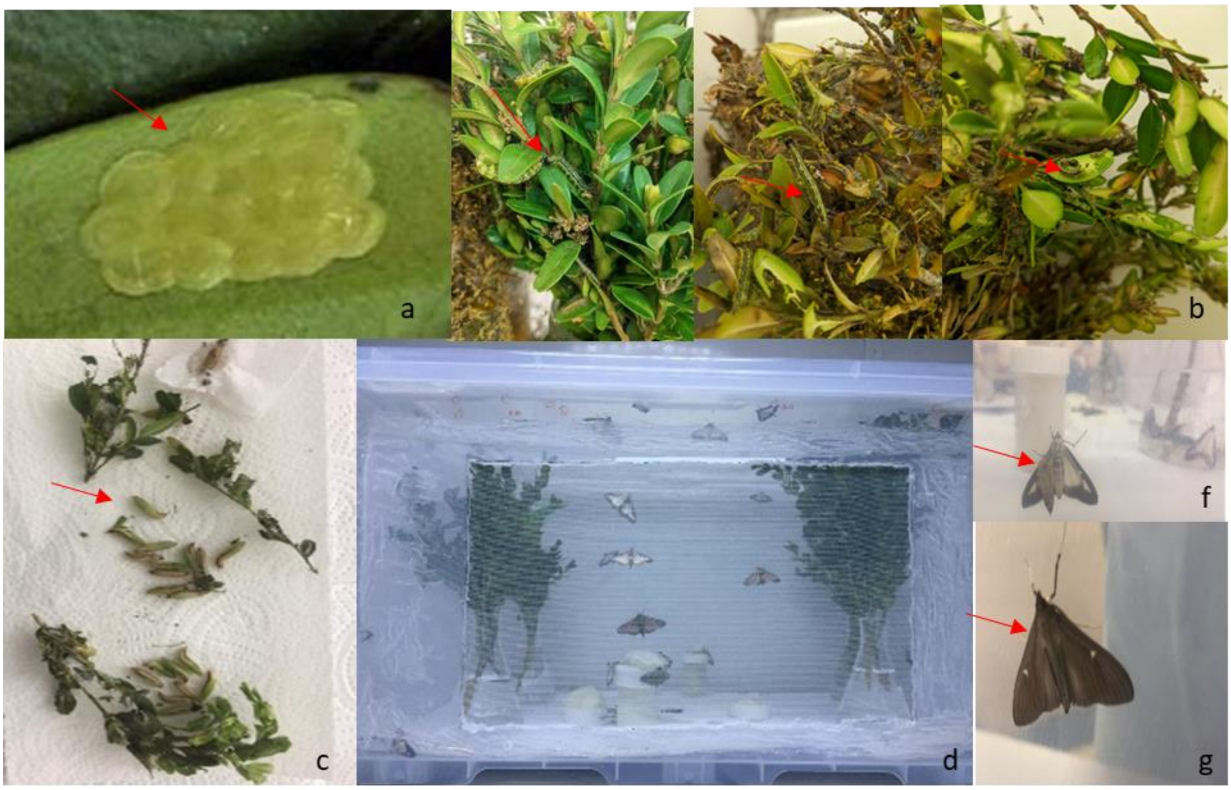

Figure 1. a) **Cydalima eggs b)** Different stages of Cydalima larvae c) * Cydalima pupae d) ** Rearing boxes with Cydalima adults f)** light colored adult moth g) *melanic adult moth (*Ankara University, Agriculture Faculty, Biocontrol Laboratory), (**INRAE UEVT Antibes Bioconcontrol Laboratory). Canon EF 100mm f/2.8 USM

Rearing of Ephestia kuehniella

Ephestia kuehniella, which was used as a host for *B. hebetor* culture, was reared in a climate room at 25±1°C, 60-70% R.H. and 16:8 (L:D). In the rearing of *E. kuehniella*, a mixture of corn flour: bran (1: 1) was used as a food. In order to prevent possible contamination in the prepared food, the prepared mixture was sterilized at 60°C for 3 hours. Other materials were sterilized with 1% sodium hypochlorite solution. 200 grams of nutrient mixture (1 corn flour :1 bran) was added to sterilized plastic rearing box (19 x 24 x 7 cm) and allowed to develop in the climatic chamber at average of 1000 host eggs. This process was repeated daily for the continuity of the *E. kuehniella* culture. The emerging moth adults were collected and taken into the egg laying box and the host eggs were daily collected (Demiray, 2021).

Rearing of Bracon hebetor

The adults of *B. hebetor*, were reared on a large scale, by using the larvae of the mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), as a host, at 25 ±1 °C, 60-70% R.H and 16:8 LD. For this purpose, a number of 40-45 4th or 5th instars *E. kuehniella* larvae were placed in 10 Petri-dishes (9 cm) with a fertilized female parasitoid. Then, droplets of 50% honey solution were applied to the paper strips and placed in the Petri- dishes to provide suitable diet for nutrition of the parasitoid adults. After 24 hours of exposure, the females were transferred to the other petri-dishes, with 40-45 4th or 5th instars larvae of the host. The parasitized larvae of the host, *E. kuehniella*, were incubated, under the controlled conditions of at 25 ±1 °C, 60-70% R.H and 16:8 LD. This process was continued every day. Female and male parasitoid adults were obtained from the culture which was used both in the experiments and for the continuity of the parasitoid culture (Demiray, 2021).

Effectiveness of Bracon hebetor on Cydalima perspectalis

In the experiments, female parasitoids tested were 2 days old, fed with honey, mated and not contacted with the host larva prior to testing and also 3rd and 5th instars of larvae of *C. perspectalis* were used. Larval stages were determined by weighing method. In the experiments, one larva of *C. perpectalis* and one mated *B. hebetor* female were placed in petri-dishes (5 cm). Larvae with parasitoid eggs were successfully parasitized. After that, each petri dish contained one *C. perspectalis* larva and one female parasitoid was left at 25±1°C, 60-70% R.H. and 16:8 (L:D) for a 24 hours of parasitism. At the end of the parasitism duration, the female parasitoid was removed. *C. perspectalis* larvae, treated with parasitoid, were examined under binocular (Leica S4). Experiments were carried out on 20 larvae (3rd and 5th instars) with three replications. At the end of the experiments this study was aimed to determine the number of paralyzed and parasitized larvae, the number of eggs laid on each parasitized larva, the larval rate, the pupal rate, the total developmental time, the parasitism rate, the emergence rate and the sex ratio.

Statistical Analysis

Independent sample T test was applied to the experiment results (number of larvae paralyzed, number of larvae parasitized, number of eggs laid, parasitism rate (%) in the Minitab Release 17 statistical program. An arcsine transformation was applied to percentage data for analysing. (Zar, 1999).

Results and Discussion

Results are shown in Table 1. According to the obtained results of the experiment, there were a significant difference between the 3rd and 5th instars larvae in terms of the number of paralyzed larvae, the number of parasitized larvae and the number of deposited eggs. It was determined that the number of paralyzed larvae was significantly higher on early larval instar (10.33) (T-Value = 7.60, P-Value = 0.017, DF = 2). Data showed that the number of parasitized larvae (14.68) was higher on the 5th instar larvae (T-Value = -6.41, P-Value = 0.003, DF = 4). When an evaluation was made in terms of the number of laid eggs by the female parasitoid, it was determined that the parasitoid lays more eggs (7.50/host-larva) on the larger (older) host larvae (T-Value = -7.71, P-Value = 0.000, DF = 56).

In this study, the first rearing method of the box tree moth was created in the laboratory for Türkiye. Similarly, data on some biological characteristics of *B. hebetor* were obtained for the first time with this study. *Bracon hebetor* females are idiobiont parasitoids that firstly inject venom in their hosts to paralyze them and then lay different numbers of eggs on/or near the paralyzed host (Antolin et al., 1995). The paralyzed host is also a food source for both adults and their larvae. It has been reported that *B. hebetor* female paralyzes several host larvae and then parasitoid female lays eggs on non-motile larvae (Ullyett, 1945; Ghimire and Phillips, 2014).

According to the results of the study, the female parasitoid paralyzed the early instar larvae, but the number of laid eggs was low. It generally, prefers late instar of *Cydalima* larvae for egg-laying. A similar result was obtained by Tunca (2010) who reported that the parasitoid paralyzed the third instar larvae of *Plodia interpunctella* (Hubner, 1813) (Lepidoptera: Pyralidae) but did not lay any or deposited a few number of eggs on *Plodia* larvae. For gregarious parasitoids, large host larvae are extremely important for the fitness of their generations (Godfray, 1987; Taylor, 1988). Studies have shown that gregarious parasitoids prefer large hosts for eggs laying. Laying eggs in large hosts ensures the healthy development of F1 (Hardy et al., 1992; Zaviezo and Mills, 2000). However, it has also been observed that host size is not very much important for laying egg preference in koinobiont parasitoids (Harvey, 2000; Harvey et al., 2004).

Considering the parasitization rates, in early instar larvae parasitism was 33.33%, and the parasitism on late instar larvae was 73.33% (Table 1) (T-Value = -6.14, P-Value = 0.009, DF = 3) (Table 1). After laying eggs on early and late instar of host larvae, it was determined that the rate of transition from egg to larva was 59.5 % and 65.3 %, respectively. The rate of transition from larval to pupal stage was found to be 26 % and 28 % in early and late instar of host larvae, respectively. It was determined that most of the parasitoid individuals could not pass from the larval stage to the pupal stage, or even if it passed to the pupal stage, it could not complete this stage. In other words, *B. hebetor* cannot complete its development from egg stage to adult stage on *Cydalima* larvae. Therefore, total developmental period, emergence rate and sex ratio could not be determined. Figure 2 shows different developmental stages of *B. hebetor* on the host *C. perspectalis*.

Table 1. Number of paralyzed and parasitized larvae by *Bracon hebetor,* number of eggs laid and parasitism rate

Host	Number of larvae paralyzed	Number of larvae parasitized	Number of eggs laid	Parasitism rate (%)
Early instar larvae	10.33±1.15 A	6.67±1.50 B	4.20±0.29 B	33.33 B
Late instar larvae	4.67±0.57 B	14.68±1.50 A	7.50±0.32 A	73.33 A

* Means in each column followed by same capital letter do not differ statistically differences (P<0.05).

Parasitoids are one of the important biological control agents and have an important role in the control to *C. perspectalis*. Göttig and Herz (2016) investigated several *Trichogramma* species on Cydalima eggs. Parasitoids species used in experiments include *Trichogramma brassicae* (Bezdenko), *Trichogramma bourarachae* (Pintureau and Babault, 1988), *Trichogramma cacoeciae* (Marchal, 1927), *Trichogramma cordubensis* (Vargas and Cabello, 1985), *Trichogramma dendrolimi* (Matsumura, 1926), *Trichogramma evanescens* (Westwood 1833), *Trichogramma nerudai* (Pintureau and Gerding, 1999) and *Trichogramma pintoi* (Voegele, 1982). *T. dendrolimi* gave the best results with 40% parasitization rate. Tabone et al., (2015) revealed that 54 strains of 17 *Trichogramma* species were studied and five strains of these were very effective on *Cydalima* eggs. Shi and Hu (2007) reported that *Exorista* sp.(Diptera: Tachinidae) caused a 32.6% and 47.5% mortality rate of larvae and pupae of *C. perspectalis* in the Xinyang region of China. Di Vitantonio (2016) reported that *E. larvarum* has a high rate of parasitism in the larval stage of the pest.

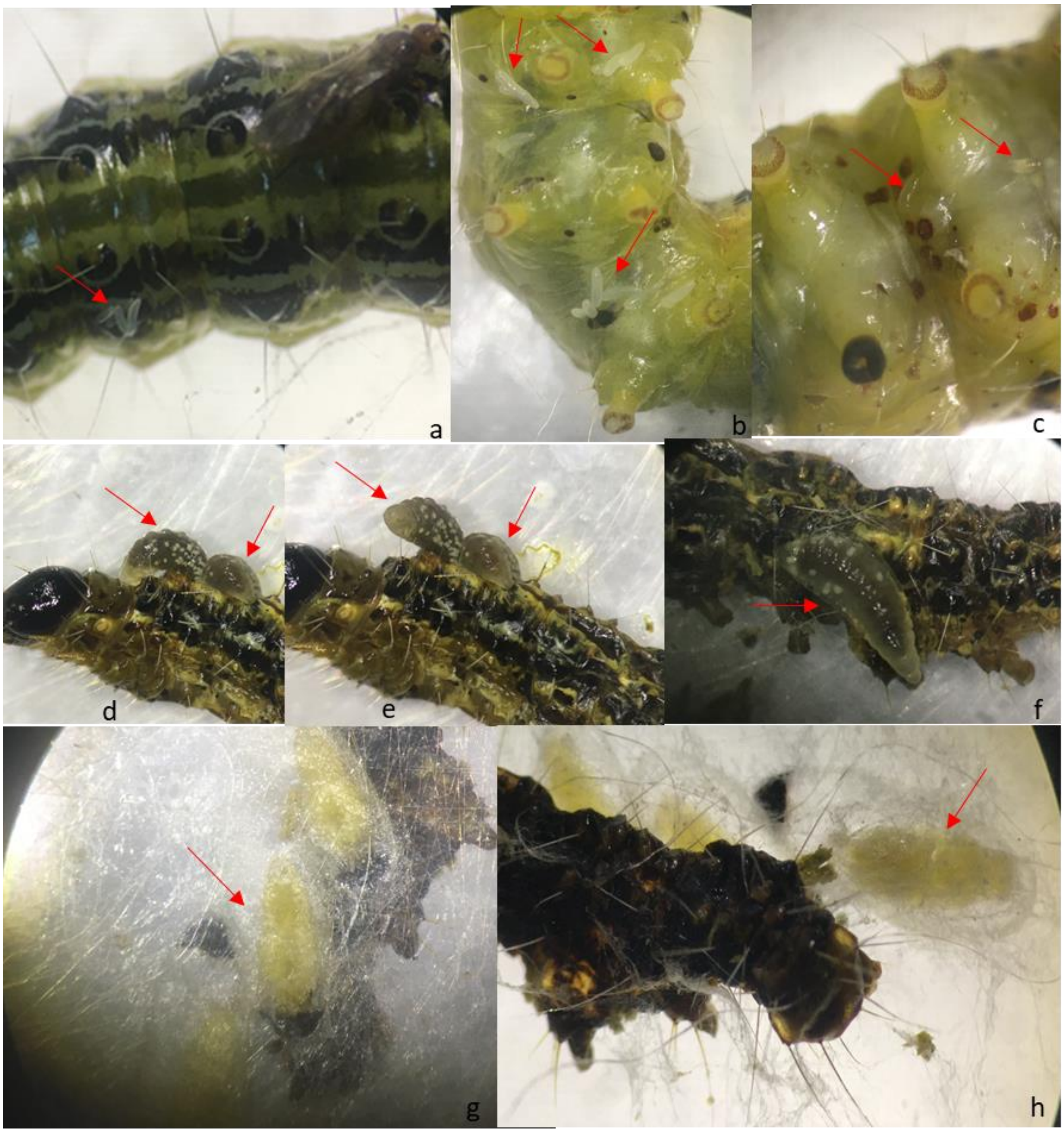

Figure 2. *Bracon hebetor* a-b)* Eggs c)* Early larvae d-e-f)* Advanced larvae g-h)* Pupae (*Ankara University, Agriculture Faculty, Biocontrol Laboratory) Leica S4 x 12.5.

In this study, it was determined that *B. hebetor* larvae could not complete their development and reach to the pupa stage on the host *Cydalima*'s larvae, thus there was no development from egg to adult stage. Similarly, it was noted that by Zimmermann & Wührer (2010) *Bracon brevicornis* (Wesmael, 1835) (Hymenoptera: Braconidae) and *B. hebetor*, was used against *C. perspectalis* in Germany, could not complete their development on the same host.

The host quality is a fundamental factor which affecting the biological characteristics of parasitoid progeny (Growth time, fertility, sex ratio, lifespan, adult size) (Vinson and Iwantsch, 1980; Charnov, 1982; Godfray, 1994). The host quality is also often related to components of host plant quality. Boxwood contains alkaloids according to different species, age and vegetative organs. For example, leaves, twigs and roots of *B. sempervirens* contains aminocycloartanoid alkaloids, which is most preferred by *C. perspectalis* for feeding. It has been stated that the total alkaloid ratio changes depending on age and season (Khodzhaev et al., 1983; Khodzhaev et al., 1984; Blaschek and Ebel 2007). Leuthardt et al., (2013) noted that the same alkaloid contents of *B. sempervirens* and *B. microphylla* were found in the tissues of *C. perspectalis* larvae. The same researchers reported that the dibasic alkaloids are stored in *C. perspectalis* larvae, whereas the monobasic alkaloids are metabolized. In addition, considering that *C. perspectalis* completes 2-3 generations per year, it has been reported that each generation is exposed to different concentrations of alkaloids on which the host larva had fed (Leuthardt & Baur, 2013). According to study, the unability of *B. hebetor* to complete its development on *C. perspectalis* larvae may be attributed to these alkaloids within the scope of tritrophic relationship.

Kaplan et al., (2016) reported that parasitoids are exposed to different plant's toxins. Firstly, some plant's toxins are not metabolised by the hosts, or these toxins are metabolised by the host and turned into toxic metabolites which retain in the hemolymph of the herbivore host. These accumulated toxins in the host insect's body provide a defense mechanism to the host for parasitoids (Futuyma and Agrawal, 2009; Nishida, 2002; Reudler and van Nouhuys, 2018). When the host took up the toxins from plants for their defense, it is ended up by decreasing the quality for the host insect (body size, etc.). In this case, it will also affect the parasitoid negatively (Ode, 2006).

Studies in which the parasitoid growth was negatively affected by the accumulated toxic alkaloids in the hemolymph of the host insect, were also studied by other researchers (Barbosa, 1988; Van Emden, 1995). Kester and Barbosa (1991) reported that high levels of nicotine in the hemeolymph of *Manduca sexta* (Linnaeus, 1763) (Lepidoptera: Sphingidae) larvae had a negative effect on the development of the parasitoid *Cotesia congregata* (Say, 1838) (Hymenoptera: Braconidae). Bukovinsky et al., (2009) noted that when the larvae of *Pieris rapae* (Linnaeus, 1758) (Lepidoptera: Pieridae) fed on with highly toxic glucosinolate, reduced the encapsulation ability of the parasitoid *Cotesia glomerata* (Linnaeus, 1758). However, venoms injected by stings of various hymenopterous parasitoids that kill the pest is reflected as the examples of the biological method. Although many studies have examined the biological control of insect pests through these venoms (Saba et al., 2017).

According to results of this study, the idiobiont larval parasitoid *B. hebetor* may be used against the main pest of box tree *C. perspectalis*, which could be found in the forests, parks and gardens of Türkiye. Forest ecosystem has its own complex relationships. The destruction of this natural system can cause other pest problems. Therefore, application of chemical insectides should be avoided. For this reason, the use of *B. hebetor* in the control of this pest becomes even more important. Additionally, it shows an highly insecticidal effect with its paralysis characteristic. *B. hebetor* biologically controls *C. perspectalis* by paralyzing it and causing its death. The parasitoid is not able to settle in the nature, because it is not able to complete its own development on *Cydalima* larvae, meanwhile pest's population could be suppressed by augmentative releases against each generation of *Cydalima* every year, periodically. In addition, it is necessary to test the effectiveness of *B. hebetor* in field conditions.

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Conflict of Interest: There is no conflict of interest.

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