

Orijinal araştırma (Original article)

Effects of artificial diets and floral nectar on longevity and progeny production of *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae)

Bitki nektarı ve yapay besinlerin *Trichogramma euproctidis* Girault (Hymenoptera: Trichogrammatidae)'in ömür uzunluğu ve döl verimi üzerine etkisi

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Summary

Trichogramma species are the most widely used biological control agents against lepidopteran pests. They prefer sugar-based substances as foods, especially plant nectar. Flowering plants have been shown to differ with regard to their attractiveness to parasitoids and nectar accessibility. The value of floral nectar and laboratory diets as food sources for *Trichogramma euproctidis* (Girault) (Hymenoptera: Trichogrammatidae) was assessed in the laboratory by testing the effects of different floral nectars (dandelion, dog-fennel, dead nettle, willow, plum) and artificial diets (honey, grape molasses, raisins, beet molasses, glucose and sucrose syrups, and egg yolk) on the longevity, capacity for parasitism and adult emergence. *Trichogramma euproctidis* females that fed on honey (10.5 days) and dandelion flowers (8.7 days) lived significantly longer than females that fed on other floral nectars and artificial diets. The females fed on raisins (2.9 days) and water alone (2.6 days) had the shortest longevity. *Trichogramma euproctidis* females that fed on sucrose syrups (30.9 eggs), honey+egg yolk+water (29.13 eggs) and honey (28.0 eggs) parasitized significantly more hosts than females that fed on other floral nectars and artificial diets. The females fed on raisins (18.5 eggs) and plum flowers (15.5 eggs) were shown to have the least parasitizing ability. The results indicate that carbohydrates such as dog-fennel nectar or honey can increase the performance of the wasp parasite. Although floral nectar qualities may be of greater importance to parasitoid longevity when selecting floral resources for conservation biological control, the artificial diets proved to be suitable foods for sustaining the development and reproduction of *T. euproctidis*.

Key words: *Trichogramma euproctidis*, nectar, artificial diet, parasitization, longevity

Özet

Trichogramma türleri lepidopter zararlılarına karşı en çok kullanılan biyolojik mücadele ajanıdır. Gıda kaynağı olarak şekerli bileşikler, özellikle nektarı tercih ederler. Çiçekli bitkiler, parazitoitleri cezbetme ve nektar uygunluğu yönünden farklılık göstermektedir. Bu çalışmada, *Trichogramma euproctidis* (Girault) (Hymenoptera: Trichogrammatidae)'in ömür uzunluğu, parazitlenme kapasitesi ve ergin çıkışı üzerine, çiçek nektarları (karahindiba, köpek papatyası, ballıbaba, söğüt, erik) ve yapay besinlerin (bal, pekmez, melas, üzüm, glikoz, sükröz şurubu ve yumurta sarısı) etkileri araştırılmıştır. Bal (10.5 gün) ve karahindiba (8.7 gün) ile beslenen *T. euproctidis* dişileri çiçek nektarı ve diğer yapay besinlerle beslenenlere göre daha uzun süre yaşamıştır. Ballıbaba (3.1 gün) ve suyla (2.6 gün) beslenen dişiler ise en kısa süre yaşamıştır. Sükröz şurubu (30.9 yumurta), bal (28.0 yumurta) ve köpek papatyası (27.6 yumurta) ile beslenen *T. euproctidis* dişilerinin parazitlenme gücü diğer besinlere göre daha yüksek bulunmuştur. En düşük parazitlenme üzüm melası (22.6 yumurta) ve erik çiçekleriyle (15.5 yumurta) beslenen bireylerde gözlenmiştir. Bu çalışmada elde edilen bulgularla parazitoitin parazitlenme performansının Karahindiba nektarı veya bal gibi karbonhidratlarla arttırılabileceği gösterilmiştir. Ayrıca her ne kadar koruyucu biyolojik mücadelede bitkisel besin kaynakları parazitoitin ömür uzunluğu için önemli olsa da, *T. euproctidis*'in gelişimi ve üremesinin devamlılığı yönünden yapay besinlerin uygun olduğu ortaya konmuştur.

Anahtar sözcükler: *Trichogramma euproctidis*, nektar, yapay besin, parazitlenme, ömür uzunluğu

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Alınış (Received): 10.06.2011 Kabul edilmiş (Accepted): 10.10.2011

Introduction

The efficacy of natural enemies as biological control agents is sometimes limited by phenological asynchrony with their host or prey populations or by climatic intolerance during parts of the season. In some circumstances, such limitations can be overcome by augmentative releases of insectary-reared natural enemies (Leppä & King 1996; Smith, 1996). It is generally accepted that most parasitoids require sugar sources, such as nectar, extrafloral nectar or honeydew, to cover their energy needs. Plant-derived foods can enhance pest suppression by parasitoids through improved nutrition (Wade et al., 2008). Protocols for the mass rearing and release of these natural enemies often take these sugar requirements into account. Nevertheless, the choice of food sources and methods of feeding are usually based on trial and error because basic information on the food ecology of beneficial insects is scarce (Wäckers, 2003). Parasitoids emerge with a limited supply of energy. The nutrients transferred from the larval stage often cover no more than 48 h of the parasitoid's energy requirements. This period is extremely brief, but sugar feeding can considerably increase the parasitoid's lifespan (Wäckers, 2003). Nectar and honeydew usually function as fuels for adult metabolism or as complementary food, rather than as complete diets; nevertheless, the fitness benefits from feeding on nectar or honeydew can be pronounced (Hainsworth et al., 1991; Fischer & Fiedler, 2001).

Several studies have screened various diets or food sources for *Trichogramma* spp. (Bourarach & Hawlitzky, 1989). Adult *Trichogramma* spp. are known to feed upon nectar (sugary plant secretions), pollen (Wellinga & Wysoki, 1989), and honeydew (excretions from attended homopterans) (Koptur, 1992). Feeding has been shown to increase the longevity of *Trichogramma platneri* Nagarkatti (Hohmann et al., 1989) and the parasitizing ability of *T. perkinsi* Girault and *T. australicum* Girault (Somchoudhury & Dutt, 1988).

Trichogramma species are egg parasitoids widely used in inoculative and augmentative release programs against many Lepidopteran pests and some 32 million hectares of agricultural and forest land are treated with *Trichogramma* spp. annually (Li, 1994). *Trichogramma* spp. are also used against different lepidopteran species on corn in the provinces of Adana, İzmir and Aydın in Turkey (Öztemiz, 2008)

In the present study, we present data on *T. euproctidis* reared on artificial diets and plant-derived food sources for several generations in a laboratory. The aim of the study was to determine the influence of natural floral nectars and artificial diets as food sources on the longevity, parasitization rate, progeny survival and sex ratio of *T. euproctidis* to explore the idea that they could serve as a supplemental food source for *T. euproctidis* in stores, warehouses and in the field.

Materials and Methods

Insect Source: The *T. euproctidis* (Girault) (Hymenoptera: Trichogrammatidae) used in this experiment were initially collected from a corn field in Adana and were continuously reared on *Ephestia kuehniella* eggs for ≈ 100 generations in the laboratory at $27\pm 1^\circ\text{C}$, $70\pm 5\%$ r.h, and a photoperiod of 14:10 (L:D). Fresh (less than 24 h old) *E. kuehniella* eggs were glued on pieces of white cardboard (2 cm x 4 cm) and were then placed in rearing vials (7.5 cm x 2 cm). These eggs were offered simultaneously to single *T. euproctidis* females for 24 hours and then were discarded. The females used in the experiments were ≈ 24 h old, mated, and lacked egg laying experience. Preliminary observations showed that males emerged earlier and females mated soon after emergence. Sex ratio was calculated by using the form of the antenna to distinguish the adult females

Host Eggs: Eggs of the Mediterranean flour moth (MFM), *E. kuehniella* were used for rearing and as host eggs in the experiment. The *E. kuehniella* adults were obtained from the Department of Plant Protection, Faculty of Agriculture of Ankara University. Adults of MFM were reared on a mixture consisting of one kg wheat flour, 5% yeast and 30 g of wheat germ. Throughout the rearing, cultures were

kept in a rearing room, equipped with a control system, at $27\pm 1^{\circ}\text{C}$, $70\pm 5\%$ r.h. and a photoperiod of 14:10 (L:D) h. Light was controlled using a 24 h time switch and fluorescent tubes. To obtain eggs for the experiments, approximately 100 mated females of MFM were collected from stock cultures and released in plastic jars. The jars was provided with fine cloth mesh strips hanging vertically from the top to provide a resting place and mating site for the adults. Deposited eggs that fell through screen were collected at the bottom of the jars in the following days. The eggs were then sifted to remove insect parts and frass, placed in petri dishes and exposed to UV irradiation (Mineralight Lamp, Shortwave UV, 254 nm, 215-250 V, 56/60 Hz, 0.12 A) to halt embryo development. The eggs of the rearing host *E. kuehniella* need to be sterilized because the voracious larvae of this species can reduce the quantity of *Trichogramma* produced by consuming parasitized eggs.

Experimental Design: The fifteen diet treatments were designated as UF for wasps that were unfed (control), AD for wasps that were given an artificial diet (honey, grapes, molasses, raisins which were rinsed with water, beet molasses, 10% glucose and 10% sucrose syrup, egg yolk+honey [1:1, w/w (weight/weight)] and egg yolk+honey+water [1:2:1, w/w (weight/weight)], ND for wasps that were regularly given whole flowers with nectar (dead, nettle, willow, dog-fennel, plum and dandelion), and W for wasps that were given water alone. One of the four treatments was applied to each glass tube as follows: (1) artificial food only (AD1-AD8), given with dilute honey on filter paper and replaced every second day; (2) flower only (ND1-ND5), a single flower was enclosed in the glass tube and replaced every second day; (3) water only, a drop of water was added to the glass tube; (4) neither food nor flower present. About 50 fresh *E. kuehniella* eggs were placed in glass vials (10 cm x 3 cm) and recently emerged (0–24 h) *T. euproctidis* females were introduced and held for 24 h. After that exposure time, the adults were removed and the number of parasitized eggs counted. All experiments were carried out in the rearing rooms under the conditions mentioned earlier. All treatments were replicated 8 times, each involving one female. Replicates were made parallel to the diet test given above. This procedure ensured that all females within the glass tubes were sufficiently and similarly exposed to the diets. The number of parasitized eggs, adults and sex ratio were determined after the larval and pupal development of the parasitoids. For each sample sheet, the number of host eggs parasitized (blackened eggs) was counted daily for 5 d after the exposure of the sheet to a parasitoid. To assess the effect of the tested diets on the longevity of *T. euproctidis*, the flowers and other diets were offered simultaneously to single *T. euproctidis* females. Treatments were replicated 10 times and the food sources and cardboard sheets were replaced daily until the parasitoids died. The longevity of each female was recorded daily as maximum days alive; for example, when a female was found dead on day 3, her longevity was recorded as 3 days.

The flowers were brought to the laboratory and cleaned of any plant parts and insects that may have fallen into the collection cylinder. We offered different whole flower with nectar to *T. euproctidis* females in glass tubes (vials). Honey, molasses and the other diets were dotted onto the paper with a sharpened dissecting probe to provide four dots no larger than 2 mm in diameter. The control tubes contained only water. Flowers were collected daily and spread on white paper under a lamp to check for insects and then offered to the parasitoids.

Data were analyzed using SPSS 10.0 Windows (SPSS, Chicago, IL). A one-way analysis of variance (ANOVA) was used to study the effects of the food sources applied as a factor and the number of parasitized eggs, adult emergence and female emergence as parasitization efficiency dependent variables (PROC ANOVA & PROC GLM) (SPSS 1999). All data were transformed to square roots before statistical analysis; when significant differences occurred, a Tukey-HSD was applied as a means of separation. Back transformed data were presented in Figures (SPSS, 1999).

Results

Longevity

The longevity of *T. euproctidis* females was influenced by the diet consumed. When only water was given, *T. euproctidis* individuals lived for only 2.6 ± 0.221 days, and had very low fecundity (24.5 ± 0.824). Honey (AD1) and dandelion flowers (ND5) significantly increased the longevity of females ($F=24.46$, $df=14,135$; $P<0.001$) of *T. euproctidis* (Figure 1). With honey (AD1) as a food source, the mean value of female longevity (10.5 ± 0.957 days) was four times greater and adults lived a maximum of 14 days, and the mean fecundity (28.0 ± 1.350 days) was one and a half times greater than that of wasps without food (UF) (Figure 2). Females lived the shortest time when fed with only water (W); all adults of *T. euproctidis* that emerged from host eggs died within 4 days.

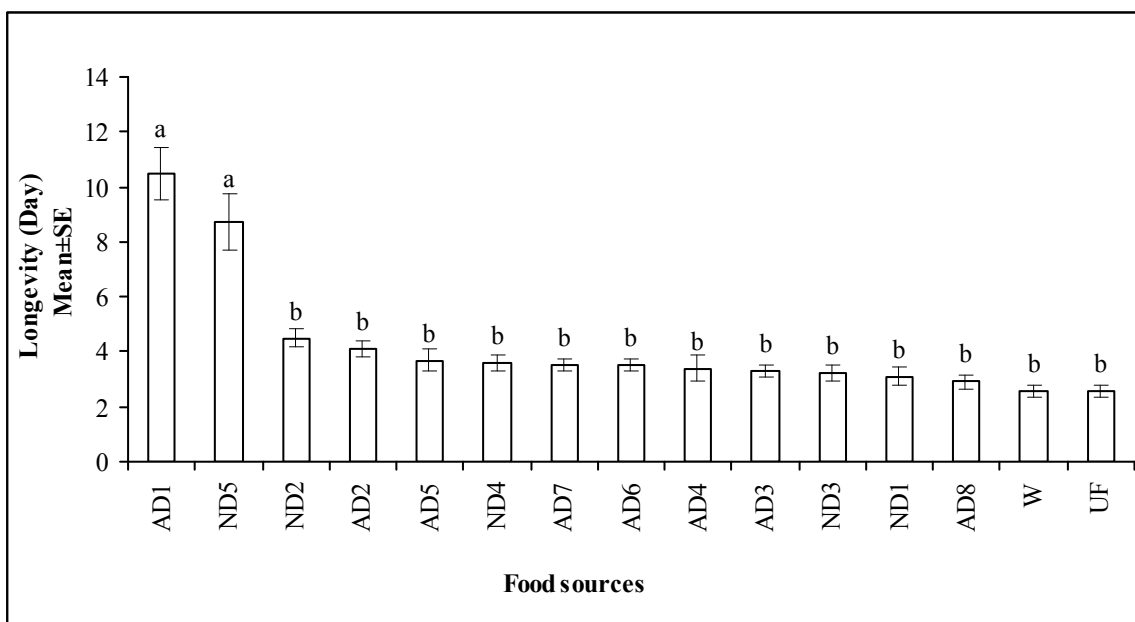


Figure 1. Mean longevity of *Trichogramma euproctidis* females when reared on the various artificial diets and flower nectars (M±SE). The same letters on the bars indicate that no significant difference at a 5% probability level was observed with the Tukey test. (AD1: Honey, AD2: Grape molasses, AD3: Beet molasses, AD4: Glucose, AD5: Sucrose, AD6: Honey+Egg yolk, AD7: Honey+Egg yolk+Water, AD8: Raisin, ND1: Dead nettle, ND2: Willow, ND3: Dog-fennel, ND4: Plum flowers, ND5: Dandelion, W: water only, UF: Unfed).

Parasitization

The one way ANOVA analysis showed that the number of eggs parasitized by *T. euproctidis* females was significantly affected by the food type. The treatments can be categorized into three groups in regards the number of parasitized eggs: the lowest (AD6, ND2, ND5, ND1, W, AD4, AD3, AD2, UF, AD8 and ND4); middle, (AD7, AD1, and ND3); and the highest (AD5) ($F=2.96$; $df=14,105$; $P=0.001$; one-way ANOVA). Within each category, no significant difference was observed among treatments ($P>0.05$; Figure 2). A large variation was found within the diets as regards fecundity. The oviposition rate decreased dramatically, especially for females on diets AD8 and ND4. The mean parasitism by wasps fed on sucrose (AD5) was significantly different from those fed on plum flowers (ND4), raisins (AD8) and those without food (UF) (Figure 2).

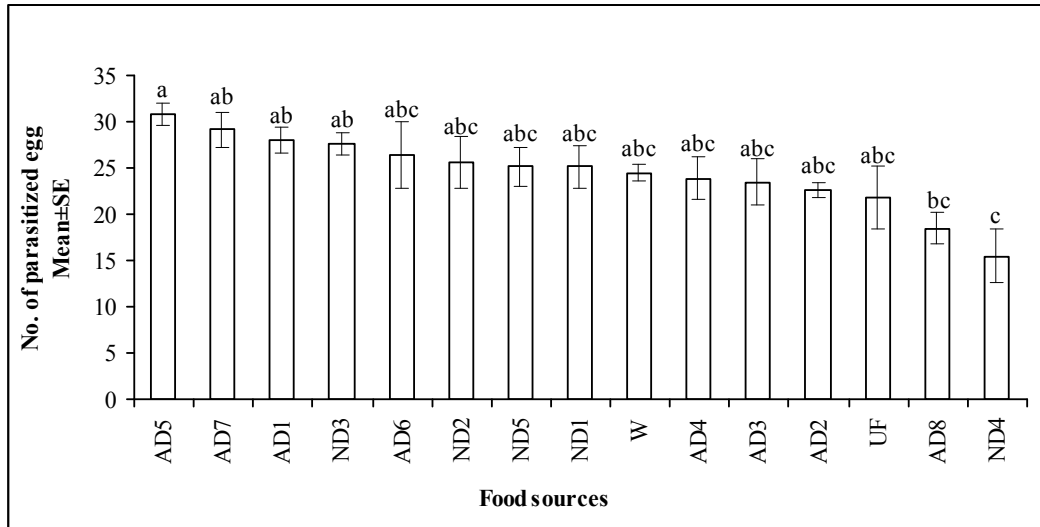


Figure 2. The number of individuals parasitized by *Trichogramma euproctidis* when reared on the various artificial diets and flower nectars ($M \pm SE$). The same letters on the bars indicate that no significant difference at a 5% probability level was observed with the Tukey test. (AD1: Honey, AD2: Grape molasses, AD3: Beet molasses, AD4: Glucose, AD5: Sucrose, AD6: Honey+Egg yolk, AD7 Honey+Egg yolk+Water, AD8: Raisin, ND1: Dead nettle, ND2: Willow, ND3: Dog-fennel, ND4: Plum flowers, ND5 Dandelion, W: water only, UF: Unfed).

Adult emergence

The parasitoid completed development on all diets tested (Figure 3). One-way ANOVA indicated that AD and ND had a significant influence ($F=57.51$; $df=14,105$; $P<0.001$) on adult emergence and the number of progeny that emerged. The number of progeny which emerged almost mirrored the pattern shown in the number of parasitized eggs and can also be divided into three groups: ND1, W, ND5, AD1, AD4, AD3, AD2, UF, AD8 and ND4 are the lowest; AD7, ND3, AD6, and ND2 are in the middle group; sucrose (AD5) is the highest ($F=57.51$; $df=14,105$; $P<0.001$; one-way ANOVA) (Figure 3). The trend of daily progeny emergence was very similar to the number of eggs parasitized daily.

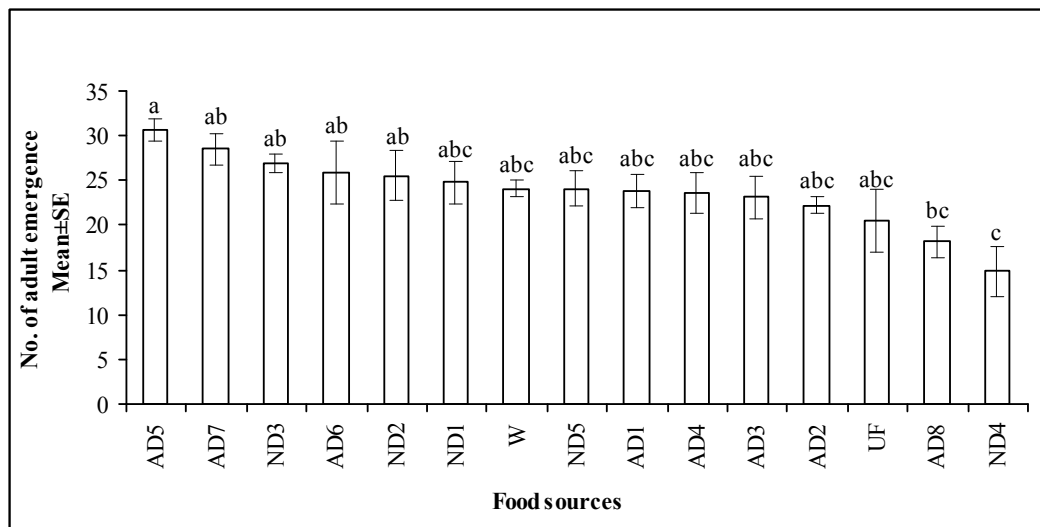


Figure 3. The number of *Trichogramma euproctidis* adults that emerged when reared on the various artificial diets and flower nectars ($M \pm SE$). The same letters on the bars indicate that no significant difference at a 5% probability level was observed with the Tukey test. (AD1: Honey, AD2: Grape molasses, AD3: Beet molasses, AD4: Glucose, AD5: Sucrose, AD6: Honey+Egg yolk, AD7 Honey+Egg yolk+Water, AD8: Raisin, ND1: Dead nettle, ND2: Willow, ND3: Dog-fennel, ND4: Plum flowers, ND5: Dandelion, W: water only, UF: Unfed).

A relationship was observed between the adult emergence of *T. euproctidis* reared on the different diets and survival rate of the F_1 generation. A large variation was found for the diets as regards fecundity. A large variation was found for within the results of the diets as regards fecundity. The greatest adult emergence was obtained on the sucrose diet (AD5) (99.51%), it was significantly different from that of wasps fed on plum flowers (ND4) and without food (UF) ($F=3.109$, $df=14$, $P<0.05$).

Female emergence

The numbers of females that emerged were significantly affected by the food given. The numbers of female progeny which emerged were different from the pattern shown in the number of parasitized eggs and can also be divided into three groups: ND4 is the lowest; W, AD3, AD4, ND5, AD2, AD8 and UF are in the middle; AD5, AD7, AD6, ND3, ND2, AD1 and ND1 are the highest ($F=4.37$; $df=14,105$; $P<0.001$; one-way ANOVA) (Figure 4). The trends of daily progeny emergence were very similar to the numbers of eggs parasitized daily.

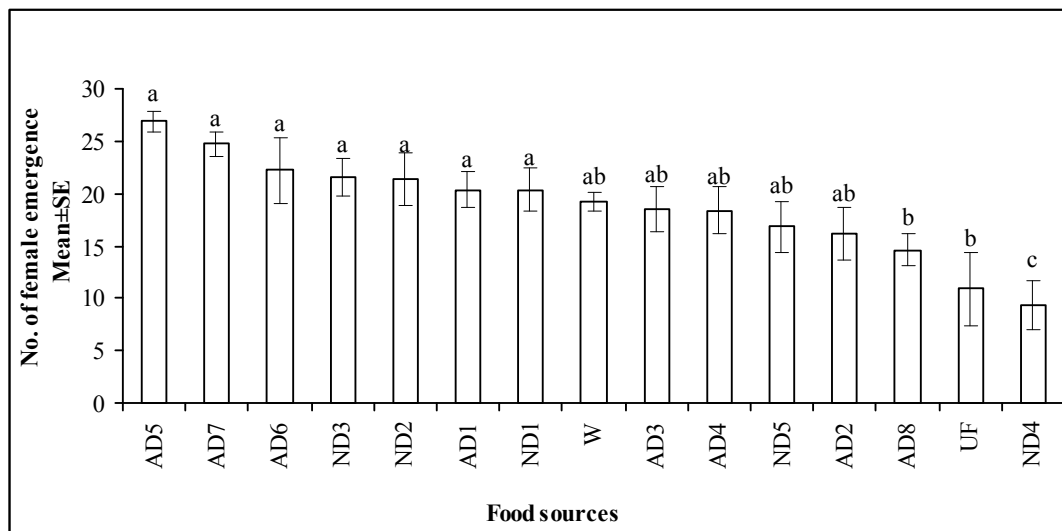


Figure 4. Mean number of female emergences of *Trichogramma euproctidis* when reared on artificial diets and flower nectars. The same letters on the bars indicate that no significant difference at a 5% probability level was observed with the Tukey test. (AD1: Honey, AD2: Grape molasses, AD3: Beet molasses, AD4: Glucose, AD5: Sucrose, AD6: Honey+Egg yolk, AD7: Honey+Egg yolk+Water, AD8: Raisin, ND1: Dead nettle, ND2: Willow, ND3: Dog-fennel, ND4: Plum flowers, ND5: Dandelion, W: water only, UF: Unfed).

The greatest female emergence was found for sucrose (AD5) and honey+egg yolk+water (AD7) diets with 87.76% and 86.84% female emergence, respectively. Female emergence differed significantly among the diets, in particular sucrose significantly differed from the raisin diet (AD8), plum flowers (ND4) and no food (UF) ($F=4.37$; $df=14,105$; $P<0.001$).

Discussion

All natural floral nectars and artificial diets allowed *T. euproctidis* to complete development. This indicates that there is a potential for rearing this parasitoid on artificial and floral nectars. The parasitization and fecundity of *T. euproctidis* females was greater on sucrose (AD5) and this value was significantly different from those obtained on plum flowers and raisins. The number of parasitization decreased to a relatively constant level from sucrose to the raisin and plum flower. The greatest female emergence was for sucrose (AD5) and honey+egg yolk+water (AD7). In contrast, some of the diets (plum flowers and raisins) produced females inferior to those on other diets.

Nectar contains a high concentration of carbohydrates that can provide energy, pollen and nectars together can generally provide a complete diet for the successful growth, development, and reproduction of many insects (Thompson & Hagen, 1999). The performance of parasitoid wasps can be strongly affected by the quality and quantity of food resources. Özkan (2007) reported that feeding with honey significantly increased egg laying of *Venturia canescens*. Other authors have documented that parasitoids are generally exploit floral nectar sources since they are innately attracted to floral colors and odors and can learn to orient to cues associated with previous feeding experiences (Lewis & Takasu, 1990; Wäckers, 1994; Patt et al, 1999). Zhang et al. (2004) showed that *T. brassicae* females fed on corn pollen plus water had significantly increased longevity and fecundity compared with those fed on water alone.

Feeding with other flowers or artificial foods significantly affected mean adult emergence. *Trichogramma euproctidis* reared on the different diets showed the high viability of the F₁ generation. not clear Somchoudhury & Dutt (1988) and Gurr & Nicol (2000) reported that feeding on flowering maize or sorghum was shown to increase the parasitizing ability of *T. perkinsi* Girault and *T. australicum* Girault. egg maturation in *Trichogramma* continues during adult life (Fleury & Bouletreau, 1993; Volkoff & Daumal, 1994) and the availability of carbohydrates significantly increases lifetime fecundity (Hohmann et al., 1989; Leatemia et al., 1995). In the same way, fructose, cotton-plant nectar, honey and beef extract in various combinations with honey were found to be equally nutritious (Ashley & Gonzalez, 1974)

In the present study, with honey as a food source, mean female longevity was four times longer, and the mean lifetime fecundity was one and half times greater than the no food diet (unfed females). When *T. euproctidis* individuals were given only water they lived for only 2.6 ± 0.221 days, but there was similar lifetime fecundity to the other diets. These results are supported by other studies which found an increase in longevity and fecundity (when it was measured) with flowers or honey as a food source, compared with water or no food (Leius, 1961; Leatemia et al., 1995; Gurr & Nicol, 2000; Johanowicz & Mitchell, 2000; Costamagna & Landis, 2004). Saljoqi & Khattak (2007) reported that adult *T. chilonis* females provided with 50% honey and water lived significantly longer than unfed females or those provided with some other kind of food. Feeding has been shown to increase the longevity of *Trichogramma platneri* Nagarkatti (Hohmann et al., 1989). *Uscana mukerjii* females fed with raw honey during their entire life span showed increases of 2.4 and 2.5 times and a 1.5 times increase in their fecundity. However, feeding on honey for a short duration (1 day/2 days) did not increase longevity or fecundity of the parasitoid significantly (Sood & Pajni, 2006). It was reported that mean time to 50% survivorship of unfed parasitoids *T. platneri*, was 2.0 ± 0.1 days, whereas honey-fed parasitoids lived significantly longer (2.6 ± 0.1 days) (Mansfield & Mills 2002). Although the availability of honey markedly affected the longevity of *T. platneri*, it did not increase fecundity (Hohmann et al., 1989). In another study, the mean fecundity of mated females fed with honey for twenty-four hours and then allowed to oviposit for five days, was similar to unfed females (Hohmann et al., 1989).

The augmentative release of mass-reared trichogrammatid egg parasitoids has been used as a promising method to reduce egg hatching and subsequent damage caused by larval pests. The availability and quality of the food available plays an important role in determining the effectiveness of parasitoids as control agents. We conclude that artificial and natural diets are effective for rearing *T. euproctidis*, except for diets without food, and with plum flowers and raisins as food sources, based on parasitization, adult emergence and female longevity.

In the current study, artificial diets proved to be suitable foods for sustaining the development and reproduction of *T. euproctidis*. Floral nectar qualities may be of importance to parasitoid longevity when selecting floral resources for conservation biological control. Nectar sugar composition may also be

crucial in determining its nutritional suitability. Our results suggest that the tested artificial diets and plant-derived food sources could serve as food sources for *T. euproctidis* in stores, warehouses and in the field.

Acknowledgements

The authors would like to thank Erciyes University in Kayseri Turkey for a supporting grant from its Research Fund through Project Number EUBAP - FBT-08-583

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