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Research Article

Age-specific oviposition behavior of *Trichogramma brassicae* on *Ephestia kuehniella* under laboratory conditions

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Abstract
Biological characteristics especially oviposition behavior of parasitoids are one of the most important factors to determine their efficiency to suppress a pest population. In this study, age-specific oviposition pattern, parasitism rate, hatch rate, and sex ratio of *Trichogramma brassicae* on *Ephestia kuehniella* were evaluated at temperature of 25±1ºC, 70±5% relative humidity and a 16 h light: 8 h dark photoperiod. The results showed that younger *T. brassicae* (1st-4th days) moved throughout the cardboard and often parasitized eggs which placed in the central part, but female parasitoid of *T. brassicae* moved straight on cardboard and parasitized closer eggs in the end days of life (5th and 6th days). The maximum daily parasitized eggs by *T. brassicae* was 13.03±0.56 at the beginning of its oviposition period. Then, it decreased significantly with adult female age. The female age influenced hatch rate (%) of *T. brassicae* eggs. Its values were ranged from 95.07±0.82% (1st day of oviposition period) to 42.78±6.43% (6th day of oviposition period) in different days of adult female age. The highest values for sex ratio were estimated to be in the first 3 days of adult female life. The female-biased sex ratio decreased significantly with increasing adult female age. For efficient mass rearing of *T. brassiace* on *E. kuehniella*, parasitoids should not be kept more than three days in cultures because the progeny becomes male-biased and parasitism rate decreased significantly after 3rd days. Results of this study show good potential of *T. brassicae* and it suggest for mass rearing and to use in augmentative biological control program.
Key words: Mass rearing, *Trichogramma brassicae*, Biological control, Sex ratio

رفتار تخمریزی وابسته به سن زنبور پارازیتویید *Trichogramma brassicae* روی *Ephestia kuehniella* در شرایط آزمایشگاهی

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چکیده
ویژگی‌های زیستی به‌ویژه رفتار تخمریزی زنبورهای پارازیتویید یکی از مهم‌ترین عوامل تعیین‌کننده کارایی آنها در مدیریت جمعیت آفت میزبان می‌باشد. در این مطالعه الگوی تخمریزی وابسته به سن، نرخ پارازیتیسم، درصد تفریخ و نسبت جنسی زنبور پارازیتویید *Trichogramma brassicae* روی *Ephestia kuehniella* در شرایط آزمایشگاهی [۱±۲۵ درجه سلسیوس، رطوبت نسبی ٪۵±۷۰ و دوره نوری ١٦: ٨ ساعت (روشنایی: تاریکی)] بررسی شد. نتایج نشان داد زنبورهای *T. brassicae* در سنین جوانی (١ تا ٤ روزه) در سراسر تریکوکارت حرکت می‌کنند و اغلب تخم‌های میزبان را که در مرکز تریکوکارت هستند پارازیته می‌کنند، اما در سنین بالاتر (روزهای ٥ و ٦) به صورت مستقیم روی تریکوکارت حرکت می‌کنند و تخم‌هایی را که در دسترس‌تر باشند پارازیته می‌کنند. بیشترین پارازیتیسم روزانه زنبور *T. brassicae*،

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۱۳/۰۳±۰/۵٦و در اوایل عمر زنبور پارازیتویید مشاهده شد. با افزایش سن زنبور پارازیتویید، میزان پارازیتیسم روزانه کاهش یافت. درصد تفریخ تخم‌های پارازیته‌شده تحت تأثیر سن زنبور پارازیتویید قرار گرفت و مقدار آن بین ۹۵/۰۷±۰/۸۲ درصد (روز اول تخمگذاری) تا ٤۲/۷۸±٦/٤۳ درصد (روز ششم تخمگذاری) متغیر بود. بیشترین مقدار نسبت جنسی در سه روز اول عمر زنبورهای ماده مشاهده شد و با افزایش سن زنبورهای ماده، نسبت ماده زایی به طور معنی داری کاهش یافت.در پرورش انبوه زنبور *T. brassicae* روی *E. kuehniella*، زنبورهای پارازیتوییدها نباید بیش از سه روز در انسکتاریوم نگهداری شوند زیرا نسبت جنسی به سمت نرزایی رفته و نرخ پارازیتیسم به طور معنی داری پس از روز سوم عمر زنبور پارازیتویید کاهش می‌یابد. نتایج این تحقیق، کارایی بالای زنبورهای پارازیتویید *T. brassicae* را نشان می‌دهد، بنابراین برای پرورش انبوه و استفاده در برنامه‌های کنترل بیولوژیک اشباعی پیشنهاد می‌شوند.

واژه‌های کلیدی: پرورش انبوه، *Trichogramma brassicae*، کنترل بیولوژیک، نسبت جنسی

دریافت: ۱٤۰۰/۰۸/۲۱، پذیرش:۱٤۰۱/۰۳/۱۵.

Introduction

Egg parasitoids of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) have been intensively studied and used in applied biological control programs (Orr *et al*., 2000; Parra, 2014) because of their efficiency, wide geographic distribution, easiness of laboratory rearing, and large numbers of hosts, more than 200 hosts belonging to 70 families and more than eight insect orders (Wu *et al*., 2018). The release of *Trichogramma* for biological control of Lepidopterous pests has been considered for more than 150 years. In the 1960s, the Europeans and Americans revitalized research on *Trichogramma* and they began their mass-rearing and release in the 1970s (Babendreier *et al*., 2003). Recently, inundative release of *Trichogramma* is doing in more than 50 countries in the world (Yan *et al*., 2020).

Trichogramma brassicae Bezdenko is a main and widely distributed species of *Trichogramma* in Iran (Ebrahimi *et al*., 1998; Shojai *et al*., 1998; Poorjavad *et al*., 2012). This species uses for biological control of many pest species; therefore, it is reared in the insectarium widely on different hosts such as *Ephestia kuehniella* (Zeller), *Sitotroga cerealella* (Olivier), *Plodia interpunctella* (Hubner), *Galeria mellonella* (L.), and *Corcyra cephalonica* (Stainton) (Smith, 1996; Ebrahimi *et al.*, 1999).

The different species of Trichogrammatid wasps have various oviposition behaviors to find their host and lay eggs in them. Their hosts are sessile and the parasitoid female examines the host at her leisure before depositing her egg (Ahmed, 2008). *Trichogramma brassicae* females almost do host feed following oviposition (Mills & Kuhlmann, 2004). They can increase their longevity and future production of eggs, or they can forgo host feeding and slowly starve, decreasing their longevity but increasing their immediate oviposition output of their current eggs (Rivero & West, 2005).

Several biological characteristics such as parasitism rate and sex ratio of parasitoids determine their ability to suppress a pest population (Kivan & Kilic, 2006). Parasitism rate is defined as ratio of the number parasitized eggs to the total number of available eggs (Mills & Kuhlmann, 2000). Sex ratio [Number of females/(number of males+number of females)]

in parasitoid cultures during mass rearing is one of the major problems affecting the success of biological control programs. Because wasps have haplo-diploid reproduction, therefore their females determine sex of the offspring through the regulation of sperm access to eggs from spermatheca where it is stored after copulation (Ode & Hardy, 2008). Female age of parasitoid is one of the main important factors known to influence sex ratio and parasitism rate of parasitoid wasps (Kivan & Kilic, 2006).

Considering in importance of biological characteristics of *T. brassicae* to estimating its efficiency in pest management program, we evaluated age-specific oviposition pattern of *T. brassicae* and age-specific parasitism rate, hatch rate and sex ratio of this parasitoid on *E. kuehniella* under laboratory condition. The results of this research can help us to determine the optimum ages of *T. brassicae* to mass rearing of this parasitoid.

Materials and methods

Insect culture

Ephestia kuehniella eggs were prepared from a culture maintained at the Insectary and Quarantine Facility, University of Tehran. The culture was kept on 75% wheat flour, 23% bran and 2% yeast at 25±1ºC, 70±5% RH, and a photoperiod 16: 8 h (L: D).

Trichogramma brassicae wasps were originally collected from Babolsar city, south of the Caspian Sea, Iran. For this study, the stock colony of *T. brassicae* came from cultures maintained by the Biological Control Department of IRIPP (Iranian Research Institute of Plant Protection). The colonies were reared on *E. kuehniella* for at least 10 generations in laboratory conditions at temperature of 25±1ºC, 70± 5% relative humidity and a 16 h light: 8 h dark photoperiod. Honey-water solution (20%) was provided for adult parasitoids.

Experiments

To study the age-specific parasitism rate and oviposition pattern of *T. brassicae*, a white cardboard (5 cm width ×5 cm length) were divided to dimensions 1 cm width ×1 cm length and created a cardboard with 25 boxes (Fig. 1). A young egg of *E. kuehniella* (< 12 h old) was placed per box on cardboard therefore a cardboard was containing 25 *E. kuehniella* eggs (< 12 h old). Then it was placed into an individual glass Petri dish (8 cm diameter) and a mated female (< 24 h old) was released into each plastic container for 24 h. A streak of water and honey solution (20%) was placed inside of the Petri dish to provide food for adult parasitoid. The female parasitoid was transferred into a new Petri dish including cardboard with 25 host eggs (< 12 h old) daily, until the death of the female parasitoid. The exposed eggs on cardboard for each day were maintained separately under laboratory condition until all parasitoid adults were emerged. Then the number of parasitized eggs was counted for each day, separately. After hatching of parasitized eggs, hatch rate was determined, too. After emergence of adults, the sex ratio of the progenies were recorded for each day. Also according to parasitized eggs of *E. kuehniella* on cardboard, ovipostional pattern of *T.*

brassicae were determined for each day. This experiment had 30 replications. These experiments were carried out at temperature of 25±1ºC, 70± 5% relative humidity and a 16 h light: 8 h dark photoperiod.

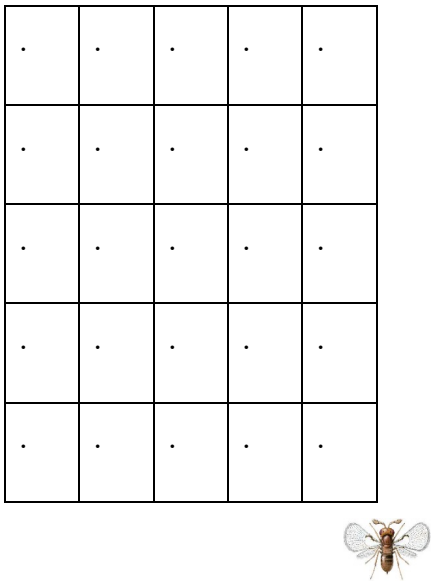

Fig. 1. White cardboard contains 25 numbers of *Ephestia kuehniella* eggs

Statistical analysis

Oviposition pattern was drawn by Excel 2016 for each day. All statistical comparisons were carried out with SPSS software version 18, using One-Way ANOVA and Tukey's test with P *value* ≤ 0.05 (SPSS, 2009).

Results

The results of relationship between parasitoid age and its oviposition pattern are presented in Fig. 2. *Trichogramma brassicae* prefer to parasitize eggs that were in center part of cardboard in a parasitoid's early days of life. In the 5th and 6th days of female life, parasitoid female selected eggs that were more available.

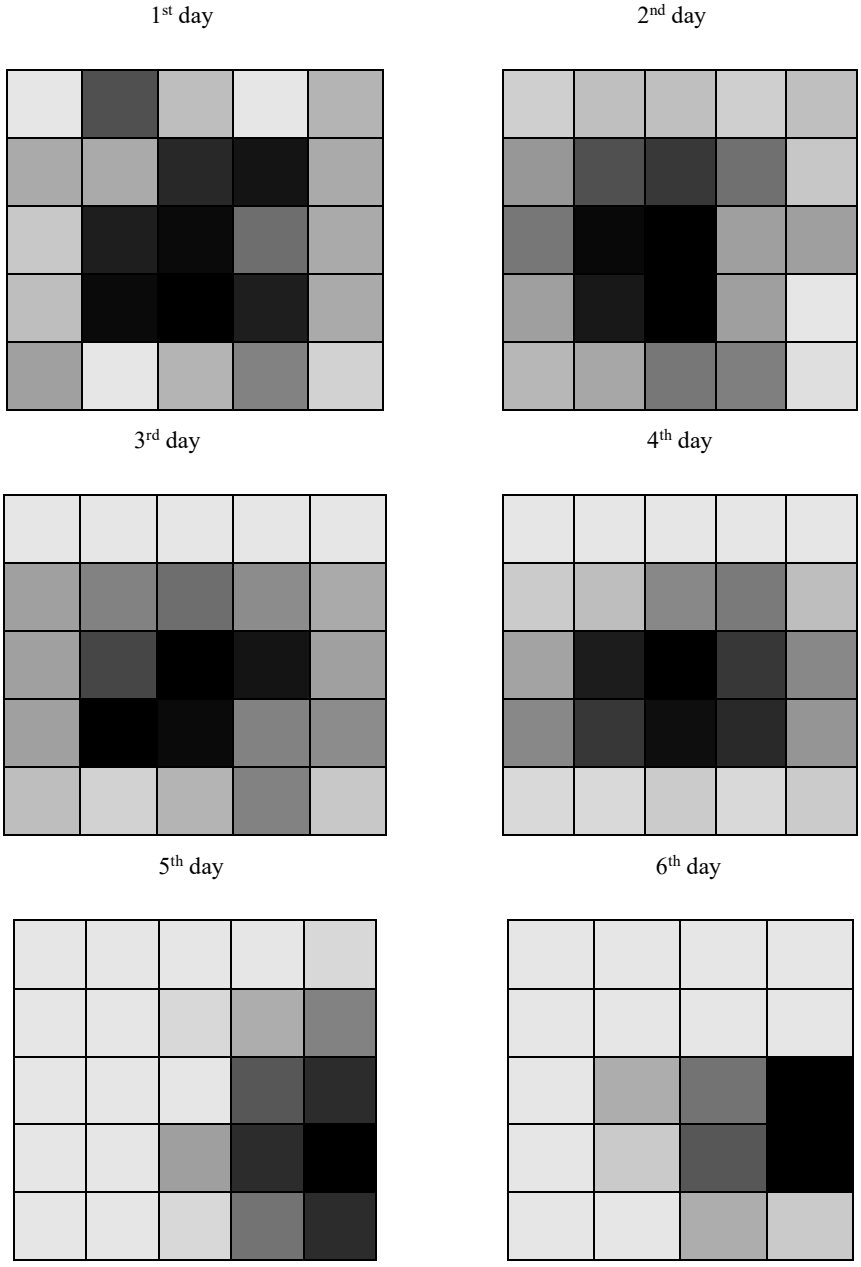

Fig. 2. Ovipostional pattern of *Trichogramma brassicae* on *Ephestia kuehniella* eggs (Highest ■ value and lowest □ value, respectively)

Age-specific parasitism rate of *T. brassicae* on *E. kuehniella* eggs are shown in Fig. 3. The maximum daily parasitized eggs by *T. brassicae* was 13.03±0.56 at the beginning of its oviposition period. Then, it decreased significantly ($F= 126.93$; $df= 5, 174$; $P < 0.001$) with adult female age. The parasitism value of *T. brassicae* was 10.67±0.44, 7.13±0.40, 4.77±0.40, 3.10±0.22, and 1.73±0.22, respectively in 2nd. 3rd, 4th, 5th, and 6th days of parasitoid age. The regression analysis showed a significant relationship between daily

parasitism with the age of female parasitoid (Y= -2.3305x+14.896, R2= 97.6%, where Y= daily parasitism rate and x= age of adult parasitoid) (Fig. 3).

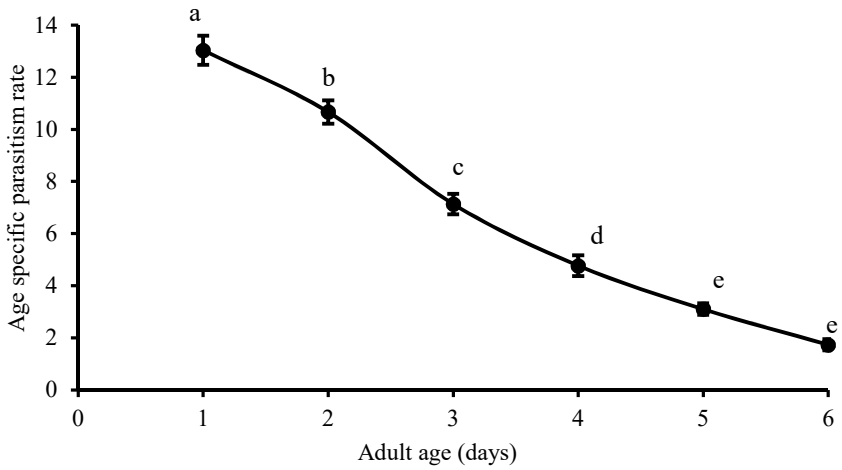

Fig. 3. Age-specific parasitism rate of *Trichogramma brassicae* on *Ephestia kuehniella* eggs

The hatch rate (%) of *T. brassicae* eggs on *E. kuehniella* ranged from 95.07±0.82% (1st day of oviposition period) to 42.78±6.43% (6th day of oviposition period) in different days of adult female age (Fig. 4). Significant differences were observed in this parameter among days (F= 35.53; df= 5, 174; P < 0.001). The hatch rate (%) of *T. brassicae* eggs was 90.66±1.24%, 86.72±1.80%, 77.09±2.78% and 54.00±4.70%, respectively in the 2nd, 3rd, 4th, and 5th days of female age.

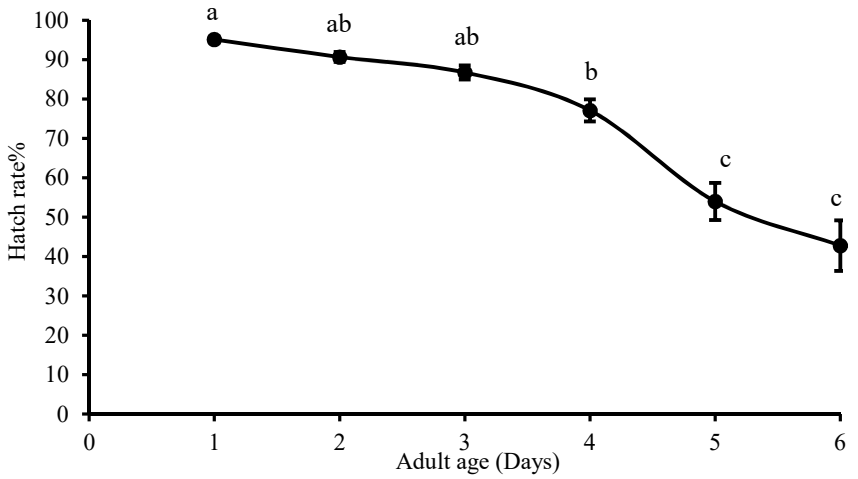

Fig. 4. Age-specific hatch rate of *Trichogramma brassicae* on *Ephestia kuehniella* eggs

Female adult age influenced sex ratio of *T. brassicae* progenies (Fig. 5). The highest values for sex ratio were estimated to be in the first 3 days of adult female life (62.76±0.90%, 58.48±0.59%, and 53.91±1.28%, respectively). The female-biased sex ratio decreased significantly with increasing adult female age ($F= 116.28$; $df= 5, 174$; $P < 0.001$). The sex ratio of *T. brassicae* was 33.17±3.38%, 15.55±3.91% and 1.67±0.99%, respectively in the 4th, 5th, and 6th days of parasitoid age.

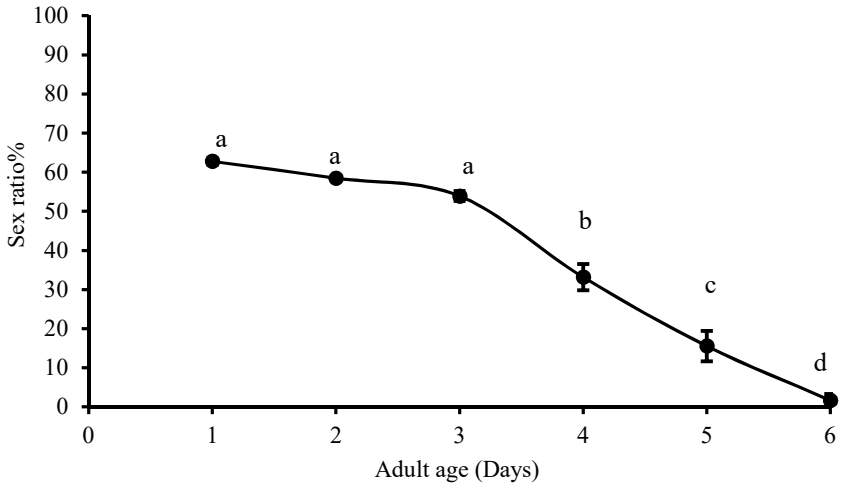

Fig. 5. Age-specific sex ratio of *Trichogramma brassicae* progenies on *Ephestia kuehniella* eggs

Discussion

Information on oviposition patterns of *T. brassicae* on host eggs on plant and especially on cardboard is lacking. At short distance, egg mass odour and visual recognition of egg masses of host play an important role to select them by parasitoid (Pak *et al.*, 1990; Mansfield & Mills, 2002; Babendreier *et al*., 2003; Colazza *et al*., 2010). The results of this study showed that parasitoid movement was not truly random. The younger *T. brassicae* moved throughout the cardboard and often parasitized eggs that are in the central part, but female parasitoid of *T. brassicae* moved straight on cardboard and parasitized closer eggs in the end days of life. It seems that the female parasitoids are highly energetic and efficient during early life only, then energy lossed and efficiency decreased in aged wasps (Nikbeen *et al*., 2014; Tazerouni *et al*., 2016).

In this research, *T. brassicae* daily parasitism, hatch rate, and sex ratio with regard to the age of female parents were determined. Age-specific parasitism rate of *T. brassicae* was higher during the first day of oviposition, and it declined as female age increased. This result is similar to those reported by Steidle *et al*. (2001) for *T. brassicae* and *T. carverae* Oatman et Pinto on *Ephestia cautella* (Walker) and *E. kuehniella* Zeller, by Garcia *et al*. (2001) for *T. cordubesis* Vargas et Cabello on *E. kuehniella* by Perera *et al*. (2015) for *T. bactrae*

Nagaraja on *Cnaphalocrocis medinalis* (Guenée) and by Mawela *et al*. (2021) for *T. lutea* Girault on *Helicovera armigera* (Hubner). Different trends were reported for *T. pretiosum* (Riley) on *Anticarsia gemmatalis* (Hübner). The number of parasitized eggs was unrelated to the age of *T. pretiosum* females (Queiroz *et al*., 2020).

The maximum hatch rate of *T. brassicae* was observed in first to third days of an adult female age and the highest its value (95.07%) was obtained in the first day of parasitoid lifetime. After third days of *T. brassicae* lifetime, the hatch rate value reduced with increasing female age and the lowest hatch rate was 42.78% in the 6th day of female age of *T. brassicae*. Jafari *et al*. (2015) obtained hatch rate of *T. brassicae* as 99.7% on *Sitotroga cerealella* Olivier and the reported value by them was more than obtained value for this parameter in this study. But, the hatching rate of *T. brassicae* on *Pieris rapae* L. was observed 76% (Pourarian *et al*., 2017). The comparisons of these researches show that host egg quality varies in different species and this can be effect on biological characteristics of *T. brassicae*. According to this, younger eggs of *S. cerealella* and *E. kuehniella* can be better host than *P. rapae*.

Parasitoid sex ratio is a key element to do successful biological control programs. Female-biased sex ratio is largely beneficial for biological control programs because adult females are responsible for attacking (include host feeding or oviposition) individual pest insects (Berndt & Wratten, 2005; Chow & Heinz, 2005). In this study the sex ratio of the offspring changed from female-biased during the first three days to male-biased between days 4 and 6. Sex allocation of *T. lutea* depended on maternal age too (Mawela *et al*., 2021). Sex ratio of this parasitoid was female-biased from first to three days, then altered to male-biased from 4 to 8 days and thereafter 100% males from days 9-16 (Mawela *et al*., 2021). Sperm depletion can increase significantly male-biased in aged female parasitoids. In other research, the sex ratio of *T. pretiosum* was similar for 5-day-old females and its value was greater than 90% (Queiroz *et al*., 2020). A sex ratio above 50% is considered adequate in biological programs (van Lenteren & Bueno, 2003).

For efficient mass rearing of *T. brassiace* on *E. kuehniella*, parasitoids should not be kept more than three days in cultures because the progeny becomes male-biased and parasitism rate decreased significantly after 3rd day. Results from this study show good potential of *T. brassicae* and it should be suggested for mass rearing and to be used in augmentative biological control program.

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