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Effect of honeybee products, as food supplements, on the biological activities of three *Trichogramma* species (Hymenoptera: Trichogrammatidae)

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Abstract

Egg parasitoids play a significant role in biological control for lepidopteran insects, where they kill the eggs (the first stage of the developmental cycle). *Trichogramma* species are the very important ones of these egg parasitoids. Under natural conditions, adult *Trichogramma* species are known to feed upon nectar, pollen, and honeydew. Here, the effects of four honey bee products; honey, pollen grains, royal jelly, propolis, their mixtures, and sugar solution, as food supplements, on longevity, fecundity, emergence rate, and sex ratio on different *Trichogramma* species were evaluated. Three species, *Trichogramma evanescens*, *T. bourarachae*, and *T. cacoeciae*, differed in their responses to the tested diets. In general, all diets containing honey improved longevity and fecundity. Honey + royal jelly and honey + propolis were the most diets improved the longevity of *T. evanescens*. Honey + royal jelly + propolis and honey alone resulted in the longest lifetime for *T. bourarachae*. While the best diets that prolonged the longevity of *T. cacoeciae* were honey + royal jelly and honey alone. The highest fecundity was obtained by honey + royal jelly + propolis, honey + royal jelly, honey alone and honey + pollen grains + royal jelly for *T. evanescens*, honey + royal jelly + propolis, honey + pollen grains + propolis, and honey + pollen grains + royal jelly + propolis for *T. bourarachae*, and by honey + royal jelly, and honey alone in case of *T. cacoeciae*. The emergence rate had not affected by most of the treatments for three *Trichogramma* species. Food supplements caused an indirect effect on sex ratio of *T. evanescens* and *T. bourarachae*, whereas long-lived females were male-biased progeny. Except when *T. evanescens* females were fed on honey + royal jelly and *T. bourarachae* on honey + royal jelly, and honey + pollen grains + royal jelly, they lived for a long time but that did not decrease the female progeny percentage. Thus, providing a suitable diet may help to enhance the biological activities for the rearing parasitoids.

Keywords: *Trichogramma* spp., Food supplements, Honeybee products, Biological parameters

Background

The genus *Trichogramma* is one of the very important genera of egg parasitoids broadly used in augmentative release programs against Lepidopterous pests in agricultural and forest. There are more than 200 known species of *Trichogramma* around the world but only 19 species have been mass-reared and released in the fields (Li 1994).

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Trichogramma cacoeciae and *T. bourarachae* were found at the olive groves in Egypt for the first time, where releases have never been applied (Hegazi et al. 2005). In Egypt, successful programs have been carried out for using *T. evanescens* in controlling many lepidopterous pests on different crops, i.e. the lesser sugarcane borer, *Chilo agamemnon* Bles. in sugarcane fields (El-Heneidy et al. 1989), the corn borers (El-Wakeil 1997), the Berry moth, *Lobesia botrana* in grape farms (El-Wakeil et al. 2009), and to control the Olive moth (*Prays oleae*) in olive groves (Agamy 2010).

Longevity, fecundity, efficacy, adult emergence, and sex ratio are important biological parameters in quality control of *Trichogramma* spp. They are influenced by nutrition (Zhang et al. 2004; Wäckers 2005, and Witting-Bissinger et al. 2008).

Adults of *Trichogramma* spp. are known to feed naturally on nectar (sugary plant secretions), pollen (Wellinga and Wysoki 1989), and honeydew (excretions from attended homopterous, i.e. aphid species) (Koptur 1992). In addition, keeping an element of carnivore by engaging in host-feeding (Jervis and Kidd 1986). Sugar-rich food provides for the parasitoid's energetic needs. Insect eggs, on the other hand, are primarily a source of proteins required for physiological processes such as egg maturation (Romeis et al. 2005). Egg parasitoids, which have been considered as pro-ovigenic, are in fact a moderately syn-ovigenic (Boivin 2009). Jervis et al. (2001) reported that pro-ovigeny was rare among parasitoid wasps and 8 species of trichogrammatids are moderately syn-ovigenic, even so, providing food resources to pro-ovigenic species that may lead to indirect improvement of parasitism rates by increasing longevity due to an extension of the amount of time available to find hosts (Thompson 1999).

Many studies were carried out to evaluate the effects of many diets on *Trichogramma* life table parameters and its performance. The presence of honey, for example, as a supplement source of food, was reported to increase the longevity and fecundity of many *Trichogramma* spp. (Malati and Hatami 2010; Tunçbilek et al. 2012; Siam et al. 2014, and Özder and Demirtas 2017). Sex ratio of *T. maxacalii* was influenced by the interaction between populations, availability of food (honey), and length of time in which the parasitoid remained without host eggs after their emergence (Oliveira et al. 2003). In addition to honey, Zhang et al. (2004) found positive effects on *T. brassicae* longevity and fecundity when fed on corn pollen grains.

Honeybee products (honey, pollen grains, royal jelly, propolis) are very rich with many important compounds. Hence, this study aimed to evaluate the effect of feeding on these four products and combinations of them on some of the *Trichogramma* spp. biological parameters.

Materials and methods

The present study was carried out at the Biological Control Laboratory, National Research Centre, Giza, Egypt.

Source of the host

The Angoumois grain moth, *Sitotroga cerealella* (Oliver), eggs were used as a factitious host for evaluating the biological aspects of the tested *Trichogramma* species. Fresh eggs were obtained from the laboratory colony of the Center of Bio-Organic Agriculture services Aswan, Egypt. The eggs were stored at 5 °C for < 7 days.

Source and rearing of the parasitoids

Three *Trichogramma* species, *T. evanescens*, *T. bourarachea* (Arrhenotokous type), and *T. cacoeciae* (Thelytokous type) were used for this study. Tested parasitoid species were obtained from an international company of bio-agriculture, Giza, Egypt, and reared on *S. cerealella* eggs (< 24 h old) glued to a self-adhesive strip (2.5 × 8 cm). The strips carrying *S. cerealella* eggs were exposed to different *Trichogramma* adults in glass jars (1 L) covered with muslin cloth, held in position by a rubber band. Egg sheets were renewed daily. Colonies of parasitoids were reared under the laboratory conditions of temperature (25 ± 1 °C), relative humidity (60 ± 10), and a 14:10 h (L:D) photoperiod.

Honeybee products

Honeybee products (honey, pollen grains, and royal jelly) were harvested from private apiary at Ismailia region, Egypt. Propolis or bee glue was collected from honeybee colonies of a private apiary at Dina farms, Cairo-Alexandria desert road, Egypt. Citrus bee honey freshly collected and unwanted materials such as wax sticks, dead bees, and particles of combs were removed by straining honey through cheesecloth before being used. Palm pollen grains were collected in March (during the flowering season) by a trap of wooden box fixed on the hive entrance throughout the pollen-collecting period (Ghoniemy 1984). Fresh royal jelly had been obtained from queen-less honeybee colony. Three days after removing mother queen, frames with natural queen cells that containing royal jelly were removed from the cell-rearing hive. The ring of wax, at the opening of each cell, was removed to expose the queen larva and royal jelly. Each larva was removed and the royal jelly was extracted by a royal jelly spoon (Wytrychowski et al. 2013). Harvested propolis was poured by alcohol (97%) into a container, then the top sealed and shaken briefly. The shaking repeated once or twice a day, but the mixture was left in a warm dark place for at least 7 days. Propolis was diluted for 1 or 2 weeks. After 2 weeks, the liquid was filtered through a clean and very fine filter paper. Alcohol solvent was evaporated, and propolis extract was refrigerated to less than 4 °C until used (Krell 1996). All honeybee products were freshly collected from honeybee colonies, pure and clean from any chemicals for controlling pests of bee colony.

Experimental procedures

Food supplements preparing

Effect of the four honeybee products: honey, pollen grains, royal jelly, propolis, their mixtures, and sugar solution, as food supplements, on the biological parameters, were conducted in comparison with no food as a control.

1. Honey (H)
2. Pollen grains (Pg)
3. Royal jelly (Rj)
4. Propolis (P)
5. Honey +pollen grains (10:5) g
6. Honey + royal jelly (10:0.1) g
7. Honey + propolis (10:0.1) g
8. Pollen grains + royal jelly (5g:10g)
9. Pollen grains + propolis (5:10) g
10. Royall jelly + propolis (5:5) g
11. Honey + pollen grains + royal jelly (10:5:0.1) g
12. Honey + pollen grains + propolis (10:5:0.1)
13. Honey + royal jelly + propolis (10:0.1:0.1) g
14. Pollen grains + royal jelly + propolis (5:0.1:0.1) g
15. Honey + pollen grains + royal jelly + propolis (10:5:0.1:0.1) g
16. Sugar solution (30%)
17. Without food (control)

Feeding of *Trichogramma* species

Fifteen newly emerged and mated parasitoid females distinguished by their antenna were used for each tested diet and placed individually in rearing glass vials (1.5 × 10 cm). Every glass vial contained a label strip (1 × 7.5 cm), which had a ½ cm disc diameter to glue approximately 120 of *S. cerealella* eggs, on the other side of the strip, a small drop of tested diet by a toothpick was added (sugar solution was added as a small drop on the bottom of the vial). The last 15 vials with adult female parasitoids and the host eggs were left without food supplement as control. The strips, carrying the parasitized eggs, were renewed daily and kept in clean vials. The longevity of each parasitoid female was recorded until death. The number of parasitized eggs (black eggs) were recorded as fecundity. After adult emergence, the percentage of emerged adults was calculated as (No. of black eggs with emergence holes/total no. of black eggs) × 100. The emerged parasitoid adults were sexed and sex ratio was calculated using the formula [No. of females/(No. of females + No. of males)] × 100

All experiments were carried out under the laboratory conditions of temperature (25 ± 1 °C), relative humidity (60 ± 10), and a 14:10 (L:D) hours photoperiod.

Statistical analysis

Data were analyzed, using one-way analysis of variance (ANOVA) and using SPSS computer program. Means were compared using Duncan's multiple range test. The relationship between longevity and fecundity was determined for the three *Trichogramma* species separately by linear regression analysis.

Results and discussion

Longevity of *T. evanescens* differed distinctly among individuals provided with different food supplements (F = 12.08, df = 16,238, Fig. 1a). Only P, Rj, and Pg + P that failed to increase the longevity over the control. The other 14 diets significantly increased longevity, whereas H + Rj and H + P were the best diets that prolonged the longevity to 12.2 ± 1.3 and 9.9 ± 1.2 days than 3.2 ± 0.5 days for control. *T. bourarachae* showed also significant differences in average longevity, when provided with different food supplements (F = 14.8, df = 16,238, Fig. 1b). Nine diets (H + Rj + P, H, H + Pg + P, H + Rj, H + Pg + Rj, H + Pg + Rj + P, H + Pg, H + P, and Rj + P) prolonged significantly the longevity, whereas H + Rj + P and H caused the longest life time (11.6 ± 0.87 and 11.5 ± 0.45 days), while the unfed females lived only for (5.2 ± 0.38 days). Females fed on the remaining eight diets did not show any increase in longevity relative to the unfed control. Longevity of *T. cacoeciae* varied also significantly among females provided with different food supplements (F = 22.6, df = 16,238, Fig. 1b). All eight diets, contained honey and sugar solution improved the female's longevity. The best ones were H + Rj and H that prolonged the longevity to (9.9 ± 0.9 and 9.6 ± 1.1 days) than (1.5 ± 0.13 days) in the control. The other diets did not improve longevity.

Feeding on carbohydrates can enhance adult parasitoid longevity (Gómez et al. (2012). Obtained results showed that honey as a source of carbohydrates had the main effect for improving the longevity of the three *Trichogramma* species, when a very small amount of royal jelly was added to it. In the case of *T. evanescens* and *T. bourarachae*, longevity increased with a factor ranged between (1.8 and 3.08, and 1.8 and 2.2), respectively. *T. cacoeciae* showed the highest improvement, with a factor of (1.9–6.4). These findings are in consistent with other researches related to the other species, i.e., *T. brassicae*, when its longevity increased about 5-folds by feeding on honey (Malati and Hatami 2010). *T. euproctidis* females lived for 10.5 days when fed on honey, while lived 2.6 days in case of feeding on water alone (Tunçbilek et al. 2012). Also, Özder and Demirtas 2017 recorded that *T. brassicae* females which fed on honey, honey + acacia nectar, honey + apple syrup lived significantly longer than those females that fed on other floral nectars and artificial diets.

The fecundity of *T. evanescens* varied significantly among different diet treatments (F = 8.5, df = 16,238, Fig. 2a). All diets improved the fecundity (especially those containing honey), except Rj, Rj + P, and P that were similar or below the control. H + Rj + P (119.3 ± 12.16 egg), H + Rj (118.87 ± 12.86 egg), H (116.87 ± 9.7 egg), and H + Pg + Rj (115.27 ± 15.6 egg) gave the highest numbers of parasitized eggs compared to 57.87 ±

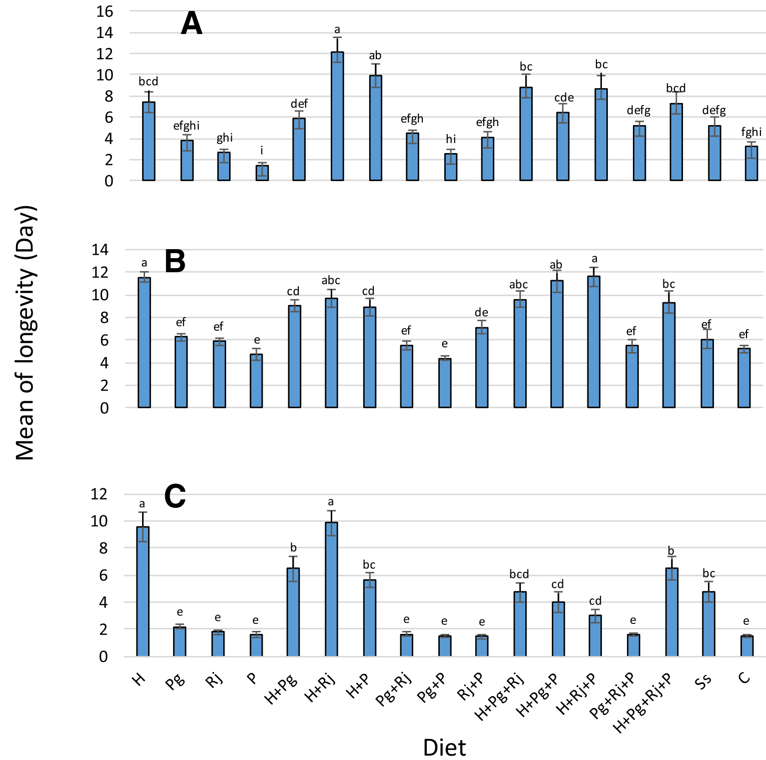

Fig. 1 Mean longevity of **a** *T.evanescens*, **b** *T. bourarachae*, and **c** *T. cacoeciae* supplied with different diets and the control

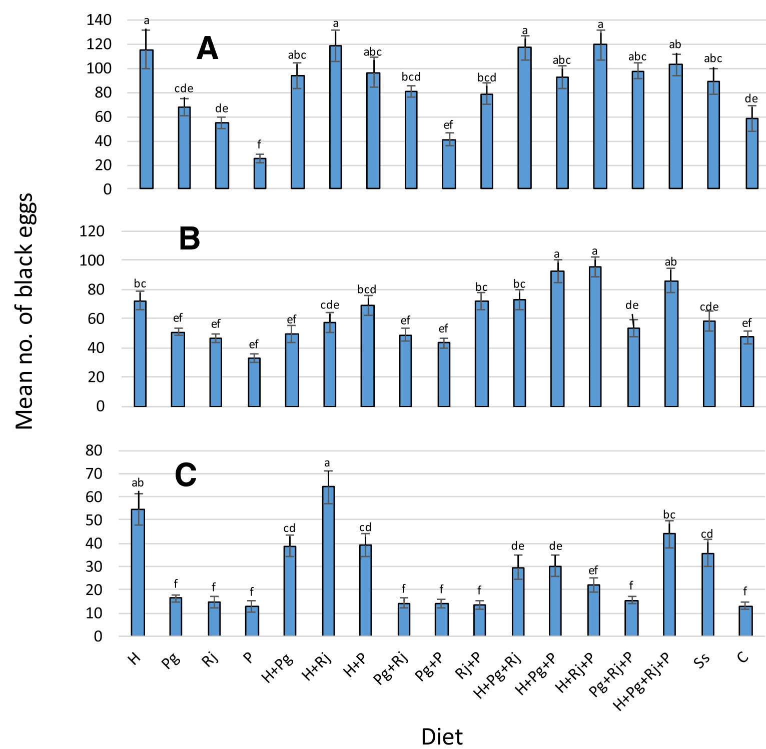

Fig. 2 Mean number of eggs laid by females of **a** *T. evanescens*, **b** *T. bourarachae*, and **c** *T. cacoeciae* supplied with different diets and the control

10.7 egg in the control. The fecundity of *T. bourarachae* also differed significantly among different diet treatments (F = 9.7, df = 16,238, Fig. 2b). The fecundity of individuals fed on Pg, H + Pg, Pg + Rj, Rj, Pg + P, and P was similar or even lower than the control. The remaining 11 diets tested improved the fecundity significantly, the female fed on H + Rj + P, H + Pg + p, and H + Pg + Rj + P laid the highest numbers of eggs (95.5 ± 6.67, 92.4 ± 7.7, and 86.0 ± 8.65) compared with (47.8 ± 4.3) eggs for unfed wasps. The fecundity of *T. cacoeciae* differed distinctly among different diets treatments (F = 15.06, df = 16,238, Fig. 2c). All diets contained honey and sugar solution improved the fecundity, but other diets had insignificant effects. H + Rj and H were the best diets that affected significantly and raised the fecundity to (64.3 ± 7.1 and 54.0 ± 6.5) eggs than (13.1 ± 1.61) eggs in the control.

Adults of different *Trichogramma* spp., like most parasitoid species, are fed naturally upon nectar, pollen as recorded by Wellinga and Wysoki (1989), and honeydew (Koptur 1992). In the laboratory, natural food sources are commonly replaced by honey or sugar. Obtained results showed that honey had the main effect on fecundity of all species, but this efficacy was improved in some cases, when pollen grains were added, propolis, and especially royal jelly (propolis and royal jelly gave a positive effect when added in a small amount to honey, but they had a harmful effect when it was used alone or in a big amount). This improvement appeared significantly in case of *T. cacoeciae* with H + Rj diet, which increased fecundity with a factor of (4.4) more than the control. This may probably due to the special food royal jelly, which responsible of the development of honeybee larvae in to queen bees as stated by Brouwers et al. (1987).

In *T. bourarachae*, fecundity was improved when Pg + Rj + P, Pg + P, and Rj + P were added to honey with a factor ranging (1.8–2.03). Obtained results are consistent with those reported by Malati and Hatami (2010) who stated that fecundity of honey-fed *T. brassicae* adults increased approximately 4-folds than the unfed females. Similar results were recorded for the same *Trichogramma* species by Özder and Demirtas (2017) that the fecundity was significantly greater when the wasps were fed on honey (106.8 ± 30.26 eggs), honey + acacia nectar (105.4 ± 12.26 eggs), and honey + *Paulownia* nectar (103.13 ± 15.34 eggs), than royal jelly + red tulip nectar (3.33 ± 1.34 eggs). In addition, Zhang et al. (2004) recorded that feeding on corn pollen increased the offspring production of *T. brassicae* females than the unfed ones, but it was lower than those fed on honey or pollen grains + honey. Paraiso et al. (2012) recommended feeding *Trichogramma* females on honey in mass-rearing programs to raise a sustainable population of *T. fuentesi* in the laboratory. Obtained results also are in conformity with the work of Siam et al. (2014) who reported the highest fecundity of *T. evanescens* females by feeding on pure sugarcane honey (58.2 ± 2.5 eggs), followed by pure bee honey (52.8 ± 2.1 eggs). Availability of honey as food supplement also increased the parasitization rate of *T. cacoeciae* as recorded by Mansour (2019). For the three *Trichogramma* species, fecundity was positively correlated to longevity, with *T. cacoeciae* benefitting much more from the increase in longevity, followed by *T. evanescens* then *T. bourarachae* (Fig. 3).

There were insignificant differences in emergence rate of *T. evanescens* females, when fed on different diets, despite the differences in averages (F = 0.752, df = 16, 238, Fig. 4a). The emergence rate in *T. bourarachae* differed significantly among the different diets (F = 2.00, df = 16,238, Fig. 4b). Most of the diets did not affect the emergence rate, except Rj + P, Pg + Rj, and H + P, which decreased the emergence rate to (86.87 ± 1.58, 89.26 ± 2.58, and 89.16 ± 1.6%) than (91.90 ± 2.07%) in the control. In the case of *T. cacoeciae*, there were significant differences in emergence rates too (F = 2.37, df = 16, 238, Fig. 4c). The greatest rate was recorded at Pg + P and Rj + P (99.2 ± 0.2%). The lowest rate was obtained by the sugar solution, which was 94.2 ± 1.5 %.

Obtained results showed that there were insignificant differences in the emergence rate of *T. evanescens* at different treatments. This result was similar to the findings of Zhang et al. (2004) and Malati and Hatami (2010) on *T. brassicae* and Fuchsberg et al. (2007) on *T. ostriniae*, who reported that no effect of feeding treatments on progeny emergence rate. In the case of *T. bourarachae* and *T. cacoeciae*, significant effects on emergence rate were found. This result agrees with that of Tunçbilek et al. (2012), who reported a significant influence of diets on *T. euproctidis* adult emergence.

The sex ratio of *T. evanescens* varied significantly among different diet treatments (F = 6.6, df = 16,238, Fig. 5a). The highest percentage of females emergence obtained by feeding on Pg (76.48 ± 3), Pg + P (74.53 ± 3.6), control (74.5 ± 3.5), and H + Rj (68.79 ± 2.4). The remaining diets decreased the percentage of females, especially H + P, which decreased the percentage to (45.5 ± 4.09). The sex ratio of *T. bourarachae* also differed significantly among different diet treatments (F = 2.5, df = 16,238, Fig. 5b). H + Pg + P, H + Rj + P, and H decreased the female percentage to (67.18 ± 3.9, 68.03 ± 2.97, and 68.17 ± 3.22) than the control (80.19 ± 2.26). *T. cacoeciae* is a thelytokus type, all progeny are females, therefore, it was not affected by different diets (Fig. 5c).

Progeny of long-lived females of *Trichogramma* species are male biased as confirmed by Malati and Hatami

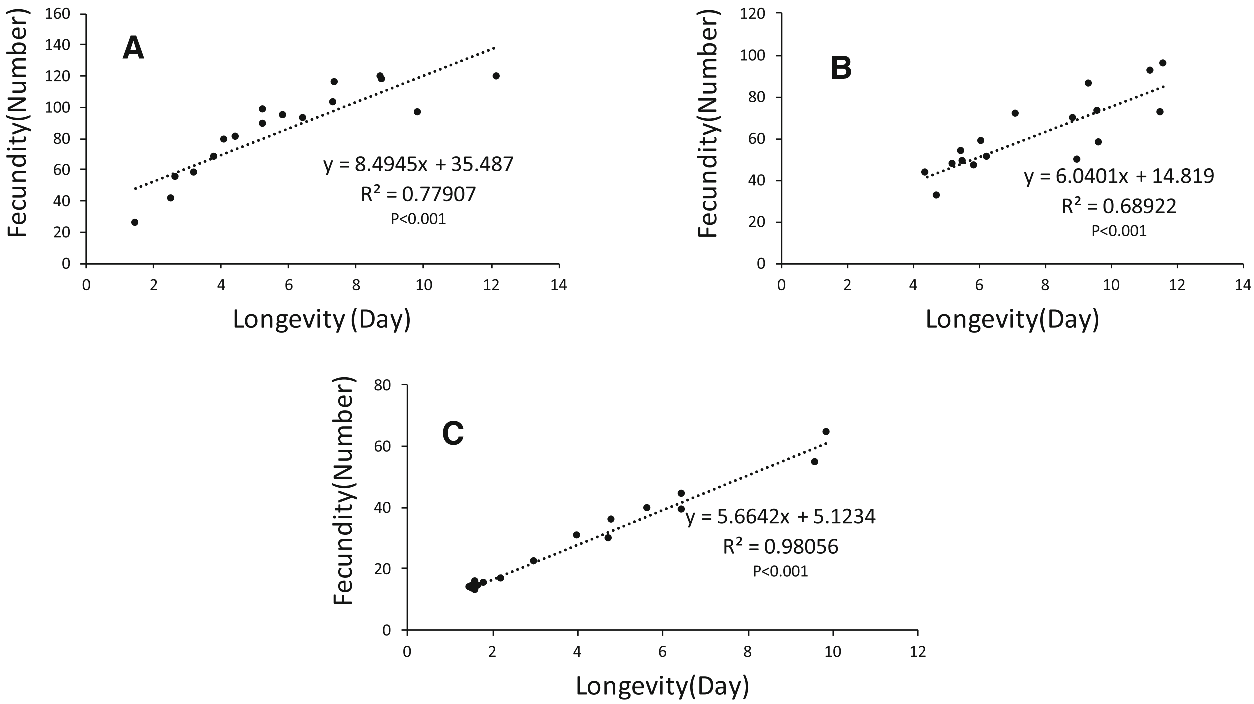

Fig. 3 Relationship between longevity and fecundity of **a** *T. evanescens*, **b** *T. bourarachae*, and **c** *T. cacoeciae*

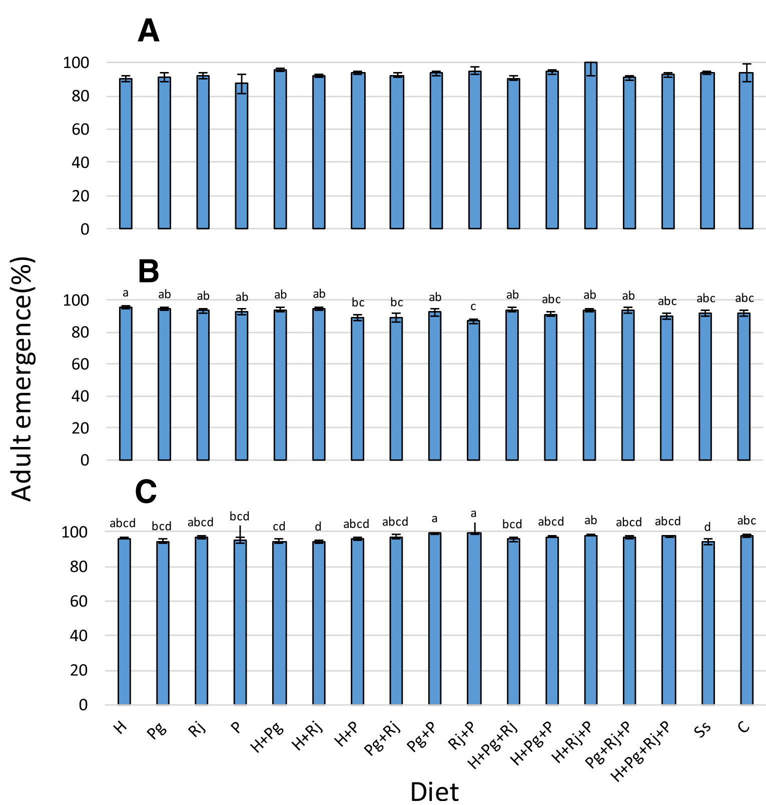

Fig. 4 Emergence rate of **a** *T. evanescens*, **b** *T. bourarachae*, and **c** *T. cacoeciae* supplied with different diets and the control

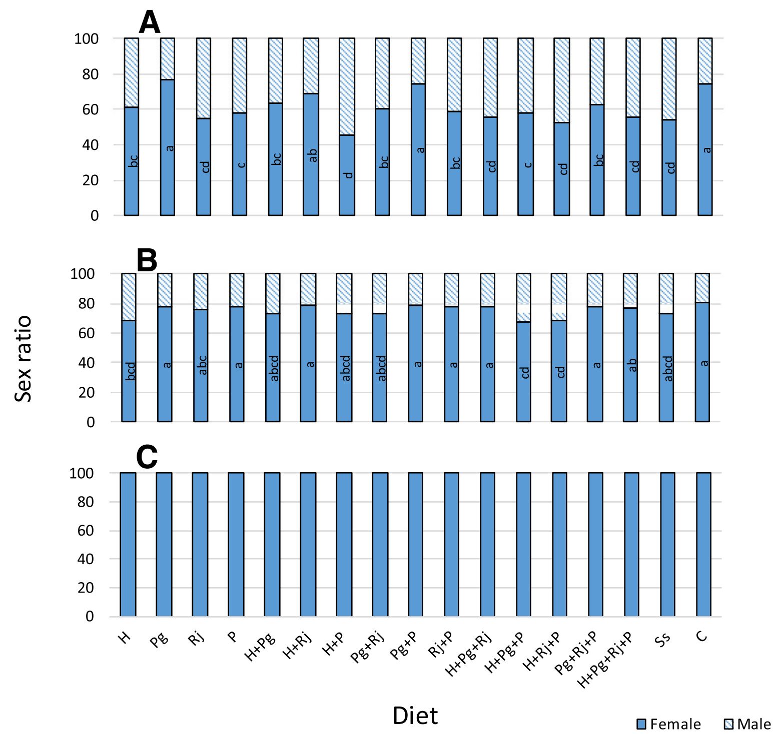

Fig. 5 Sex ratio of **a** *T. evanescens*, **b** *T. bourarachae*, **c** *T. cacoeciae* supplied with different diets and the control

(2010). In these results, long-lived females of *T. evanescens* and *T. bourarachae* had lower female offspring percentages, except when *T. evanescens* was fed on H + Rj, which caused the longest lifetime, higher fecundity and did not decrease female percentage. When *T. bourarachae* was fed on H + Rj and H + Pg + Rj, it lived a longer time, but did not decrease the female percentage. This was probably due to royal jelly. Lower proportion of female progeny in case of some of the supplemental diets, which caused a long living, does not mean that adult nutrition decreases parasitoid potential. In fact, the total number of female progeny per fed female was significantly more than the unfed ones. Therefore, it should be considered that adult nutrition is not omitted because of lower proportion of female progeny. Higher fecundity in fed parasitoid can compensate for this limitation.

Conclusion

Nutrition is the main factor of life of any organism. Obtained results indicated that wasp nutrition type could improve the parasitoid's quality parameters. This improvement varies in different species depending upon their responses to the tested diets. Therefore, providing the right diet from honey, pollen grains, royal jelly, and propolis, at suitable concentrations, for *Trichogramma* spp. will enhance their biological activities during mass rearing and their efficacy throughout field application.

Abbreviations

C: Control; H: Honey; P: Propolis; Pg: Pollen grains; Rj: Royall jelly; Ss: Sugar solution

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Authors' contributions

All authors designed the experiments. EA, HA-b, TE, and HE supervised and coordinated the laboratory work, results analysis, and manuscript drafting. ShM performed the experiments and wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

The data and material used during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Ethical approval and consent to participate are not required for this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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