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The effect of royal jelly on some biological and morphological characteristics of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae)

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Abstract

The feeding of parasitoid wasps is a forcible factor in ensuring the efficiency of biological control agents. The quality of natural resources can affect different aspects of morphology, biology and physiology of parasitoids which are linked to their fitness and fecundity. The logic behind the experiment was to study the effect of different concentrations of royal jelly (RJ) solution, on the biology of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) with focus on rate of parasitism and morphology traits (wing size and shape variation) under laboratory conditions. Results showed that feeding with 0.5% gml^{-1} royal jelly/honey water solution have increased rate of parasitism (mean= 75.06%± SE 6.03, n=15). The lowest rate of parasitism occurred in females fed without RJ (mean= 46.26%± SE 8.03, n=15). There were significant differences in weight matrices as shape variables, but according to independent sample t-test, we did not find any significant difference in centroid size (as size variable). Enhancing nutritional supplements has been able to influence some biological characteristics of *T. brassicae*. We argue that there was beneficial effect in royal jelly on morphological and biological traits in parasitoid wasp.

Key words: *T. brassicae*, royal jelly, parasitism rate, geometric morphometric, biological control

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Trichogramma brassicae'nin (Hymenoptera: Trichogrammatidae) bazı biyolojik ve morfolojik özellikleri üzerine arı sütünün etkisi

Özet

Parazitoid arıların beslenmesi biyolojik kontrol ajanlarının etkinliğini sağlamada zorunlu bir faktördür. Doğal kaynakların kalitesi, zindelik ve doğurganlıklarına bağlı olarak parazitoitlerin morfolojilerini, biyolojilerini ve fizyolojilerini farklı yönlerden etkileyebilir. Deneyin arkasındaki mantık, laboratuvar şartlarında farklı konsantrasyonlarda arı sütü (RJ) çözeltisinin parazitizm oranı ve morfolojik özelliklerine (kanat büyüklüğü ve şekil varyasyonu) odaklı olarak Trichogramma brassicae Bezdenko (Hym .: Trichogrammatidae) biyolojisi üzerindeki etkisini araştırmaktır. Sonuçlar, % 0.5 gml-1 arı sütü / bal suyu çözeltisi ile beslenmenin parazitizm oranını arttırdığını göstermiştir (ortalama = % 75.06 ± SE 6.03, n = 15). En düşük parazitizm oranı, RJ'siz beslenen dişilerde görülmüştür (ortalama = % 46.26 ± SE 8.03, n = 15). Ağırlık matrislerinde şekil değişkenleri olarak önemli farklılıklar vardı, ancak bağımsız örneklem t-testine göre, ağırlık merkezi boyutunda (boyut değişkeni olarak) anlamlı bir fark bulamadık. Besin takviyelerinin arttırılması, T. brassicae'nin bazı biyolojik özelliklerini etkileyebilmiştir. Arı sütünün parazitoid arının morfolojik ve biyolojik özelliklerine faydalı bir etkisi olduğunu savunuyoruz.

Anahtar kelimeler: *T. brassicae*, arı sütü, parazitizm oranı, geometrik morfometrik, biyolojik kontrol

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1. Introduction

Egg parasitoids, *Trichogramma* Westwood, are efficient biological control agents and widely used in inundative releases against a number of lepidopterous pests in many crops [1]. More than 150 different species of *Trichogramma* are known as various biotopes [2]. Species of *Trichogramma* are distributed around the world as biological agents because they are highly specialized and beneficial [3]. The commercial use of several species of egg parasitoids *Trichogramma* is carried out in over 30 countries to control key pests of 34 crops through inundative releases over 32 million hectares [4].

Various factors can impact the stage of development of *Trichogramma* [5], but some of them directly affect the process of mass rearing of *Trichogramma* species. The power of fertility, the rate of parasitism, the ability to find host patches in fields and storage conditions are the desirable features for biological control programmes [1]. Among these factors, the power of fecundity is an important characteristic that reduces the number of releases. Not only does it save time, but also reduces production costs per generation. Some materials can be used to enhance the reproductive quality and quantity of *Trichogramma* wasps in fields. Honeydew (as well as nectar) becomes viscous and finally solidifies as its water evaporates when exposed to the atmosphere, which might pose a problem for parasitoid uptake. For example, *Trichogramma platneri* Nagarkatti was found to live longer (10–13 fold) when provided daily with 43% fructose and glucose solutions compared to those receiving the same food sources only on the 1st day of the trial [6].

Royal jelly (RJ) is a creamy product secreted by the hypopharingeal and mandibular glands of worker bees, and is primarily used for developing and maintaining the queen bee although young larveas fed on RJ in the first days of life [7, 8, 9, 10, 11]. The longer life of the queen bee over other bees is linked to RJ [9]. The Royal Jelly is a yellowish-white, acidic secretion, with a slightly sharp odour and taste, which has been used centuries for extraordinary traits and its effects on health [7, 9]. Honey has been found to be a beneficial food source for many organism [12] such as insects, especially parasitoids. For example, royalactin, which is the protein in royal jelly produced by honeybees, was shown to increase body size, ovary development, and shortened developmental time in both honeybee, *Apis mellifera* L. (Hymenoptera: Apidae), and common fruit fly, *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) [13]. Royal jelly has lots of beneficial effects but was not studied as well as other hive products like honey, propolis and bee pollen.

The object of this study was to appraise the effects of Royal Jelly on biological and morphological traits of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) because not only is RJ important for its remarkable nutritional properties, but also for its functional and biological features [9, 11, 14].

For the study of biological trait of the parasitoid, the rate of parasitism was selected. Previous studies stated that RJ has complex of compositions such as water, carbohydrates, proteins, lipids, amino acids, vitamins, enzymes and natural antibiotics, so has been efficient for developing the larvae stages in blood cells and maintaining its ovulatory properties over whole lifespan [15, 11]. This feature is related to fertility and so RJ has always been used as a stimulator of fertility [16, 17]. In humans, RJ can improve hormonal equilibrium and fertility by increasing ovules and sperm quality [18]. Also, RJ has several fortifying and tonic effects on health. Owing to its perfect composition, it is a complex nutritional food source that stimulates growth [9, 16].

In order to study effect of RJ on morphological characteristic, size and shape variation were considered. Geometric morphometric (G.M.) analysis is one of the robust morphological methods, which helps to distinguish insect populations based on anatomical landmarks. Detecting accurate shape variations at species level makes it a powerful tool [19, 20, 21]. This method is defined according to anatomical Landmarks with Cartesian coordinates (x and y). The coordinates will be compared after removing the effects of orientation, size and position [22]. In different literature, wing morphometric analysis has been used to clarify very close taxa relations [23, 24].

Therefore, the evaluation of effect of RJ on the biological and morphological characteristics of *T. brassicae* is considered as the logic of all experiments.

2. Materials and methods

2.1. Parasitoid

The present work was conducted in the Biosystematic Laboratory at The University of Tehran, Karaj, Iran. The *Trichogramma brassicae* used in this experiment was originated from the insectarium of the Agricultural Jihad Organization of Golestan province, Iran. It had been reared from the eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). Experimental conditions were in an incubator at 25±1ºC, 60±10% RH and 16:8 L: D photoperiod.

2.2. Rate of parasitism

To determine the effect of different concentrations of royal jelly/honey water (RJ/H) solution on the rate of parasitism (%), a mated naive (no previous oviposition experience) one day old female was used. This was done by feeding of the wasps by four diets of the solution, including 0.5%, 1%, 1.5% gml^{-1} royal jelly/ honey water solution and the other one without any concentration of jelly as control treatment for one day. Fifty new eggs of *E. kuehniella* were supplied and treatments (different concentrations of royal jelly/honey solution) were rubbed with one white card (5×1 cm) by a soft painting brush placed in a plastic cylinder (18×8 cm). The female was released in it. All the containers prepared (four treatment×15 female individuals = 60) were kept in the growth chamber at 25±1ºC, 60±10% Rh and 16:8 L: D. After 24 h, the female parasitoid wasps were collected from the container and the eggs were left at the growth chamber until they turned black.

2.3. Preparation of slides

To avoid any problem associated with asymmetry [25], only the right forewing of adult females *T. brassicae* were selected in all specimens. Samples included two populations: 1) population fed with 0.5% gml^{-1} royal jelly/ honey water solution during five generation and 2) none fed with royal jelly/ honey water solution. Wings were mounted in Hoyer's medium on clean glass microscope slides and were checked under 100 magnification on ZEISS light microscope. In order to protecting toward moisture intrusion and crystallization, coverslips were installed on all slides. Afterwards all of them were placed in oven (45°C) for 4 to 5 days to be completely dried. Fifteen replicates were considered for two population. Digital images of the right forewings were captured using CCD video camera (Sony, Dinolite 2).

2.4. Geometric morphometric: data acquisition

We defined five landmarks in types I and II, according to Bookstein (1990). Figure 1 shows distribution of all landmarks in right forewing of female *T. brassicae*. Landmarks concluded using software tpsDig.2 v.2.16 [26] and its resulted coordinates were sorted by software tpsuUtil v 1.52. Partial warps were emanated from these coordinates in tpsRelw v1.49 [27]. Relative warp analysis (RW) was performed based on partial warps in order to assessing of individuals distribution on RW axis. Wing relative variations in negative and positive of RW1 were procured. Weight matrices of partial warps and Centroid size (CS), the sum of squared distance of landmarks, were obtained as shape and size variables, respectively.

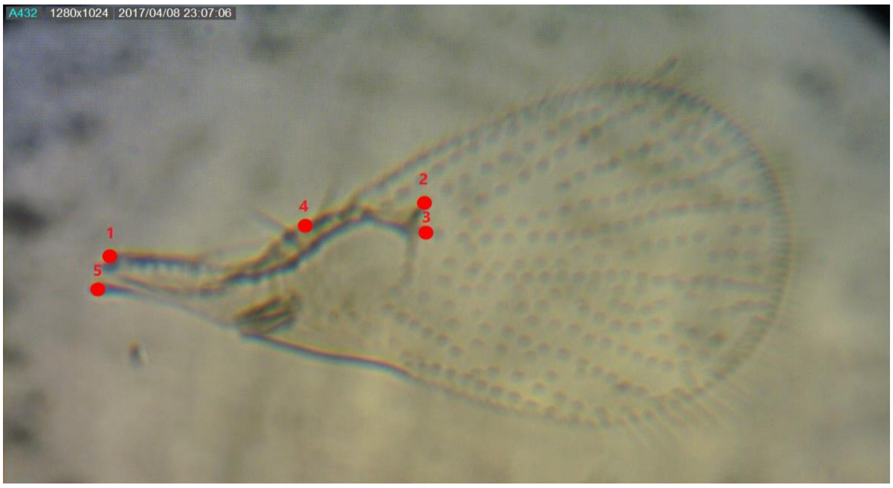

Figure 1. Location of five landmarks on right female forewing of *T. Brassicae*

2.5. Statistical analysis

Data of experiment of rate of parasitism was submitted to ANOVA and group differences were found by using Tukey Data of experiment of rate of parasitism was submitted to ANOVA and group differences were found by using Tukey post hoc comparisons.

Multivariate analysis of variance (MANOVA) based on shape variables, weight matrices, was carried out to proving significance.

Independent sample T test was performed to size compression of the populations.

All statistical analysis were took place by R 3.3.1 Statistics software.

3. Results

There were significant differences in the rate of parasitism *T. brassicae* ($F=5.739$; $df= 4, 55$; $P<0.001$). The highest rate of parasitism was for 0.5% gml^{-1} royal jelly/honey solution (75.06%) and the lowest was when the wasps were fed without any concentration of royal jelly (46.26%) (Figure 2).

Figure 2. The effect of the present different concentrations of royal jelly/honey solution on the rate of parasitism *T. brassicae*. The bars in each group with the same letters are not significantly different (P>0.05). Diets 1, 2, 3 show 0.5, 1, 1.5% gm^{-1} jelly/honey solution and control as diet without any concentration of jelly.

Principal Component Analysis (PCA) based on informations from the shape of right forewings of *T. brassicae* recorded on Table 1. Two first PCA axis were obtained from the covariance matrix, showed 62.31% of the shape variance (Figure 3).

Table 1.Principal Component Analysis (PCA) of wing shape in two population of *T. brassicae* included fed with 0.5% gml^{-1} jelly/ honey solution and another one without any concentration.SOV: Source of Variation, Cum: Cumulative Variance.

Components	SOV	Variance (%)	Cum (%)
1	1.44	34.63	34.63
2	1.28	27.68	62.31
3	1.08	19.65	81.96
4	0.77	9.99	91.95
5	0.55	5.16	97.11
6	0.41	2.88	100

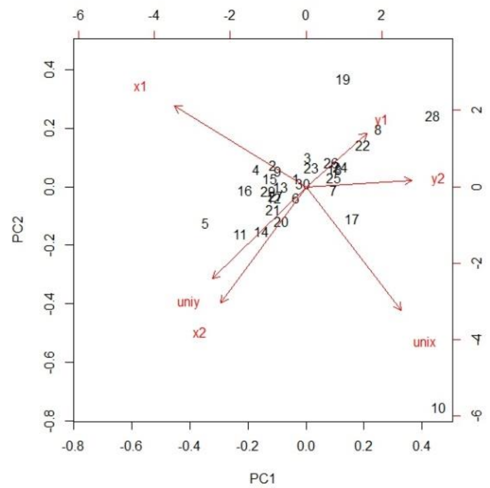

Figure 3. Diagram of Principal Component Analysis of the covariance matrix.

Weight matrices on test populations showed significant differences based on multivariate analysis of variance (MANOVA) (Table 2).

Table 2. MANOVA on weight matric of right forewing of *T. brassicae*

entries	Pillai	Df	F	probability

Unix	0.66704	1	11.5196	<0.001
Uniy	0.30440	1	2.5162	<0.05
Unix.uniy	0.10021	1	0.6404	ns

Analysis of variance of centroid size manifested no significant differences between two populations of *T. brassicae*. Distribution of all individuals including parasitoid fed 0.5% gml^{-1} jelly/ honey solution and another one without any concentration (30 individuals) based on partial warps showed on figure 4.

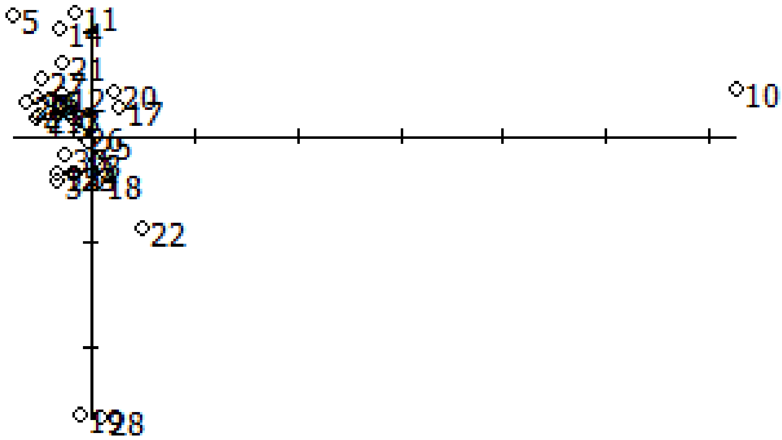

Figure 4. Distribution of individuals based on partial warps in Rw1 and Rw2. (1-15: fed with 0.5% gml^{-1} jelly/ honey solution and 16-30: none fed with jelly/ honey solution).

Figure 5 demonstrated the relative variations of right forewing in two population of *T. brassicae* that were detected by using geometry coordinate of all landmarks. These results showed positive and negative extreme of right forewing female *T. brassicae* so that in negative extreme of forewing shape variation along the RW axis was more than an the other.

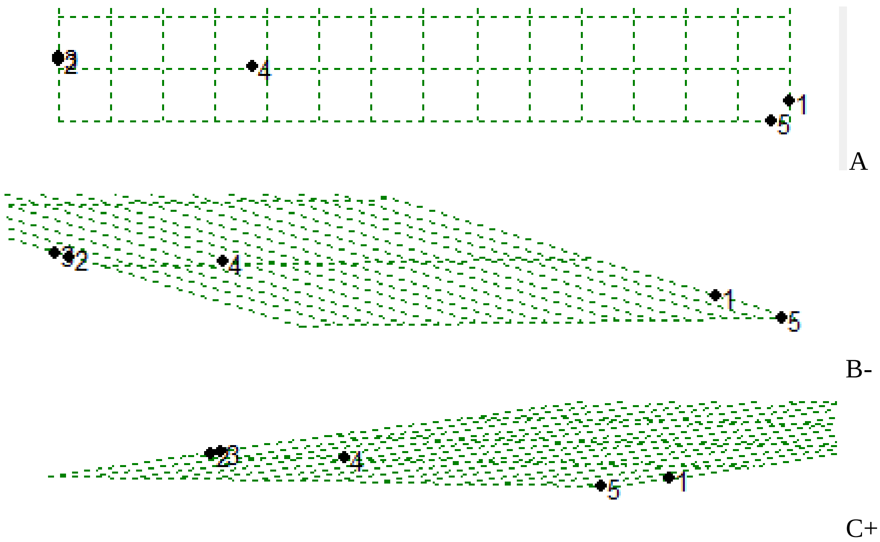

Figure 5. Reference shape of front wing (A), relative variation in positive and negative direct, respectively (B, C).

4. Conclusions and discussion

The current results are in concordance with our hypothesis, as RJ may affect morphological and biological traits of *T. braasicae*. Based on the results two viewpoints can be discussed. First, the results showed the rate of parasitism *T. brassicae* was increased with 0.5% gml^{-1} jelly/honey water solution. Second, our results about effect of RJ on shape variation showed significant differences.

In several studies, it has been noted that in the field, parasitoids should be more efficient and have longer lifespans with floral resources than other variables agreed with our profits [28]. Undoubtedly, there is evidence that floral resources can increase the rate of parasitism in agricultural systems [28]. Many of the parasitoids require to have carbohydrate-rich food in order to maximize their longevity and potential reproductive ability [29]. Other studies stated that nutrition with nectar and pollen can increase the lifetime and fecundity in parasitoids and predators [30]. The role of feeding in increasing the lifetime in different species of *Trichogramma* has been studied by Hohmann et al. (1988) [31].

The effect of nutrition on fecundity and longevity of *T.minutum* Riley have been evaluated that it was greater with honey than fructose and sucrose [32]. Olson and Andow (1998) [33] stated that *T. nubilale* Ertle & Davis, fed with honey, had increased fecundity and longevity. Regardless of the presence or absence of host eggs, females of *T. pretiosum* Riley fed with honey/nectar had more longevity than those were not fed with this mixture [34]. In the present study, the effect of royal jelly was tested for the first time as a multiplicative factor on the parasitism rate of *T. brassicae*. El-Wahab et al. (2016) [35] showed the results of feeding royal jelly + bee honey diets were the highest number of eggs/females in the ectoparasitoid *Bracon hebetor* (Say.) (Hymenoptera: Braconidae). Adding a certain concentration of jelly (0.5% gml^{-1}) to honey solution caused a major change in the parasitism rate. Considering the fact that previous studies evaluated the variability of the parasitism rate of *Trichogramma* species, as related to the presence or absence of the host [36], the current study has observed an increase in the parasitism rate related to the 0.5% mgl^{-1} concentration of RJ. The existence of some chemical stimuli such as 10-hydroxyl-2-decenoic acid, antibacterial protein and peptids [11] and the presence of complex nutrients in the royal jelly may play a role in increasing the parasitism rate of *T. brassicae*. In men and women, RJ can affect fertility by raising ovules and sperm quality [18] and because it is a rich source of para-aminobenzoic acid, in women who consume this product regularly at least for six months, RJ increases fertility [9]. Therefore, with the approval of the few studies that have been conducted on some of the organisms as well as human and mice fertility, this study suggested that the consumption of RJ as a dietary supplement in the diet of parasitoids because of its rich composition can be effective on biological and morphological traits.

In parasitoids, there is a positive relationship between size and female reproductive success [37]. Several studies have mentioned the effects of nutrition on the pest morphological variations. Nutrition plays an important role in living organism's differentiation and morphological variation and shape plasticity in result of food sources would be predictable. The quality of food sources for *T. brassicae* has been proved to increase biological parameters such as longevity and fecundity. Although, the Wing loading was affected by host quality [38]. Some of the morphological features acquired by feeding, can influence other biological characteristics of *Trichogramma* species. The length and width of the ovipositor in *Trichogramma* spp. has a decisive role in the selection of the host [39].

In four species of *Diplodus*: Sparidae, with switching of feeding habits, the shape will be differentiated. In fact, there are positive correlation between shape variation and efficiency of food resources in these taxa [40]. The influence of host species on genitalia capsule of some *Trichogramma* species is well studied by Querino et al. (2002) [41]. The bigger genitalia is belonged to *Trichogramma* populations feed on egg hosts with larger size. These differences are illustrated by Relative Warp Analysis in geometric morphometric method.

It is notable that nutrition in addition to the morphological parameters, can affect fertility and reproductive futures. The result of this study underline the impact of nutrition quality on the morphological and biological opportunities of *T. brassicae*.

Obviously the positive results obtained, can be used as an advantageous issues in the mass rearing of this polyphagous egg parasitoid. Changes in morphological futures will affect their fitness. This phenomenon will help insects to benefit in different environments.

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