



Parasitism Efficiency of *Trichogramma evanescens* Westwood and *Trichogramma pintoi* Voegelé on *Ephestia kuehniella* Zeller Eggs Reared on Different Larval Diets^A

Ali Kürşat ŞAHİN^{1*}, Ali ÖZPINAR², Besime GÜR³, Asude TÜRKOĞLU KEPEZ⁴

Abstract: This study aimed to evaluate the effects of incorporating fish meal and corn meal at varying proportions into the diet of the host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) on the parasitism efficiency of *Trichogramma evanescens* Westwood and *T. pintoi* Voegelé (Hymenoptera: Trichogrammatidae). Eggs from *E. kuehniella* females, reared for two generations on five experimental larval diets and a standard control diet (50% wheat flour + 50% wheat bran), were parasitized by both parasitoid species. The number of parasitized eggs and adult emergence rates were quantified. Statistical analysis revealed that host larval diets significantly influenced parasitism efficiency and that adult emergence rates differed between species. The highest number of parasitized eggs was recorded in Diet 4 for *T. evanescens* (53.3 eggs) and in Diet 2 for *T. pintoi* (50.75 eggs). Adult emergence rates peaked at 94.37% for *T. evanescens* and 85.56% for *T. pintoi*. Eggs from *E. kuehniella* reared on fish meal-containing diets exhibited parasitism rates comparable to those from the control diet. Fish meal did not impair parasitism efficiency, indicating its suitability as a component in host larval diets.

Keywords: *Ephestia kuehniella*, *Trichogramma evanescens*, *Trichogramma pintoi*, Larval diets.

^A Research and Publication Ethics were followed in this study. This study did not require an ethical committee permit. Authors have contributed equally and there isn't any conflict of interest between authors.

* **Sorumlu yazar/Corresponding Author:** ¹Ali Kürşat ŞAHİN, Çanakkale Onsekiz Mart Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Çanakkale, Türkiye, aksahin@comu.edu.tr, [OrcID 0000-0002-2721-8822](https://orcid.org/0000-0002-2721-8822)

² Ali ÖZPINAR, Çanakkale Onsekiz Mart Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Çanakkale, Türkiye, aozpinar@comu.edu.tr, [OrcID 0000-0003-4512-8027](https://orcid.org/0000-0003-4512-8027)

³ Besime GÜR, Ardahan Ziraat Karantina Müdürlüğü, Ardahan, Türkiye, besime.gur@tarimorman.gov.tr [OrcID 0009-0007-7033-7492](https://orcid.org/0009-0007-7033-7492)

⁴ Asude TÜRKOĞLU, Gökçebey İlçe Tarım ve Orman Müdürlüğü, Zonguldak, Türkiye, turkogluasude@gmail.com [OrcID 0000-0001-6587-4778](https://orcid.org/0000-0001-6587-4778)

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***Trichogramma evanescens* Westwood ve *Trichogramma pintoi* Voegelé'nin Farklı Larva Diyetlerinde Gelişen *Ephestia kuehniella* Zeller Yumurtalarını Parazitlenme Etkinliği**

Öz: Çalışmada farklı oranlarda konukçu *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae)'nin besinine eklenen balık unu ve mısır ununun, konukçunun yumurtalarını parazitleyen *Trichogramma evanescens* Westwood ve *T. pintoi* Voegelé, (Hymenoptera: Trichogrammatidae)'nin parazitlenme etkinliği üzerindeki etkilerinin belirlenmesi amaçlanmıştır. Bu amaçla standart (%50 buğday unu +%50 buğday kepeği) besin kontrol olacak şekilde 5 farklı larva diyeti üzerinde 2 generasyon üretilen *E. kuehniella* dişilerinden elde edilen yumurtalar parazitoit türleri tarafından parazitlenmiş ve parazitlenen yumurta sayıları ile ergin çıkış oranları belirlenmiştir. Sonuçlar ile yapılan analiz sonucunda farklı konukçu larva besinlerinin parazitoitlerin parazitlenme etkinliği üzerinde istatistiksel olarak anlamlı bir etkisi olduğu ve parazitli yumurtalardan ergin parazitoit çıkış oranları arasında türler arasındaki farklılık olduğu görülmüştür. En yüksek sayıda yumurta *T. evanescens*'de 53,3 ile 4 nolu diyet, *T. pintoi*'de ise 50,75 ile 2 nolu diyetle belirlenmiştir. En yüksek ergin çıkış oranı *T. evanescens*'de %94,37, *T. pintoi*'de ise %85,56 olarak tespit edilmiştir. Balık unu içeren larva diyetlerinden elde edilen *E. kuehniella* yumurtaları kontrol besininden elde edilen yumurtalarla benzer oranlarda parazitlenmiştir. Balık unu parazitlenme etkinliği üzerinde bir dezavantaj yaratmamış ve larva diyetlerinde kullanılabileceği görülmüştür.

Anahtar Kelimeler: *Ephestia kuehniella*, *Trichogramma evanescens*, *Trichogramma pintoi*, Larva diyetleri.

Introduction

For over a century, egg parasitoids belonging to the genus *Trichogramma* have been employed as biological control agents through mass rearing and release strategies targeting lepidopteran pests (Wang et al., 2014). These parasitoids have been utilized to manage at least 28 pest species across approximately 32 million hectares of agricultural and forested land (Wu et al., 2015; Taghikhani et al., 2019; Tayat and Özder, 2023). In Türkiye, 8 *Trichogramma* species, including *Trichogramma evanescens* Westwood (Hymenoptera; Trichogrammatidae) and *T. pintoi* Voegelé, were identified on different cultivated plants and host pests (Öztemiz et al., 2013). *T. pintoi* was first identified on *Helicoverpa armigera* eggs on tomato plants in Aegean region (Koçlu and Karsavuran, 1998). The more commonly encountered species, *Trichogramma evanescens*, has been reported parasitizing the eggs of various lepidopteran pests, including *Ostrinia nubilalis* Hübner (Özpinar et al., 1996; Öztemiz et al., 2013).

Host selection for the mass production of *Trichogramma* species is influenced by several factors, including the characteristics of the host egg, suitability for large-scale rearing, storage capabilities, and the potential to yield a high number of parasitoids. From 200 *Trichogramma* species only 19 species are being mass produced on a host in different countries (Davies et al., 2011). *Ephestia kuehniella* Zeller (Lepidoptera; Pyralidae) is a pest of

grains and stored grain products and it is preferred in mass production of *Trichogramma* species as host due to its easy and cheap production on wheat flour and other similar food products (Locatelli et al., 2008; Kara and Özder, 2017; Moghaddasi et al., 2019; Özder and Tayat, 2019; Pehlivan, 2021; Ghaemmaghami et al., 2022; Özpınar et al., 2023).

The effectiveness of a biological control agent depends on the strategic planning of host production using an appropriate and cost-effective larval diet. Optimum protein content is important for optimum insect development and reproduction. Thus, herbivorous insects prefer plants with high nitrogen content as their protein source (Firidin et al., 2013). General predators like coccinellids fulfil their protein requirements from a wide selection of preys (Yalçın and Ülgentürk, 2023). In addition to proteins insects also need fats for optimal reproduction and development (Candy and Kilby, 1975). Insects can synthesize fats from stored carbohydrates and proteins, besides receiving them from their food (Werren, 1987). According to Ayvaz and Karabörklü (2008), *E. kuehniella* larval diet with low amounts of protein and carbohydrate causes decreased egg quantity in adulthood.

The protein and fat content of insect rearing diets play a critical role in reproductive quality. To maintain continuous mass production while ensuring high reproductive capacity and minimizing rearing costs, various larval diets have been evaluated by researchers (Tarlack et al., 2015; Moghaddasi et al., 2019; Pehlivan, 2021). Among these, fishmeal has been identified as a suitable dietary component that fulfills these criteria, despite its relatively higher cost (Özek, 2016). A portion of the waste generated by fish processing factories can be repurposed into various products. Although fish meal is a commonly utilized product, in this study, it was selected as a protein source to represent fish waste broadly. Future studies could explore the potential use of various fish by-products, particularly those typically unsuitable for conventional applications, as components in host diets.

The aim of this study was to determine the parasitism efficiency of *Trichogramma evanescens* and *T. pintoi* on *E. kuehniella* eggs reared on diets with fishmeal made of fish waste from a fish processing factory (Dardanel Ton) and cornflour made from unusable corn of a seed growing company.

Materials and Methods

Biological Materials

Ephesia kuehniella was reared in an insect rearing chamber under controlled conditions of 24 ± 1 °C, 50–60% relative humidity, and a photoperiod of 18:6 (L:D), using a 1:1 mixture of wheat flour and wheat bran as the larval diet. The eggs used in the experiment were obtained from adults that had been reared for two generations on either this control diet or alternative larval diets.

Similarly, *Trichogramma evanescens* and *T. pintoi* were maintained in a climate chamber under the same environmental conditions and reared on *E. kuehniella* eggs.

Diets

The larval diets used in the experiment were formulated by modifying the control diet through the addition of fishmeal and corn flour in proportions equal to the amount of wheat bran removed, while maintaining a constant quantity of wheat flour across all diets (Table 1). Each diet was weighed to 5g and their macronutrient compositions were analyzed using a near-infrared (NIR) spectrometer operating in the 1200–2400 nm range.

Diet 1 exhibited the highest carbohydrate content (57.80%) and the lowest protein content (7.95%), even lower than that of the control diet. As the proportion of fishmeal increased across the diets, carbohydrate content decreased, whereas protein and fat contents showed a corresponding increase.

Table 1. Food materials and nutritional values of *Ephestia kuehniella* larval diets

Diets	Food Material Amount (%)				Nutritional Value (%)			
	Wheat Flour	Wheat Bran	Corn Flour	Fishmeal	Carbohydrate	Fat	Protein	Dry Matter
Control	50	50.00	-	-	53.90	2.42	9.51	89.41
Diet 1	50	25.00	25.00	-	57.80	0.69	7.95	89.71
Diet 2	50	22.50	22.50	5.00	51.10	0.24	10.17	89.50
Diet 3	50	21.25	21.25	7.50	39.00	0.73	11.60	89.96
Diet 4	50	20.00	20.00	10.00	34.20	2.21	14.52	90.52
Diet 5	50	18.75	18.75	12.50	25.80	2.80	17.03	90.71

Wheat flour and wheat bran used in the diets were commercially bought, fishmeal was obtained from a fish processing plant in Çanakkale and sweet corn was obtained from MAY Seed Growing Company. Corn was grinded in a modified coffee mill to create suitable sized granules.

Experimental Design

The experiment was conducted in a climate chamber with 24±1°C temperature, 50-60% R.h. and 18:6 L:D photoperiod conditions with 10 repetitions. Fifty eggs obtained from *E. kuehniella* adults reared on different larval diets for 2 generations were glued on 1x10 sized egg cards using 10% arabic gum solution. Then, egg cards were placed in 1.7x15 cm sized glass tubes. 1 female and 2 male adults of *T. evanescens* and *T. pintoi* were placed in each glass tube and honey was supplied by drawing a thin line inside surface of the tubes. The tubes were closed with cotton, and they were placed with the ends facing the lights to encourage the parasitoids to go towards the eggs. Parasitoids were given new egg cards with fresh *E. kuehniella* eggs after 24 hours and this procedure was repeated for 3 times. These egg cards were placed into their own tubes and followed daily to determine the parasitized eggs per female parasitoid, number of adults emerged from host eggs and the number of emerged male and female parasitoids.

Data was analyzed with two way ANOVA and the differences between diets were analyzed with TUKEY Multi comparison test by MINITAB 17 statistical software.

Results and Discussion

Parasitism Efficiency of *Trichogramma evanescens*

The parasitism efficiency of *Trichogramma evanescens* on *Ephestia kuehniella* eggs obtained from individuals reared on the control and experimental larval diets for two generations is presented in Table 2. The number of parasitized *E. kuehniella* eggs did not differ significantly among the diets, including the control ($p = 0.742$) (Table 2). Pehlivan (2021) reported the highest parasitism rates in both the F_0 (38.82 eggs) and F_1 (46.22 eggs) generations on a diet composed of 95% corn flour and 5% brewer's yeast. These findings are consistent with our results for Diet 1, which had a similarly high corn flour content. Conversely, a study by Moghaddasi et al. (2019) examining the impact of host egg familiarity found that parasitism by *T. brassicae* increased over successive generations. In their study, the number of parasitized eggs rose from 90.70 in the first generation to 136.20 by the seventh generation.

Table 2. Parasitism efficiency of *Trichogramma evanescens* on *E. kuehniella* eggs obtained from adults reared on different larval diets (Mean±Standart Error)

	Control	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Number of Parasitized Eggs*	45.6±1.49 ^a	47.3±4.21 ^a	48.0±4.31 ^a	48.0±1.38 ^a	53.3±4.26 ^a	48.9±1.94 ^a
Adult Emergence Rate	91.48±2.39 ^{ab}	84.14±2.94 ^b	88.25±2.69 ^{ab}	92.70±1.95 ^{ab}	88.04±2.39 ^{ab}	94.37±1.85 ^a
Number of Males	19.5±2.75 ^a	20.90±5.34 ^a	31.10±5.30 ^a	24.40±2.36 ^a	22.20±3.58 ^a	26.90±2.55 ^a
Number of Females	22.10±1.84 ^a	18.90±4.43 ^a	11.00±4.37 ^a	20.10±2.04 ^a	24.70±5.62 ^a	19.10±2.14 ^a
Number of Adults	41.60±1.46 ^a	39.80±4.24 ^a	42.10±3.69 ^a	44.50±1.59 ^a	46.90±4.03 ^a	46.00±1.66 ^a
Female %	0.53±0,03 ^a	0.47±0,01 ^a	0.26±0,01 ^a	0.45±0,02 ^a	0.53±0,03 ^a	0.42±0,02 ^a

*Parasitized *Ephestia kuehniella* eggs from the initial 3 days of parasitism were used.

Note: The differences between means at the same row with different letters are significant ($p < 0.05$)

Adult emergence rates from parasitized eggs differed significantly among the diets ($p = 0.034$). The highest emergence rate was observed in Diet 5 (94.37%), which contained the highest proportion of fishmeal. Although no statistically significant differences were found between the control and most experimental diets, Diet 1 which contained no fishmeal and the highest level of corn flour (25%) resulted in a significantly lower number of emerged adults compared to both the control and the other diets. In contrast, Pehlivan (2021) reported no significant differences in adult emergence across three tested diets. However, Moghaddasi et al. (2019) observed

significant differences in *T. brassicae* adult emergence from *E. kuehniella* eggs when comparing a standard diet to alternative larval diets.

No significant differences were found in the total number of emerged adults, as well as in the number of male and female individuals, between the control and experimental diets. Nevertheless, Diet 4 yielded the highest number of parasitized eggs (53.3) and the greatest number of emerged females (24.70), both of which are critical parameters for assessing the parasitism efficiency of egg parasitoids. These findings suggest that further detailed studies are required to better understand the underlying factors influencing female emergence and parasitism potential.

Parasitism Efficiency of *Trichogramma pintoi*

The parasitism efficiency of *Trichogramma pintoi* on eggs of *Ephestia kuehniella* reared on a control diet and four alternative larval diets over two generations is presented in Table 3.

The mean number of parasitized *E. kuehniella* eggs was lower for the control diet compared to the alternative diets; however, no statistically significant difference was observed between the control and other diets ($P = 0.849$). Diets 2 and 3 yielded higher numbers of parasitized eggs, whereas diets 4 and 5 resulted in fewer parasitized eggs, despite their higher fish meal content. According to İşgören (2021), the mean number of *E. kuehniella* eggs parasitized by *T. pintoi* per female over its lifetime was 120.56. The diet used in İşgören's study was comparable to diet 1 in the present study; however, the parasitism rate observed here was lower.

Table 3. Parasitism efficiency of *Trichogramma pintoi* on *E. kuehniella* eggs obtained from adults reared on different larval diets (Mean±Standart Error)

	Control	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5
Number of Parasitized Eggs*	35.44±1.92 ^a	37.16±2.19 ^a	50.75±2.23 ^a	50.50±1.83 ^a	46.11±2.86 ^a	42.55±1.58 ^a
Adult Emergence Rate	79.46±7.4 ^a	81.69±4.75 ^b	74.68±7.7 ^{ab}	78.21±6.6 ^{ab}	84.57±3.48 ^a	85.56±3.57 ^a
Number of Males	10.00±2.79 ^a	17.08±6.37 ^a	20.50±4.82 ^a	23.50±7.84 ^a	17.11±4.02 ^a	19.20±6.90 ^a
Number of Females	18.78±5.47 ^a	13.26±3.77 ^a	17.13±3.28 ^a	16.00±3.21 ^a	22.00±4.02 ^a	17.20±2.85 ^a
Number of Adults	28.78±5.16 ^a	30.34±10.05 ^a	37.63±6.73 ^a	39.50±9.88 ^a	39.00±7.17 ^a	36.40±9.05 ^a
Female %	0.65±0,04 ^a	0.45±0,03 ^a	0.47±0,03 ^a	0.41±0,02 ^a	0.56±0,04 ^a	0.47±0,03 ^a

*Parasitized *Ephestia kuehniella* eggs from the initial 3 days of parasitism were used.

Note: The differences between means at the same row with different letters are significant ($p<0.05$)

Significant differences were observed in adult emergence rates of *T. pintoï* across various larval diets ($p = 0.049$). Diet 5 yielded the highest emergence rate at 85.56%, followed closely by Diet 4 (84.57%) and Diet 1 (81.69%). Notably, Diet 1, characterized by the highest corn flour content, exhibited a lower adult emergence rate compared to the control. Consistent with findings for *T. evanescens*, these results suggest that increased fish meal content in larval diets may enhance adult emergence rates.

No significant differences were detected in the number of male *T. pintoï* emerging from *E. kuehniella* eggs across diets ($p = 0.500$). Similarly, while Diet 4 produced the highest number of adult females, no statistically significant variation was observed among diets ($p = 0.907$).

Both *Trichogramma* species exhibited comparable parasitism activity. However, the number of parasitized eggs, a critical performance metric for parasitoids, varied across the adult lifespan (see Figure 1). In *T. pintoï*, females demonstrated peak parasitism on the first day of adulthood across all diets, with a progressive decline on the second and third days. This pattern of elevated parasitism during initial days is advantageous for biological control applications, as it facilitates rapid pest suppression, thereby reducing labor and time requirements.

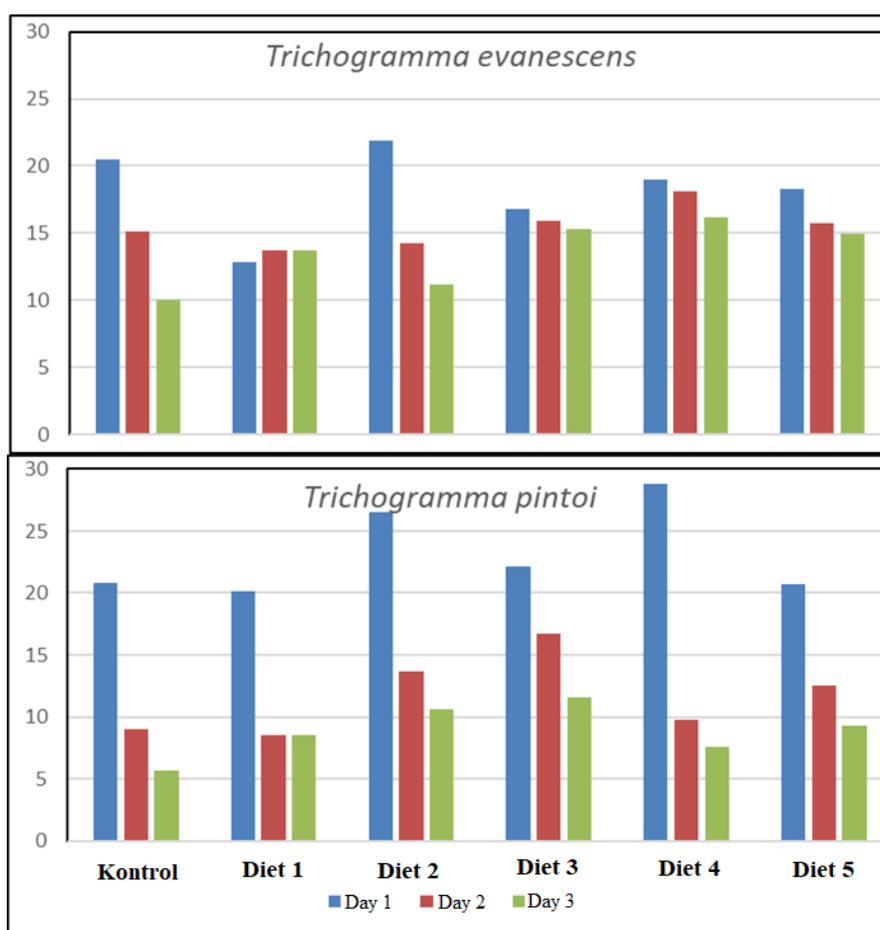


Figure 1. Number of *Ephestia kuehniella* eggs parasitized by *Trichogramma evanescens* and *Trichogramma pintoï* in the first 3 days of their adult lives

The number of eggs parasitized by *T. evanescens* on the first day was lower than on the second and third days in Diet 1, which contained the highest corn flour content. Conversely, parasitism rates were higher on the first day in the control diet and Diet 2. Diets 3, 4, and 5 exhibited consistent parasitism rates across the first, second, and third days. These findings align partially with Pehlivan (2021), who reported higher first-day parasitism across two generations of *T. evanescens* females, and Achiri et al. (2020), whose results for *T. evanescens* more closely resembled our observations for *T. pintoii*.

Host diet quality significantly influences parasitoid performance. Nathan et al. (2006) found no significant differences in parasitism of *Corcyra cephalonica* (Stainton) by *Trichogramma chilonis* across diets. However, Moghaddasi et al. (2019) linked higher egg deposition by *T. brassicae* in *Ephestia kuehniella* to larger host eggs resulting from high-quality diets. Similarly, González-Teuber et al. (2008) reported smaller *Callosobruchus maculatus* (Fabricius) eggs from females reared on low-quality larval diets. These findings underscore the role of diet quality in producing healthier, larger host insects, thereby enhancing the efficacy of parasitoids as biological control agents.

Parasitoid emergence rates for *T. evanescens* and *T. pintoii* were comparable across control and experimental diets, with Diet 5, containing the highest fish meal content, yielding the highest emergence rates for both species. No significant differences were observed in sex ratios or adult numbers across diets. Farahani et al. (2016) suggest that improved host diets enhance parasitoid fecundity, size, and sex ratio, thereby increasing biological control efficacy. Similarly, Liu et al. (2013) emphasize host quality as a critical determinant of parasitoid development and parasitism success.

Conclusions

In this study, eggs of *Ephestia kuehniella* reared on five experimental diets and a control diet were parasitized by *T. evanescens* and *T. pintoii* females. Adult emergence rates were significantly higher in Diets 4 and 5, which contained elevated fish meal content, compared to other diets and the control.

No significant differences were observed in the number of parasitized eggs across larval diets. However, *T. pintoii* parasitized more host eggs on the first day than *T. evanescens*. For *T. evanescens* in Diet 1, which had the highest corn meal content, parasitism was lower on the first day compared to the second and third days. This may be attributed to the prolonged rearing of *T. evanescens* on control diet eggs, potentially limiting adaptation to novel diets. In contrast, Diet 2 exhibited higher first-day parasitism, while Diets 3, 4, and 5, with increased fish meal, showed consistent parasitism across days.

Incorporating fish meal into *E. kuehniella* larval diets did not adversely affect parasitism efficiency in either parasitoid species. These findings suggest that fish meal is a viable protein and fat source for host diets. However, further research is needed to determine optimal fish meal concentrations, accounting for parasitoid adaptation periods.

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This study did not require an ethical committee permit. Contents of this article is suitable to publication ethics. Authors have contributed equally and there isn't any conflict of interest between authors.

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