



Erciyes University Journal of the Institute of Science and Technology

Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi

ISSN 1012-2354

Cilt (Volume): 31, Sayı (Issue): 4, Aralık/December-2015

<http://fbe.erciyes.edu.tr/>



Trichogramma embryophagum Hartig (Hymenoptera: Trichogrammatidae)'in ömür uzunluğu, parazitlenme performansı ve verimliliği üzerine *Wolbachia* spp.'nin etkisi

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ÖZET

Anahtar Kelimeler:
Trichogramma embryophagum,
Wolbachia spp.,
Partenogenez,
Ömür uzunluğu,
Telitoki.

Wolbachia cinsi endosimbiontlar *Trichogramma* türlerinde yaygın olarak görülmektedir. *Wolbachia* spp. bazı *Trichogramma* türlerinde tam partenogenez (telitoki) uyaran zorunlu hücre içi bakteridir. Telitoki sonucu döllenmemiş yumurtalardan sadece dişi *Trichogramma*'lar oluşmakta bu da biyolojik mücadele için bir avantaj sağlamaktadır. Bu çalışma parazitoidteki *Wolbachia* spp. kaynaklı bakteriyel enfeksiyonunu *Wolbachia* yüzey protein-PCR yöntemi kullanarak tanımlamak ve bu enfeksiyonun *Ephestia kuehniella* Zeller yumurtalarında yetiştirilen *Trichogramma embryophagum* Hartig'in ömür uzunluğu ve parazitlenme performansı üzerindeki etkilerini belirlemek amacıyla yapılmıştır. Kontrol grubu olarak ısı ile muamele edilmiş hat (arhenotoki) kullanılmıştır. *Wolbachia* spp. *T. embryophagum*'un verimliliği üzerinde herhangi bir negatif etki göstermemiştir. Isı ile muamele edilmiş arıların sadece erkek döl ürettiği görülmüştür. Bu arıların, *Wolbachia*-enfekteli olanlara göre daha uzun yaşadığı ancak bu farklılığın istatistiksel açıdan önemli olmadığı görülmüştür.

The effect of *Wolbachia* spp. on the longevity, parasitization performance and fecundity of *Trichogramma embryophagum* Hartig (Hymenoptera: Trichogrammatidae)

ABSTRACT

Key Words:
Trichogramma embryophagum,
Wolbachia spp.,
Parthenogenesis,
Longevity,
Thelytoky.

Endosymbionts of the genus *Wolbachia* are commonly found in *Trichogramma* species. *Wolbachia* spp. is obligatory intracellular bacteria that induced complete parthenogenesis (thelytoky) in some *Trichogramma* species. The result of Thelytoky, only *Trichogramma* females occurs from unfertilized eggs and this provides an advantage in biological control. This study was conducted to define the *Wolbachia* induced bacterial infection in parasitoid using *Wolbachia* surface protein-PCR method and to determine the effects of bacterial infection on the longevity and parasitization performance of *Trichogramma embryophagum* Hartig reared on *Ephestia kuehniella* Zeller eggs as a host. *Wolbachia* spp. had not showed any negative effect on fecundity of *T. embryophagum*. Heat treated lines (arrhenotokous) were used as control group. Heat treated wasps produced only male progeny. These wasps survived longer than *Wolbachia*-infected ones, however this difference was not statistically significant.

1. Giriş

Trichogramma species are the most frequently used natural enemies for controlling many lepidopteran species in inundative biological control programs in the world. These wasps lay their eggs into the host eggs and so they prevent the development of the pest. The mass rearing of these parasitoids are very easy due to their short generation time. Therewithal, they can attack many important crop insect pests (Li, 1994). These characteristics make them ideal insects to be investigated. These important natural enemies are haplo-diploid egg parasitoids that generally produce males originated from unfertilized eggs and females from fertilized eggs. This phenomenon called as arrhenotoky. Nevertheless there are some strains of *Trichogramma* contain thelytokous females that produce only daughters from unfertilized eggs (Luck et al., 1992).

Thelytoky in *Trichogramma* is typically caused by maternally inherited bacterium *Wolbachia* that caused only female broods (Stouthamer and Kazmer, 1994). These bacteria infect approximately 40 species of parasitic wasps that contain *Trichogramma* (Stouthamer, 1997). *Wolbachia* are an obligate, intracellular and maternally inherited Gram negative bacteria relating to Rickettsiaceae (Lo et al., 2007). *Wolbachia* strains can affect their host reproduction in different ways such as thelytokous parthenogenesis, cytoplasmic incompatibility, male killing and feminization of males (Stouthamer et al., 1999a). Thelytokous parthenogenesis induction (PI) is an advantage for *Wolbachia* because the bacteria are transmitted only through females (Islam, 2007).

Wolbachia have positive and negative effects on *Trichogramma*; increasing in progeny production (Girin and Bouletreau, 1995) and reduction in egg-laying and hatch rate, respectively (Huigens et al., 2000). On the other hand, *Wolbachia* infected females have found that less fecund and higher immature mortality than their uninfected counterparts (Hohmann and Luck, 2000). Thelytokous females produce only females, but if such females are exposed to >30 °C during their larval development, males are seen in their offspring (Bowen and Stren 1966). Theoretically, thelytoky provides advantages in a biological control program because of all of them are female offspring. Selection of the most effective species and strains of *Trichogramma* is the first step of a successful biological control program (Smith, 1996). Some biological characteristics such as fecundity, longevity and sex ratio are decisive for determining the efficacy of a parasitoid. At the same time, searching ability and walking activity of parasitoid have been used to define the effectiveness of parasitoid, because these features are related with host finding in the field (Bigler et al., 1988). Arrhenotokous and thelytokous females of some *Trichogramma* species were compared according to these features in previous studies (van Hezewijk et al., 2000; Silva et al., 2000; de Almeida, 2004).

T. embryophagum belonging to the pretiosum group (Pintureau, 1994) and has two different reproductive systems; thelytokous parthenogenesis in some individuals and bisexual reproduction in most individuals. At the present study, we have compared the some important parameters of *Wolbachia* infected (thelytokous) and heat treated (arrhenotokous) *T.*

2. Materyal ve metot

Host rearing

Mediterranean flour moth (MFM), *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), was obtained from the Adana Plant Protection Research Institute. *E. kuehniella* adults were reared on a mixture consisting of 1 kg wheat flour, 5% yeast, and 30 g wheat germs (Tuncbilek et al., 2009). Throughout the experiments, cultures were kept at constant temperature (27 ± 1 °C), relative humidity ($70 \pm 5\%$) and under a light regime of 16 h light followed by 8 h darkness (Tuncbilek et al., 2012). Adults from these cultures were collected and placed in plastic jars with screen bottoms. Eggs that fell through the screen were collected daily and used for rearing of parasitoid and experiments.

Trichogramma stock

The *Trichogramma* culture used in the study was obtained from the Adana Plant Protection Research Institute. *E. kuehniella* eggs were used as a host for rearing of parasitoid. Rearing was conducted in test tubes (180 mm long and 18 mm in diameter) by glued on the egg cards and adult parasitoids were fed by diluted honey solution. Females that emerged from parasitized flour moth eggs were used to initiate isofemale lines. Throughout the rearing, cultures were kept in the rearing room at 25 ± 1 °C and $70 \pm 5\%$ RH, and under a light regime of 16h L:8 h D.

DNA extraction and ITS2 PCR amplification

DNA was extracted from one *Trichogramma* (randomly from male or female individuals). It was ground in 60µl 5% Chelex-100 and 2µl Proteinase K (20mg/ml). Then it was incubated for 1h at 55 °C, followed by 10 min at 96 °C (Ercan et al., 2011). For ITS2 PCR amplification, ITS2 forward (5'-TGT GAA CTG CAG GAC ACA TG-3') and ITS2 reverse (5'-GTC TTG CCT GCT CTG CTC TGA G-3') primers were used according to Stouthamer et al., (1999b). Cycling program were: pre-denaturing step of 3 min at 95 °C, followed by 33 cycles of 45 s at 92 °C, 45 s at 53 °C, and 45 s at 72 °C with a final extension step of 3 min at 72 °C.

Wolbachia spp. detection

DNA was obtained as described above. The presence of *Wolbachia* was determined in female broods of thelytoky *T. embryophagum* population. *Wolbachia* surface protein-PCR (wsp-PCR) method was used for detecting of *Wolbachia*. PCR reactions were performed by using wsp-Forward primer 5'TGGTCCAATAAGTGATGAAGAAAC-3' and wsp-Reverse 5'-AAAAATTAACGCTACTCCA-3' primers (Braig et al., 1998). Cycling program were: 3 min. at 94°C followed by 40 cycles of 1 min. at 94°C, 1 min. at 50°C and 1 min. at 72°C with 5 min at 72°C after the last cycle.

Experimental procedure

The one group *Wolbachia* infected females of *Trichogramma* exposed to temperature >30 °C during their larval development for obtain arrhenotokous individuals. To determine the effect of *Wolbachia* on parasitoid's longevity and parasitization, 10

embryophagum lines.

had no contact with host eggs, only they fed with honey and were followed in controlled conditions until death. For determining the effect of bacteria on parasitization of cured and infected lines, 50±5 host eggs were glued to lightweight cardboard cards as described by Tuncbilek et al. (2009) and were placed in tubes along with single female of each line. After 24 h, the egg cards were removed from the tubes and incubated in rearing conditions.

newly emerged cured (=heat treated, arrhenotokous) and infected (=thelytokous) females were randomly selected. Each individual female was taken to test tubes (180 mm long and 18 mm in diameter) for experiments. For longevity test, all females. The parasitization was evaluated by counting the number of black eggs after 5 days of development of parasitoid. Adult emergence, female and male offspring numbers also were recorded.

Statistical Analysis

Longevity and parasitization data obtained from cured and infected lines of *T. embryophagum* were assessed using analysis of Mann-Whitney U Test and T-Test (SPSS, 1999).

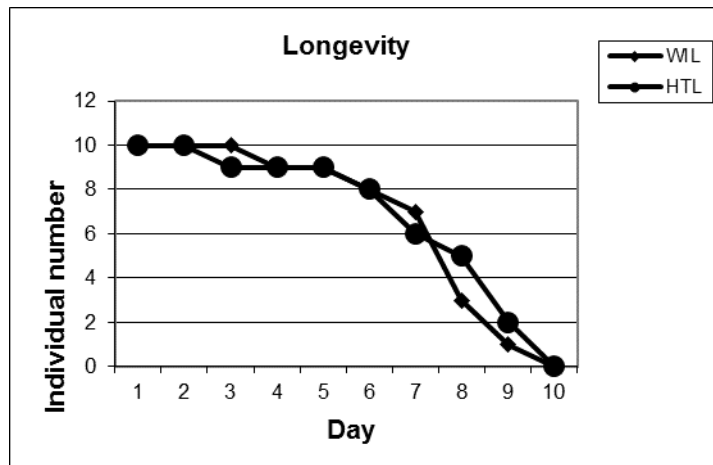


Figure 1. Longevity of heat-treated and *Wolbachia*-infected lines of *T. embryophagum* (WIL: *Wolbachia*-infected line, HTL: Heat-treated line). Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate SD of means

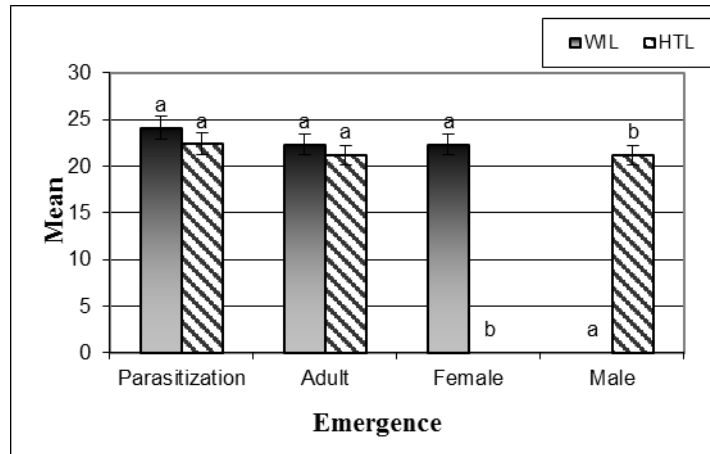


Figure 2. Mean numbers of parasitization, adult, female and male emergences (\pm SD) of heat-treated and *Wolbachia*-infected lines of *T. embryophagum* reared on eggs of *E. kuehniella* (WIL: *Wolbachia*-infected line, HTL: Heat-treated line). Letters above bars indicate significant differences between concentrations. Bars with the same letter are not significantly different. Error bars indicate SD of means

3. Bulgular

ITS2 gene sequence of the *Trichogramma* spp. was successfully determined. After sequencing, a BLAST search analysis in National Center for Biotechnology was carried out for finding the sequence similarity rates between *Trichogramma* sequences in database. As a result of ITS2 sequence studies, *Trichogramma* species determined as *T. embryophagum* (GenBank Accession Nos. AY244465).

In some *Trichogramma* species, parthenogenesis caused by endosymbiotic bacterium *Wolbachia* which induced gamete duplication in the haplodiploid organisms, resulting in completely female offspring in the next generations (Stouthamer and Kazmer, 1994). This phenomenon called as thelytoky and thelytokous strains can be cured from *Wolbachia* infection by antibiotic or heat treatments (Stouthamer et al., 1990). This situation may result in the production of males.

Here, amplification of *wsp* gene showed that *T. embryophagum* strain was infected with *Wolbachia* spp. *Wsp* gene sequence is a general marker for *Wolbachia* spp. detection and it provides informative characters for determining the relationships between strains. The *wsp* sequence obtained from our study can be found on GenBank under Accession Nos. AF245165. It was reported that *Wolbachia* strain infected *T. embryophagum* belonged to the supergroup B and subgroup Sib (van Meer et al., 1999). In 190 described *Trichogramma* species, at least 17 (include supergroup B) of them *Wolbachia* infection showed and thelytoky detected (de Almeida, 2004).

In our study, longevity was differed between two lines of *T. embryophagum* and heat treated females lived longer (6.80 ± 3.49) than their infected counterparts (6.70 ± 3.89). But

More recently, Poorjavad et al. (2012) was detected *Wolbachia* in Iranian *Trichogramma* by using *wsp* primers. We used the same primers for detection of *Wolbachia* in *T. embryophagum*. *Wolbachia* cause parthenogenesis in *Trichogramma* (supergroup B). In our study, *Wolbachia* spp. infection was determined in different *T. embryophagum* groups (supergroup B and subgroup Sib.). The *wsp* sequence provides an informative character for detection relationships between different strains (Braig et al., 1998).

In *Trichogramma* species, *Wolbachia* infection causing thelytokous parthenogenesis can be converted to normal bisexual reproduction using specific antibiotics or high rearing temperatures (Stouthamer et al. 1990). Thus, influence of *Wolbachia* on the parasitoid's reproductive biology such as egg load, survival and sex ratio can be investigated.

Hohmann et al. (2001) showed that antibiotic-treated *T. kaykai* Pinto and Stouthamer females produced more offsprings but they survived less than others. Stouthamer et al. (1990) reported that thelytoky can be changed to arrhenotoky in four *Trichogramma* species by using >30 °C temperature or antibiotic applications. In *T. evanescens* Westwood, there was not any difference between antibiotic treated and non-treated females in terms of progeny production. Likewise longevity was not significantly different between the 5 strains of *T. pretiosum* (Grenier et al., 2002). In another study, authors revealed that *Wolbachia* had a negative effect on the fecundity of *T. deion* Pinto ve Oatman (Silva et al., 2000). *Wolbachia* infection did not affect the productivity in *T. cordubensis* (Neto, 1996), although it had a positive effect on *T. oleae* Voegelé and Pointel (Silva, 1999). Stouthamer et al. (1990) proposed that thelytokous females produce male offspring with high temperature treatment

this differences was not statistically important ($P>0.05$) (Figure 1). The differences of the number of parasitized eggs and adult emergences between two lines were not statistically important ($P>0.05$). But only female progeny emerged from host eggs parasitized by *Wolbachia*-infected females during 24 h. Furthermore, heat treated females produced only males in first generation (Figure 2). This situation can be explained by two alternative hypotheses; first, high temperature or certain antibiotics may signal to the female about the environmental changing and so arrhenotokous reproduction may be preferred. Second, high temperature or certain antibiotics may induce some chromosomal damages that change reproduction mode from thelytoky to arrhenotoky (Stouthamer et al., 1990).

ITS2 sequence is a highly conserved region within species, but it varies fairly between species. Consequently, they are most useful tools for detecting and distinguishing *Trichogramma* species (Ercan et al. 2013). In our study, we identified our *Trichogramma* species by using ITS2-PCR as *T. embryophagum*.

Wolbachia is an intracellular bacterium that manipulates the reproduction of their hosts in different ways, such as complete parthenogenesis (thelytoky) in several parasitoid species and nematodes (Werren and Windsor, 2000). Detection of *Wolbachia* infection has been generally based on amplification of bacteria DNA by using polymerase chain reaction. Because *Wolbachia* cannot be cultured in a defined media and determining of it in infected gonadal cells is time consuming (Jeyaprakash and Hoy, 2000).

during their larval development, nevertheless, when these females exposed to lower temperature subsequently, they start to produce female offspring again.

In our study, we detected that *Wolbachia* spp. had not showed any positive or negative effect on fecundity of *T. embryophagum*. Moreover, in the cured *T. embryophagum* females did not showed any difference interms of reproductive capacity compared with their *Wolbachia*-infected counterparts. On the other hand infected females produced only female offsprings and this situation could increase the success of the biological control. At the same time, differences between longevity of two lines (infected (thelytokous) and heat treated (arrhenotokous)) did not found to be statistically important ($P>0.05$).

Additionally, there were not significant differences between two lines of *T. embryophagum* under laboratory conditions. Understanding of *Wolbachia* spp. effects, on parasitoid effectiveness, like longevity and fecundity, could be a major importance in improving biological control with *Trichogramma*. Correct species usage is an important step for a successful pest management. *Wolbachia* infection inducing thelytokous reproduction in *Trichogramma* may supply an advantage in biological control by producing only females.

Acknowledgments

This work is a part of the 2012FBE/T11 project and financially supported by the Bozok University Research Fund. We express our sincerest gratitude to them for their support.

Kaynaklar

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