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Mahmoud Nazeri[a], Ahmad Ashouri[a] & Mojtaba Hosseini[b]
[a] Department of Plant Protection, College of Agriculture, University of Tehran, Karaj, Iran
[b] Department of Plant Protection, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
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Can *Wolbachia* infection improve qualitative characteristics of *Trichogramma brassicae* reared on cold stored eggs of the host?

Mahmoud Nazeri[a]*, Ahmad Ashouri[a] and Mojtaba Hosseini[b]

[a]*Department of Plant Protection, College of Agriculture, University of Tehran, Karaj, Iran;* [b]*Department of Plant Protection, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran*

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The mass production cost of parasitoids is an important limiting factor in the development of successful biological control program. Parasitoids cannot be stored for a long time, thus should be produced shortly before use. Storage of host in low temperatures is an alternative technique to increase time flexibility of parasitoids production, thus contribute to reduction of production expenses. In this study, the influence of cold storage time (1 to 16 days) of host (*Ephestia kuehniella* Zeller) eggs on some qualitative characteristics of the egg parasitoid, *Trichogramma brassicae* Bezdenko (Hym.: Trichogrammatidae), was studied under laboratory conditions. The stored eggs were exposed to two different strains of *T. brassicae*, which were infected or uninfected with the endosymbiotic bacteria, *Wolbachia*, and the interactions between the host storage time and *Wolbachia* infection was studied. Increased host storage time negatively affected some qualitative characteristics of *T. brassicae*, such as fecundity, longevity, wing deformity and sex ratio. Some of these effects (e.g. fecundity, longevity and sex ratio) were different among *Wolbachia*-infected and uninfected strains. Given that the *Wolbachia*-infected strain produces higher proportion of females, they may compensate for other negative effects of *Wolbachia* as well as prolonged storage time of the host from biological control point of view.

Keywords: biological control; egg parasitoid; *Trichogramma*; parthenogenesis induction; *Ephestia kuehniella*, cold storage

1. Introduction

The egg parasitoids of the genus *Trichogramma* (Hym: Trichogrammatidae) are the most widely studied biocontrol agents, which have been successfully used for inundative biological control of many lepidopteran pests for more than 120 years (Smith 1996; Van Lenteren 2000). This genus includes about 200 species, 11 of which have been already reported form Iran (Ebrahimi 1999). The most widely distributed species in this country, *T. brassicae* Bezdenko (Poorjavad 2011), has been successfully released against some lepidopteran pests, such as the European corn borer, *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), the carob moth *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae), and the stripped rice borer, *Chilo suppressalis* Walker (Lepidoptera: Crambidae) (Ebrahimi 1999).

For development of a successful inundated biological control program, it is necessary to reduce costs, facilitate mass production process, and establish efficient methods for providing a large number of beneficial insects at the appropriate time (Colinet & Boivin 2011). Unlike pesticides, most biological control agents, including predators and parasitoids, cannot be stored for a long time, thus must be produced shortly before application. Development of efficient techniques for storage of these agents can reduce the cost of biocontrol programs (Colinet & Boivin 2011). A convenient exclusive technique for parasitoids is to retain their host eggs in low temperatures. As this technique does not require the host survival during storage, wider temperature ranges as well as longer storage periods can be used compared to when the own parasitoids are stored. Host storage has been promised as an efficient method for mass rearing of different *Trichogramma* species, such as *T. dendrolimi* (Liu et al. 1998), *T. chilonis* (Nadeem 2010), and *T. evanescens* (Tuncbilek et al. 2005).

Like many other species in the order Hymenoptera, *Trichogramma* wasps display a haplo-diploid sex determination, where females develop from fertilized diploid eggs, while males arise from unfertilized haploid eggs (Martel et al. 2008). However, females infected with the endosymbiotic bacteria, *Wolbachia*, are enabled to produce diploid female progeny from both fertilized and unfertilized eggs, a process known as parthenogenesis induction (Pintureau et al. 2002). *Wolbachia*-induced parthenogenesis has been already reported in at least 18 species of *Trichogramma* wasps (Pinto and Stouthamer 1994; de Almeida 2004; Farrokhi 2010), including the Iranian strains of *T. brassicae* (Poorjavad et al. 2012; Karimi et al. 2010). The use of thelytokous strains has been proposed to provide some advantages for biological control, especially because they produce a higher proportion of females. However, the fitness of *Wolbachia*-infected versus uninfected strains plays a major role in selection of appropriate strain for use in biological control programs (Russell & Stouthamer 2010).

This study was conducted to explore the effects of cold storage time of host eggs (the Mediterranean flour

*Corresponding author. Email: mahmoud_nazeri@ut.ac.ir

moth, *E. kuehniella* Zeller (Lep: Pyralidae)), on qualitative characteristics of *T. brassicae* under laboratory conditions. Thanks to the identification of *Wolbachia*-infected (hereafter thelytokous) and uninfected (hereafter arrhenotokous) strains of this species by Poorjavad (2011), we were also able to compare these effects among thelytokous and arrhenotokous strains of *T. brassicae*.

2. Materials and methods

2.1. Sample collection and identification

The two parasitoid strains studied here, the *Wolbachia*-infected and *Wolbachia*-free strains were derived from cultures maintained by the Biological Control Research Department (BCRD) of the Iranian Research Institute of Plant Protection (IRIPP). These strains were collected from parasitized *Ostrinia nubilalis* (Hübner) egg batches laid on *Xanthium strumarium* L. (Asterales: Asteraceae) in Mazandaran province (north of Iran). Emerging *Trichogramma* wasps were reared separately in laboratories of the BCRD of the IRIPP. A stock colony was established using the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lep: Pyralidae) sterile eggs as host. After adult emergence, a single-mated *Wolbachia*-free or virgin *Wolbachia*-infected female was allowed to establish an isofemale line and these lines were used in the study.

2.2. Detection of Wolbachia

The *wsp* gene was used to check the presence of *Wolbachia* in *Trichogramma* specimens. The PCR reaction was performed in 25 μl reaction volumes using an Eppendorf thermocycler. Each reaction mixture contained 3 μl DNA template, 2.5 μl (10×) *Taq* buffer, 0.75 μl MgCl2, 0.5 μl dNTPs, 0.5 μl forward and reverse primer (10 picomoles/μl), and 0.3 μl *Taq* polymerase (5 U). The Braig et al. (1998) primer set was used, which amplifies a 590−632 bp segment of the *wsp* gene. The temperature profile consisted of an initial denaturation step at 94 °C for 30 s followed by 36 cycles (denaturation at 94 °C for 30 s, annealing at 50 °C for 45 s, and extension at 72 °C for 60 s), with a final extension at 72 °C for 5 min. For each DNA extraction, three control extractions were performed using a *Drosophila melanogaster* Meigen (Diptera: Drosophilidae) *Wolbachia*-positive line, a *Trichogramma Wolbachia*-negative line, and a non-DNA sample. The PCR product was electrophoresed on 0.8% agarose gels along with a size ladder. DNA was sequenced with the BigDye Terminator Kit (Macrogen Inc. Korea). Both DNA chains of each sample were sequenced separately with the corresponding primers. Each chromatogram was checked for double or multiple peaks. ABI files of forward and reverse sequences were edited using the BioEdit software (Hall 1999). MEGA 5 (Tamura et al. 2011) software was used for conceptual translation of *wsp* sequences into protein. The sequences were edited and aligned with the software BioEdit and compared through NCBI with available data to characterize its identity.

2.3. Experiment procedure

The *Wolbachia*-infected and uninfected strains were reared as separate lines on sterilized eggs of *E. kuehniella*. Host eggs were collected daily and glued (200 eggs) with diluted honey onto cardboard strips (10 × 60 mm). The eggs were sterilized by exposing them to UV-C lamp (15 w Philips Holland) with a wavelength of 280 nm with a distance of 30 cm for 30 minutes, then stored separately at 4 °C, 60 ± 10% relative humidity (RH) and a dark photoperiod until used.

The eggs were removed from the cold conditions after 1, 2, 4, 8, 12, and 16 days and placed in glass tubes (16 cm length × 1 cm diameter) containing either a newly emerged arrhenotokous or thelytokous wasp and a diluted honey drop as food. The prepared vials were kept at 25 ± 1°C, 70 ± 10% RH and 16:8 L:D. The wasps were removed from the vials after 24 hours and their eggs were left at the same environmental conditions until adult's emergence.

The qualitative characteristics, including rate of parasitism, adult longevity, fecundity, wing deformity, length of hind tibiae, and sex ratio were recorded for *Wolbachia*-infected and *Wolbachia*-uninfected wasps. Each treatment (cold stored eggs of the host) had 12 replications.

The rate of parasitism was defined as the number of eggs parasitized by a single female during 24 hours. Immediately after adult emergence, one female per tube was isolated to assess fecundity and longevity. Each isolated female was provided with 200 sterile eggs of *E. kuehniella* (<24 h) glued on a cardboard strip. The cardboards were removed daily and incubated for six days when the parasitized eggs with black colour were counted. The total number of eggs parasitized by individual females was considered as fecundity. To assess longevity, the isolated females were observed daily until death. The sex ratio was checked for all parasitic wasps based on dimorphic antennal setation (Knutson 1998). The occurrence of aptery (lack of wings) and brachyptery (very small or stubby wings) was recorded for all progeny of individual females (Knutson 1998). The length of hind tibia was used as a reliable index for comparison of female body size (Waage & Ming 1984). Adult females were mounted on a glass microscope slide in Hoyer's medium, and the length of their tibia was measured using a scaled microscope. The effects of host storage time on offspring qualitative characteristics were also assessed. Parasitism rate, longevity, fecundity, wing deformity, and sex ratio were evaluated in offspring generation with the same above-mentioned methods. Parental and offspring generations, hereafter called the F1 and F2, respectively.

2.4. Data analysis

All data were tested for normality, (Kolmogorov−Smirnov test) and met the assumptions of analysis of variance (ANOVA). Some parameters showed significant deviation from a normal distribution, thus analyzed using non-parametric statistical tests (Mann−Whitney U test or Kruskal−Wallis H). The influences of wasp strain and host storage time on qualitative characteristics of

Table 1. Effect of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs cold storage on qualitative characteristics of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), in F1 and F2. Rows followed by the same letter are not significantly different by Tukey test ($P \leq 0.05$).

	F1			F2		
Day	Parasitism rate	Longevity	Hind tibia length	Parasitism rate	Longevity	Fecundity
1	33.2 ± 1.8 a	16.7 ± 1.09 a	267 ± 1.3 a	32.9 ± 2.1 a	15.9 ± 1 a	123.3 ± 6.2 a
2	31.5 ± 1.6 ab	17.1 ± .097 a	266.9 ± 1.3 a	31.5 ± 3 a	14.2 ± 1.1 ab	116.9 ± 7.2 a
4	27.4 ± 1.8 ab	15 ± 0.8 ± ab	264 ± 2.1 a	30.4 ± 2.7 a	13.5 ± 1.1 ab	112.2 ± 6.9 ab
8	30.4 ± 1. ab	13 ± 1.1 b	262.4 ± 2.4 a	25.3 ± 2.8 a	12.5 ± 1 b	95.3 ± 6 b
12	29 ± 1.2 ab	12 ± 1.1 b	261.3 ± 1.2 a	27.9 ± 2 a	12.2 ± 1 b	108.6 ± 6.2 ab
16	29.7 ± 1.1 ab	13 ± 1.2 b	262.1 ± 2 a	27.5 ± 2.7 a	11.5 ± 1.1 b	95.2 ± 5.8 b

T. brassicae were analyzed by two-way full factorial ANOVA (wasp strains × host egg storage time). When ANOVA indicated a significant effect, Tukey's honestly significant difference test was used to determine the significance between means values. If the interaction of two factors was not significant, the relationship between the host storage time and qualitative characteristics of *T. brassicae* were analyzed by linear regression. All data were analyzed using SAS computer software, version 9.1 (SAS Institute, 2004).

3. Results

To prove that the differences between *T. brassicae* strains are effectively linked to the *Wolbachia* infection, we must use strains that have the same genetic background. The genetic similarly of these two isolines have been previously demonstrated by Farrokhi (2010). Farrokhi (2010) showed that differences between strains are in 3 base in ITS2 gene, and concluded that this difference refer to individual diversity, and cannot be attributed to higher level. In addition, according to Mayr et al. (1953) sympatric species that do not have reproductive isolation and live in the same host do not have genetical difference. Since all the wasps emerged from the egg patch were not thelytokous parthenogenetic and unisexual and bisexual parasitoids coexist in the same patch, and regard to the effect of antibiotics on microorganisms of *Trichogramma*, instead of individuals treated with antibiotics, bisexual parasitoids were used in this study, and the observed differences on biological characteristics between strains could be attributed to the *Wolbachia* infection.

Amplification of *wsp* gene in collected *T. brassicae* revealed a *Wolbachia*-infected strain. The *wsp* sequence is available in GenBank with an accession number of JX131628. The phylogenetic tree based on *wsp* sequences of *Wolbachia* strains in *Trichogramma* wasps indicated that this strain belonged to the supergroup B (Sib group) (Farrokhi et al. 2013).

The parasitism rate of female *T. brassicae* was not significantly different among cold stored hosts in both F1 ($F = 1.71$, d.f. = 5, 131, $P = 0.13$) (Table 1) and F2 ($F = 1.13$; d.f. = 5, 131, $P = 0.35$) (Table 1). There was no statistical difference between the parasitism rates of thelytokous and arrhenotokous wasps in both F1 ($F = 1.93$, d.f. = 1, 131, $P = 0.16$) (Table 3) and F2 ($F = 2.17$, d.f. = 1, 131, $P = 0.14$) (Table 3). Similarly, we found no significant effect of cold storage time ($F = 1.88$, d.f. = 5, 131, $P = 0.1$) (Table 1) and *Wolbachia* infection ($F = 7.29$, d.f. = 1, 131, $P = 0.1$) (Table 3) on length of the hind tibiae in F1.

Interaction of host cold storage times and strain of *T. brassicae* on fecundity was significant in F1 ($F = 8.29$, d.f. = 5, 131, $P > 0.01$). The arrhenotokous wasps grown on the host with shorter storage time had a significantly higher fecundity compared to those wasps grown on hosts with longer storage period (Figure 1). The interaction between these two factors, however, was not statistically significant in F2 ($F = 1.23$, d.f. = 5, 131, $P = 0.3$). Nevertheless, the effect of cold storage time on fecundity of *T. brassicae* was significant (Table 1) with a negative relationship that was revealed between the fecundity and the cold storage time ($R^2 = 0.26$, $Y = 119.75 - 1.56$ X). The arrhenotokous strain was also significantly more fecund than the thelytokous strain ($F = 17.02$, d.f. = 1, 131, $P > 0.01$) (Table 3).

Effect of cold storage time on the longevity of *T. brassicae* were significant in both F1 ($F = 3.02$, d.f. = 5, 131, $P < 0.01$) (Table 1), and F2 ($F = 2.33$, d.f. = 5, 131, $P = 0.04$) (Table 1). A negative relationship was found between the longevity and the cold storage time of host eggs ($R^2 = 0.28$, $Y = 16.75 - 0.28$ X and $R^2 = 0.36$, $Y = 15.12 - 0.24$ X for F1 and F2, respectively). The longevity of thelytokous strain in both F1 and F2 was significantly higher than that of arrhenotokous strain ($F = 7.08$, d.f. = 1, 131, $P > 0.01$, and $F = 16.23$; d.f. = 1, 131; $P > 0.01$ (Table 3) for F1 and F2, respectively).

We also found significant effects of cold storage on the sex ratio of arrhenotokous *T. brassicae* in both F1 (Kruskal–Wallis H Statistics = 31.522, $P < 0.001$) (Table 2) and F2 (Kruskal–Wallis H Statistics = 24.032, $P < 0.001$) (Table 2). As expected, the thelytokous wasps produced no male offspring, thus the female ratio was significantly higher in thelytokous strain in both F1 and F2 (Mann–Whitney U Statistics = 8844.000, $P = 0.001$ and Mann–Whitney U Statistics = 25806.000, $P = 0.001$ for F1 and F2, respectively) (Table 4).

Effect of cold storage time on wing deformity of *T. brassicae* adults was also significant in both F1 (Kruskal–Wallis H Statistics = 20.552, $P < 0.01$)

Table 2. Effect of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs cold storage on wing deformity and sex ratio of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), in F1 and F2. Numbers followed by the same letter in each column do not differ significantly (Kruskal–Wallis test, $P \leq 0.05$).

	F1		F2	
Day	Wing deformity	Sex ratio	Wing deformity	Sex ratio
1	0.04 ± .0.4 b	0.88 ± 0.01 a	0 b	0863 ± 0.008 a
2	0.08 ± 0.05 b	0.84 ± 0.01 b	0.014 ± 0.04 b	0.891 ± 0.012 a
4	0.12 ± 0.06 b	0.83 ± 0.01 b	0.12 ± 0.08 b	0.855 ± 0.016 a
8	0.20 ± 0.10 a	0.78 ± 0.04 c	0.20 ± 0.09 a	0.74 ± 0.051 c
12	0.20 ± 0.10 a	0.78 ± 0.01 c	0.16 ± 0.09 b	0.825 ± 0.018 b
16	0.20 ± 0.10 a	0.75 ± 0.04 c	0.54 ± 0.24 a	0.787 ± 0.034 c

(Table 2) and F2 (Kruskal–Wallis H Statistics = 24.032, $P < 0.01$) (Table 2). There was no statistical difference in the number of deformed adults among thelytokous and arrhenotokous wasps in both F1 (Mann–Whitney U Statistics = 2592.000, $P = 1.00$) (Table 4) and F2 (Mann–Whitney U Statistics = 522.000, $P = 0.051$) (Table 4).

4. Discussion

Results of this study revealed that the storage time of host eggs as well as the presence of the endosymbiotic bacterium, *Wolbachia* may affect some biological characteristics of the parasitic wasp, *T. brassicae*. The parasitism rate of *T. brassicae* on *E. kuehniella* eggs was not affected by either storage time of host eggs or infection by *Wolbachia*. These findings are in accordance to those of Liu et al. (1998), de Almeida (2004), Farrokhi (2010), and Nadeem (2010). Increased duration of egg storage caused significant reduction in fecundity of *T. brassicae*. Similarly, *Wolbachia* infection was found to negatively affect the fecundity of *T. brassicae*. The reduced fecundity of *Wolbachia*-infected lines has been previously reported for different *Trichogramma* species in both laboratory and greenhouse conditions (Stouthamer & Luck 1993; Wang & Smith 1996; Silva 2000; Hohmann et al., 2001). Apparently, differences in the ovarian development underlies the differential fecundity of *Wolbachia*-infected and uninfected strains (Wang & Smith 1996). As Figure 1 shows, duration of host storage had the greatest effect on fecundity of uninfected strains, while no significant differences was found in the fecundity of thelytokous wasps grown on host eggs, which were stored for 1 and 16 days. In the laboratory experiments, fecundity is considered as the total number of offspring produced under no limitations of host and time. However, in field conditions, where both host and time are limited, *Wolbachia*-infected parasitoids produced more daughters than antibiotic-cured females (Stouthamer & Luck 1993). Calculations confirm that by releasing equal number of both strains of parasitoids, more eggs are parasitized by the *Wolbachia*-infected wasps (Silva et al. 2000).

The storage time of host eggs led to an increased longevity of *T. brassicae*. These observations are in line with the results obtained by Nadeem (2010). On the other hand, in accordance to the results obtained by Hohmann et al. (2001) and Miura and Tagami (2004), *Wolbachia* infection was found to cause significant increase in the longevity of *T. brassicae*. Therefore, by using the long-period stored eggs for rearing of thelytokous strain, the negative effects of host storage on longevity may be compensated. In contrast to these results, Stouthamer and Luck (1993) and Wang and Smith (1996) found positive effects of *Wolbachia* infection on the longevity of *Trichogramma*.

Since only female wasps have the potential for control of pests, the control of sex ratio is of special importance in mass rearing and release of parasitoids in biological control programs (de Almeida 2004). In *Trichogramma* wasps, host size, age, nutritional suitability, and previous parasitism of females have been shown to affect the sex ratio of offspring (Schmidt 1994). Host quality has been repeatedly shown to affect the oviposition patterns of parasitic wasps where female eggs are laid on higher quality hosts, while male eggs are laid on lower quality hosts

Table 3. Comporisson of qualitative characteristics of two arrhenotokous and thelytokous strains of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) in F1 and F2. Rows followed by the same letter are not significantly different by Tukey test ($P \leq 0.05$).

	F2			F2		
Strain	Parasitism rate	Longevity	Hind tibia length	Parasitism rate	Longevity	Fecundity
+W	29.42 ± 0.86 a	15.89 ± 0.57 a	262.98 ± 0.82 a	30.97 ± 1.79 a	15.18 ± 0.66 a	98.35 ± 3.74 b
−W	31.16 ± 0.96 a	13.62 ± 0.67 b	265.96 ± 1.22 a	27.74 ± 1.16 a	11.68 ± 0.57 b	118.76 ± 3.61 a

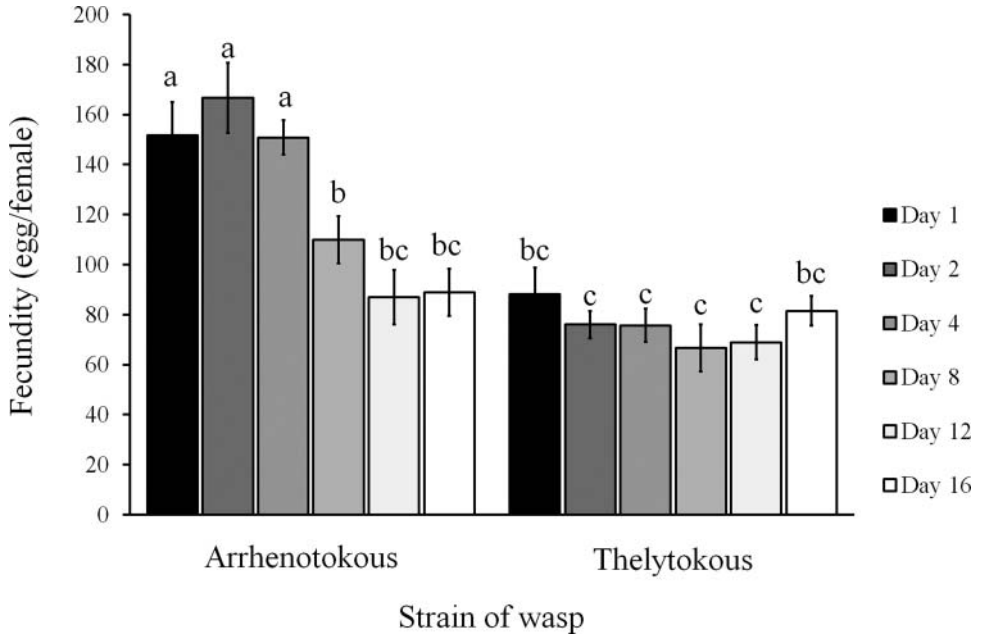

Figure 1. Fecundity of thelytokous and arrhenotokous strain of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) reared on cold stored eggs of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) in F1. Columns followed by the same letter are not significantly different by Tukey test ($P \leq 0.05$).

(van den Assem 1971). Since the increased period of egg storage may decrease their quality, we hypothesized that by the increase in egg storage duration, the proportion of female offspring would reduce. Our results, however, revealed no effects of the storage time on the sex ratio of *T. brassicae*.

Our results showed negative effect of host storage time on wing deformity of *T. brassicae.* As flight plays a key role in host finding of parasitic wasps, wing deformity would lead to a significant decrease in parasitism rate and efficiency of biological control agents (Knutson 1998, Kölliker-Ott et al. 2003). The precise mechanism involving in wing deformity induction remains to be cleared.

It has been repeatedly suggested that the fitness of *Trichogramma* species is positively associated with their body size (McDougall & Mills 1997; Boivin & Lagacé 1999). Given the high correlation of hind tibia length and body size (van Lenteren & Bigler 2010), in this study, we used the length of hind tibia as a reliable index of body size in *T. brassicae*. The storage time of host eggs did not affect the length of hind tibiae of adult parasitic wasps. Accordingly, we found no significant difference between the length of hind tibiae among arrhenotokous and thelytokous strains, implying that *Wolbachia* does not affect its host size. These results are in line with those of Neto (1996) and de Almeida, (2004). However, there are evidence that *Wolbachia* can reduce its host body size [e.g. *Trichogramma* (Silva 1999), and *Drosophila* (Fytrou et al. 2006)].

Altogether, results of this study showed that different biological characteristics of *Trichogramma* wasps, including fecundity, longevity, wing deformity, and sex ratio are negatively affected by increased duration of host eggs. Some of these effects are different among thelytokous and arrhenotokous strains of *T. brassicae*. Since *Wolbachia* influences on qualitative characteristics of the *Trichogramma*, it may be essential to investigate their occurrence in strains selected for biological control programs. Given that *Wolbachia* is one of the most common parasitic symbiont in insects, especially parasitoids (Braconidae, Ichneumonidae, Aphelinidae, Encyrtidae etc), it would be sensible to explore the presence and probable effects of *Wolbachia* in other species of biocontrol agents before their selection for use in biological control programs.

Table 4. Comporisson of wing deformity and sex ratio of two arrhenotokous and thelytokous strains of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae) in F1 and F2. Numbers followed by the same letter in each column do not differ significantly (Mann–Whitney test, $P \leq 0.05$).

	F1		F2	
Strain	Wing deformity	Sex ratio	Wing deformity	Sex ratio
+W	0.26 ± 0.06 a	1 a	0.25 ± 0.09 a	1 a
−W	0.02 ± 0.01 a	0. 82 ± 0.01 b	0.11 ± 0.05 a	0.81 ± 0.009 b

Selection of an efficient species or strain of *Trichogramma* is one of the most important parts of a successful augmentative biological control program (Van Driesche & Bellows 1996). This selection is based on certain biological aspects, such as parasitism rate, development time (egg to adult), life cycle viability, sex ratio, and longevity (Cerutti & Bigler 1995, Pratissoli & Parra 2001). Therefore, by selection of suitable strain, it would be possible to reduce some negative effects of host storage time. According to our results, the native thelytokous strain of *T. brassicae* may be considered as a good candidate for biological control of lepidopteran pests in Iran. It should be held in mind that *Wolbachia* may differentially affect a variety of fitness-related components from biological control point of view, thus one should not predict the performance of any strain without considering all of these properties. For example, despite having lower fecundity, the *Wolbachia*-infected strain may compensate for this deficiency by production of higher proportions of female offspring compared to uninfected strain thus, represent more efficiency in biological control (Silva et al. 2000). Therefore, a comprehensive, preferably field study, would be needed to evaluate all aspects of *Wolbachia* infection and host storage in biological control agents.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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