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SOME BIOLOGICAL PROPERTIES OF *TRISSOLCUS SEMISTRIATUS* NEES (HYMENOPTERA: SCELIONIDAE) ADULTS STORED AT DIFFERENT TEMPERATURES

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Abstract

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The purpose of this study is to evaluate the suitable storage temperature and duration for an important egg parasitoid *Trissolcus semistriatus* Nees (Hymenoptera: Scelionidae). Adults of the parasitoid used in these experiments were obtained from 26 ± 1°C and were then stored at 6, 9, 12, and 15ºC from 1 to 6 months. Survival rates, longevity, oviposition period, lifetime fecundity, female progeny and sex ratio of *T. semistriatus* were determined under the laboratory conditions. The results of this study showed that the highest rate of vitality was obtained in the first month of storage at all temperatures; and the lowest rate of vitality was obtained in the sixth month of storage. Depending on the storage temperature, the average longevity of the *T. semistriatus* adult changed, the longest longevity of 16.7 ± 1.28 days belonged to control individuals. The lowest longevity of 9.5 ± 1.51 days belonged to the adult individuals stored at 15°C. The longest oviposition period was calculated as 12.7 ± 1.00 days for control and the lowest oviposition period was calculated as 7.2 ± 1.16 days for *T. semistriatus* stored at 9°C. Lifetime fecundity of *T. semistriatus* stored at various temperatures were 49.8 ± 6.56 at 9°C, 57.7 ± 5.77 at 12°C and 53.6 ± 6.92 at 15°C, and was 89.0 ± 5.46 for control. The highest sex ratio was 73.0 ± 0.90 for control; the lowest was 62.4 ± 0.87 for females stored at 15°C.

Key words: fecundity, storage, sunn pest, temperature, *Trissolcus semistriatus*

Introduction

Sunn pest (SP), *Eurygaster* spp. (Heteroptera: Scutelleridae) are the most important harmful insect pests on wheat in Turkey. Overwintered adults of SP attack the leaves and stems of young, succulent wheat plants, causing them to wither and die prior to spike formation. The SP is univoltine and the adults rest under bushes and litter on mountains around cereal fields during aestivation and hibernation. In spring, when soil surface temperature reaches 15°C at overwintering sites, adults migrate to cereal fields. Overwintered adults appear in the wheat fields for a period of 1 – 4 weeks (Lodos, 1961 and 1986). They also feed at the base of the spike during the early growth period, resulting in grayish white spikes without kernels, called white spikes. Fourth and fifth nymphal instars and new-generation adults of the SP feed on grains (Lodos, 1986; Memisoglu and Ozer, 1992). Yield losses are estimated at 50 - 90% in wheat (Lodos, 1986). In addition to direct yield reduction, during feeding, the insect injects digestive enzymes into the grain, reducing the baking quality of the dough. If as little as 2 - 3% of the grain has been fed on, the entire grain lot may be rendered unacceptable for baking purposes because of poor-quality flour (Lodos, 1986; Kinaci and Kinaci, 2007).

To suppress SP population to acceptable levels in Turkey, chemical control by aerial application was carried out from 1954 to 2004. This method had a negative impact on human health and the environment; therefore, resulting in chemical control by ground equipment as of 2005. In addition, various natural enemies attack SP in Turkey. It was found that natural enemies have the greatest effect among the factors restraining SP population (Lodos, 1961; 1986 and Rosca et

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al., 1996). *Trissolcus semistriatus* Nees (Hymenoptera: Scelionidae) is the most common natural enemy of *Eurygaster integriceps* Put. (Heteroptera: Scutelleridae) in Turkey. This parasitoid gives three generations during oviposition of SP, and especially in the third generation reach up to 100% of eggs of parasitisim (Zwölfer, 1942). The species of scelionid parasitoids have been used against SP in both inundative releases and classical biological control programs in Iran, Morocco and the former USSR (Voegelé, 1961; Laraichi and Voegelé, 1975; Shumakov, 1978). Mass rearing and inundative releases of the egg parasitoid attempts in Turkey started in 1990 and continued until 1997. However, it could not succeed because of timing of the releases (Akinci and Soysal, 1996). Afterwards, new releasing studies started in 2001. *T. semistriatus* species have been used in the National SP Project in Turkey for biological control of SP and continued up to the present. It was determined that parasitoid efficacy changes depending on the years and regions (Islamoglu et al, 2010; Islamoglu 2012).

The SP is not a suitable host for year-round production of egg parasitoids, because it belongs to the univoltine species. Many pentatomid species, such as *Dolycoris baccarum* (L.), *Eurydema ornatum* (L.), *Graphosoma lineatum* L. and *Holcostethus vernalis* Wolf. (Heteroptera: Pentatomidae), exist in alternative hosts for egg parasitoids, but until now, it has been impossible to produce sufficient host eggs for mass culture of these parasitoids. The release of parasitoids in the field should be managed at the initial onset of SP oviposition (Tarla and Kornosor, 2009). Mass production of egg parasitoids, based on adults of SP, was collected from infested wheat fields. After they fed on wheat and laid eggs under laboratory conditions, these eggs were parasitized by *T. semistriatus* and released into infested wheat fields. However, enough adults were not collected and the inability to obtain eggs has a negative impact on success. One of the most important factors affecting the success of mass production is the creation of a culture of *T. semistiatus* in early spring. When the temperature reached 13°C in early spring, under natural condition a sweeping net collected the scelionid parasitoids from newly planted wheat fields, from the plants around those fields and from *T. semistriatus* culturing in the laboratory. However, the formation of this culture requires a period of at least 1 - 1.5 months; consequently this situation leads to late release in some regions of Turkey.

There are no studies concerning the storage adults of *T. semistriatus* and reproductive biology, but many laboratory studies at low temperatures on cold storage conditions have been conducted about the Scelionidae species (Bayram et al., 2005; Foerster and Doetzer, 2006; Foerster et al., 2004). Storage at low air temperatures, at or below the developmental threshold, called cold storage, is useful for regulating the supply of natural enemies (Colinet and Hance, 2010; Colinet and Boivin, 2011). Long-term cold storage of natural enemies is highly desirable because it reduces production costs through adjustment of the production and preserves a natural enemy population by compensating for periods of low production or high demand. Additionally, cold storage also provides an extended shelf life for natural enemies (Van Lenteren and Tommasini, 1999).

This study was aimed to constitute the next years' culture from the stored *T. semistriatus,* one of the most commonly present SP parasitoids in Turkey, at low temperatures from 1 to 6 months.

Material and Method

Collection and storage of *Trissolcus semistriatus*

A large amount of parasitized egg masses of SP were collected by hand from the wheat fields located in Nurdağı (37º 11' N, 36º 50' E) and Islahiye (37º 07' N, 36º 40' E), districts of the Gaziantep province in late May. Each of the parasitized egg masses (1 egg mass = ca. 14 eggs) was transferred separately into cotton-plugged glass tubes (1.5 cm diameter, 9 cm long). The inner areas of the tubes were streaked with a honey solution diluted by 10% water to provide a food source for the hatching parasitoids. The tubes were kept in an incubator at 26 ± 1°C, 65 ± 5% relative humidity (RH), and under a light: dark (L : D) cycle of 16 : 8 hours (hrs) until the parasitoids emerged from the eggs. After emergence, the parasitoids were separated by species within the laboratory. The 40 glass tubes, each one including 14 *T. semistriatus,* were separated to four equal groups. Each group was stored at 6, 9, 12 and 15ºC under dark conditions from 1 to 6 months. Stored parasitoids were removed from the incubator at the end of each month in order to be fed and counted at the room temperature for 15 - 20 minutes in laboratory conditions. Living and dead individuals were recorded. These trials were carried out at the laboratory of Adana Biological Management Research Institute in 2011.

Some biological Properties of *Trissolcus semistriatus* stored at different temperatures

Experiments were carried out to determine biological characteristics of stored *T. semistriatus* at different temperatures on SP eggs in an incubator at 26 ± 1°C, 65 ± 5% RH, L16 : D 8 hour photoperiod. To determine reproduction characteristics and longevity of stored *T. semistriatus* and F1 generations, eighteen females were tested. Parasitoids were stored at different temperatures taken in the glass tubes. In the first two days, four egg masses, and the remaining days, two egg masses (one day old) of *E. integriceps,* glued to a labeled index card strip (1 x 3 cm), were exposed to one female per treatment until the parasitoid females died. Honey diluted by 10% was given to adult parasitoids as a food. Egg masses were provided every 24 hrs and then the old egg masses were transferred to new tubes under the same conditions until their fate could be determined. When nymphs of *E. integriceps* hatched from un-parasitized eggs, they were removed from the glass tubes. The mortality of female parasitoids was recorded twice daily. The sex of the progeny that emerged from the egg masses was determined and recorded. Males and females are distinguishable by the shape of their antennae. The eggs from which no parasitoids emerged were controlled through dissection by a needle under a stereomicroscope to make clear whether they were parasitized or not. In this way, life longevity, oviposition period, the number of SP eggs parasitized during the parasitoids life and the number of the females were determined and recorded. The F1 cultures of the parasitoids obtained under laboratory conditions were used as the control.

Statistical analysis

Different temperatures and different lengths of time data were tested using analysis of variance (two - way ANOVA) and others data were tested using analysis of variance (one - way ANOVA). To reduce variance differences, data concerning the sex ratio (proportion of females) were transformed to arcsin $\sqrt{(x)}$ before analysis (Zar, 1984). Statistical differences were separated by using the Duncan test (P = 0.05). In the figures, non-transformed means and standard errors are presented. The software SPSS 16.0 for Windows (SPSS 16.0, SPSS Inc., Chicago, IL) was used for statistical analysis.

Results and Discussion

Collection and storage of *Trissolcus semistriatus*

The survival rates of *T. semistriatus* stored at different temperatures are given in Figure 1. While the highest survival rate was detected at the end of storage in the first month with 72.9%, the lowest survival rate was determined at the end of storage in the third month with 25.0%. Survival rates of adults stored at 6ºC decreased rapidly from the third month and when calculating the living individuals at the end of the fourth month, it was determined that individuals were not alive.

While the highest survival rate of *T. semistriatus* adults stored at 9ºC was 84.3%, in the first month, the lowest survival rate was 33.6% in the fifth and sixth months. While the highest survival rate of *T. semistriatus* adults stored at 12ºC was 85.0%, in the first month, the lowest survival rate was 46.4% in the fifth and sixth months. While the highest survival rate of *T. semistriatus* adults stored at 15ºC was 89.3%, in the first month, the lowest survival rate was 37.1% in the fifth and sixth months (Figure 1).

When statistically evaluating the results of the survival rates of *T. semistriatus* adults stored at different temperatures and different lengths of time, temperature and time interaction was found to be significant ($F_{15,225} = 14.672$, P= 0.00). At all temperatures, while the highest survival rate was observed in the first month of storage, the lowest survival rate was in the sixth month. In the statistical grouping carried out, *T. semistriatus* adults were determined to be in different groups.

Foerster and Nakama (2002) reported as follows that female longevity and fecundity of *Trissolcus basalis* (Wollaston) and *Telenomus podisi* Ashmead (Hymenoptera: Scelionidae) were evaluated at 25°C after storage periods ranging from 1 to 140 days at 15°C, in intervals of 10 days. Females of both species survived for more than five months when stored at 15°C. That the increased longevity and reduced fecundity of *T. basalis* and *T. podisi* obtained when the adults were stored at 15°C, showed that both species hibernate, and this arrestment can be useful as a tool for mass production and storage of *T. basalis* and *T. podisi* during autumn and winter. Foerster et al. (2004) reported as follow that pupae of *T. basalis* and *T. podisi* were stored at 12 and 15°C for 120 - 210 days after different periods of parasitism at 18°C to evaluate adult emergence, longevity and ovipositional capacity. The rates of emergence of parasitoids transferred to 15°C at the beginning of the pupal stage were 1.5 and 26.3% for *T. basalis* and *T. podisi* respectively; whereas those parasitoids transferred one day before the expected date of emergence at 18°C showed 86.4% of emergence for *T. basalis* and 59.9% for *T. podisi*.

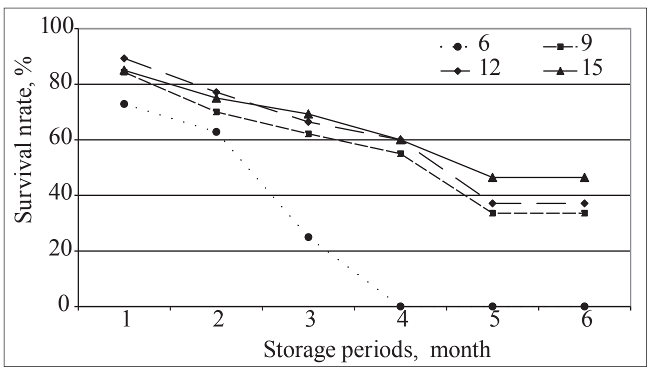

Fig. 1. Survival rates of *Trissolcus semistriatus* adults stored at different temperatures

Some biological Properties of *Trissolcus semistriatus* stored at different temperatures

Age-specific fecundity and survival of females of *T. semistriatus* at different temperatures are given in Figure 2.

Female individuals of *T. semistriatus* stored at 9ºC under laboratory conditions started parasitizing as soon as giving the SP eggs, and parasitizing ended in the 14th day. Dying of female individuals began on the second day. The survival rate continued until the 20th day decreasingly, and it was determined that there were not any living parasitoids. The number of eggs parasitizing daily reached 12.6 on the first day, and decreased continuously until the last day of the oviposition period, ending on 14th day. The parasitoids stored at 12ºC parasitized SP eggs until the 16th day and parasitizing ended on the 17th day. The number of eggs parasitizing daily reached 11.5 on the first day, even though a relative increase occurred, continuous decrease took place until the last day of the oviposition period. Death of the adult individuals began on the second day and the survival rate continued decreasing until the 22nd day. The parasitoids stored at 15ºC began to parasite SP eggs since the first day, and parasitizing ended on the 18th day. The number of eggs daily parasitized reached the highest level on the first day with 11.5 eggs and continued decreasingly until the end of the oviposition period. Parasitizing ended on the 14th day. Death of the adult individuals started on the second day and the survival rate continued decreasing until the 19th day.

F1 progeny of *T. semistriatus* females that were used as control began parasitizing since the first day, ending on the 19th day. Natural death in female adults started on the 7th day, and the viability rate continued decreasing until the 29th day. The highest number of parasitized eggs was determined on the first day with the number of 15.6, then continued to decrease until the end of oviposition period. It decreased to zero on 20th day. In the research carried out in Turkey; Kodan (2007) reported that the rates of *Graphosoma lineatum* L. (Heteroptera: Pentatomidae) eggs, *T.semistriatus* and *T. grandis* Thomson parasitized in the first six days of their lives, were 48.2% and 52.8% respectively, and decreased with age. Tarla (2002) reported that *T. semistriatus* parasitized 52.8% of the eggs at 18°C in the first 7 days of its life, 55.0% of the eggs at 22°C in the first 8 days of its life, 52.7% of the eggs at 30°C in the first 3 days of its life, 60.0% of the eggs at 34°C in the first 2 days of its life, and Memişoğlu (1990) reported that *T. semistriatus* parasite had 12.6 eggs in the first 3 days of its life. Tarla (2002) reported that *T semistriatus* parasitized

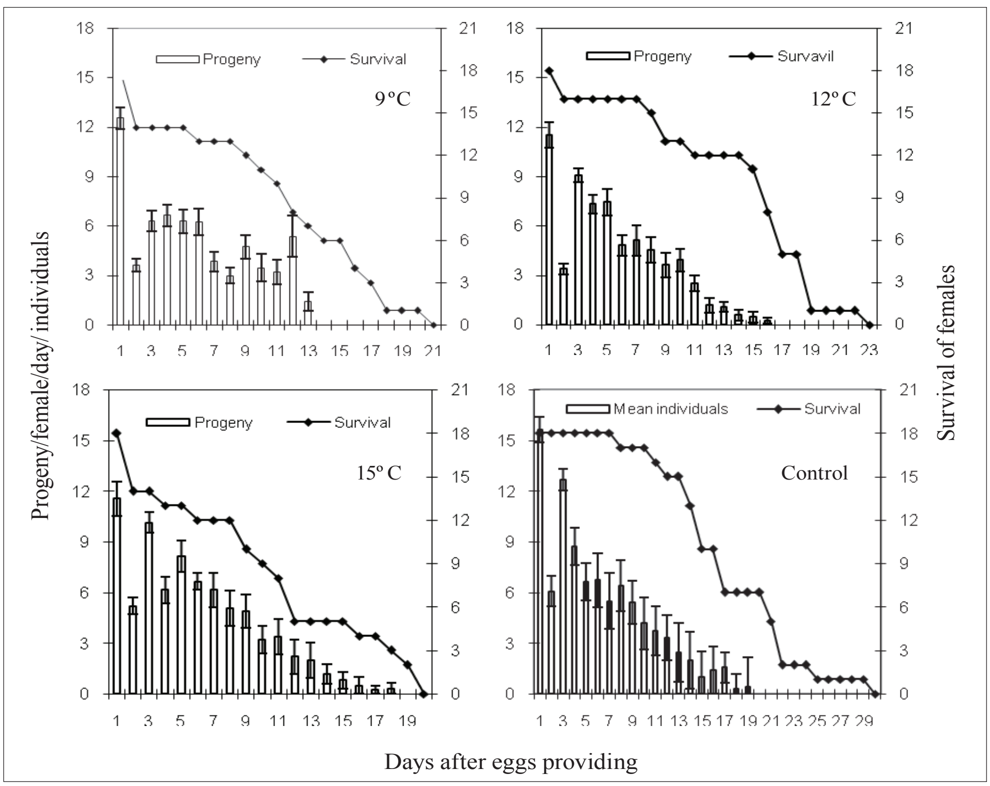

Fig. 2. Age-specific fecundity (mean ± S.E.) and survival females of *Trissolcus semistriatus* at different temperatures

57.0% of the total eggs in the first 6 days of its life in the case of giving a packet of SP eggs every 48 hours at 25°C. In a study carried out by Sabbatini et al. (2012), it was determined that *Gryon pennsylvanicum* (Ashmead) (Hymenoptera: Scelionidae) parasitized the most eggs of *Leptoglossus occidentalis* Heidemann (Heteroptera: Coreidae) on the first day, it decreased in the second day, and although increased fifth day again, after this point it decreased gradually. Also in this study, it was reported that 50.0% of parasitizing occurred in the first 6 days, 90.0% of parasitizing occurred in the first 15 days, and parasitizing ended after the 17th day. Canto - Silva et al. (2006) reported that *G. pennsylvanicum* started parasitizing the host's eggs as soon as it came out from the egg, and other *Gryon* species reached the maximum parasitizing level on the second and third days. Dasilao and Arakawa (2004) reported that *Gryon clavigrallae* Mineo parasitized half of the eggs in the first week of its whole life. The result obtain from our study were similar to these other examples.

The data for mean oviposition period, life longevity, fecundity, and sex ratio of *T. semistriatus* stored at different temperatures on SP eggs are shown in Table 1.

While the longest oviposition period was 12.7 ± 1.00 days for control, the lowest oviposition period was 7.2 ± 1.16 days for parasitoid females stored at 9^{0}C. The life longevities of the individuals stored at 9 and 12 °C were 9.3 ± 1.22 days and 7.7 ± 1.21 days respectively. The differences between oviposition periods of *T. semistriatus* stored at different temperatures were found to be statistically significant ($F_{3,68}$ = 4.216, P: 0.09). In terms of the oviposition period longevity, while the adults stored at 9, 12 and 15°C were in the same group, control was in another group. Tarla (2002) reported that oviposition and post-oviposition period longevities of *T. semistriatus* at 18, 22, 26, 30 and 34°C, 65 ± 5% RH and L16 : D 16 hour photoperiods were 37.9, 24.9, 15.30, 10.7 and 10.1 days and 30.0, 6.5, 2.0, 4.2 and 0.8 days respectively, and male and female life longevities at the same temperatures were 67.9, 31.14, 16.2, 14.9, and 10.9 days and 34.8, 20.5, 11.7, 12.9 and 8.8 days respectively.

It was determined that the average adult longevity of *T. semistriatus* changed depending on the storage temperature. The control individuals had the longest longevity with 16.7 ± 1.28 days, and the adult individuals stored at 15°C had lowest longevity with 9.5 ± 1.51 days. The life longevities of the adults stored at 9 and 12°C were 10.2 ± 1.45 and 13.1 ± 1.39 days respectively. The differences between life longevities of *T. semistriatus* adults stored at different temperatures were found statistically significant ($F_{3,68}$ = 5.283, P: 0.02,). While life longevities of the adults stored at 9 and 15°C spaced in the same group, the adults stored and control located in another group. Memisoglu (1990) reported that at 26°C, 65 ± 5% RH and L18 : D 18 hour photoperiod, life longevity of *T. semistriatus* females was 12.41 day and post-oviposition longevity was, on average, 1 (0 - 4). Kodan (2007) reported that longevities of the female parasitoids of *T. semistriatus* and *T. grandis* on the eggs of *G. lineatum* were 34.8 ± 2.85 days and 38.7 ± 2.34 days respectively under the laboratory conditions.

While lifetime fecundity of *T. semistriatus* stored at 9, 12 and 15°C were 49.8 ± 6.56, 57.7 ± 5.77 and 53.6 ± 6.92 respectively, lifetime fecundity of control was 89.0 ± 5.46. Statistical analysis showed that while lifetime fecundity of *T. semistriatus* stored at different temperatures were at the same group and fecundity of control was in another group ($F_{3,68}$ = 8.361, P: 0.00). Kodan (2007) reported that while *T. semistriatus* parasitized an average of 173.70 ± 10.69 eggs of *G. lineatum, T. grandis* parasitized an average of 151.6 ± 14.66 eggs of *G. lineatum* during their whole lives under the laboratory conditions. Memisoglu (1990) reported that *T. semistriatus* parasitized an average of 85.4 ± 6.59 eggs of *E. maura* L., and Kıvan (1998) reported in his study carried about the biology of *T. semistriatus* in 1996 and 1997 that wasp parasitized an average of 101.5 and 57.4 eggs. Foerster and Nakama (2002) reported as follows that female fecundity of *T. basalis* and *T. podisi* were evaluated at 25°C after storage period ranging from one to 140 days at 15°C, in intervals of 10 days; fecundity was significantly reduced by exposure to the low temperature. Despite the reduction in fecundity, both species were able to parasitize host eggs at 25°C after spending 140 days at 15°C. Although parasitism was observed at 15°C, only 3.1% and 0.2% of the exposed eggs were parasitized by *T. basalis* and *T. podisi* respectively. Foerster et al. (2004) reported that

Table 1
Statistics results for the mean oviposition period, life longevity, fecundity, and sex ratio of *Trissolcus semistriatus* stored at different temperatures on Sunn pest eggs

Temperatures, °C	Oviposition period, day	Life longevity, day	Fecundity	Sex ratio, % [a]
9 °C	7.2±1.16 **b**	10.2±1.45 **b**	49.8±6.56 **b**	63.9±1.80 **b**
12 °C	9.3±1.22 **b**	13.1±1.39 **ab**	57.7±5.77 **b**	65.3±1.19 **b**
15 °C	7.7±1.21 **b**	9.5±1.51 **b**	53.6±6.92 **b**	62.4±0.87 **b**
Control	12.7±1.00 **a**	16.7±1.28 **a**	89.0±5.46 **a**	73.0±0.90 **a**

[a] Data transformed using arcsin $\sqrt{(x)}$ before statistical analysis.

females emerged after storage and maintained for 120 - 210 days at 15°C parasitized host eggs after transference to 25°C; however, fecundity of *T. podisi* was decreased by 80% after cold storage.

The highest sex ratio was 73.0 ± 0.90 for control and the lowest sex ratio was 62.4 ± 0.87 for females stored at 15°C. The differences were found to be statistically significant between the sex ratio of *T. semistriatus* stored at different temperatures ($F_{3,68}$ = 14.036, P: 0.00). At grouping, while the numbers of female individuals given by *T. semistriatus* stored at different temperatures were at the same group and control individuals were in another group. Kodan (2007) reported that the female rate of the *E. maura* parasite by *T. semistriatus* through the whole life was 80.4%, and it was 82.8% for *T. grandis*. Nakama and Foerster (2001) reported as follows that how the egg parasitoids *T. basalis* and *T. podisi* survive during winter in southern Brazil, at mean temperatures 12 - 15°C. The sex ratio was not significantly affected by storage at 15°C for 30 days. However, only *T. podisi* females were able to emerge after remaining at 15°C beyond 30 days in the egg stage or 35 days in the larval and pupal stages. Foerster and Nakama (2002) reported as follows that fecundity of *T. basalis* and *T. podisi* were evaluated at 25°C after storage periods ranging from one to 140 days at 15°C, in intervals of 10 days. The proportion of males was significantly increased in the progeny of *T. basalis* females, maintained at 15°C, for any of the storage periods investigated. Similar results were obtained for *T. podisi* stored at 15°C up to 50 days; however the females of this species, maintained at the lower temperature for longer periods, either laid no eggs or produced progenies with sex ratio similar to the control insects, kept at 25°C.

Relationship between the lifetime fecundity and longevity of *T. semistriatus* stored at different temperatures is given in Figure 3.

In the results of statistical analysis, a linear relationship was found between lifetime fecundity and female longevity of stored parasitoids and control. Tarla and Kornoşor (2009) reported that the differences in the reproductive biology of overwintered and F1 generation of *T. semistriatus* and *T. festivae* were found to be statistically significant under laboratory conditions. Sabbatini et al. (2012) reported that the highest mean parasitization of *G. pennsylvanicum* on *L. occidentalis*

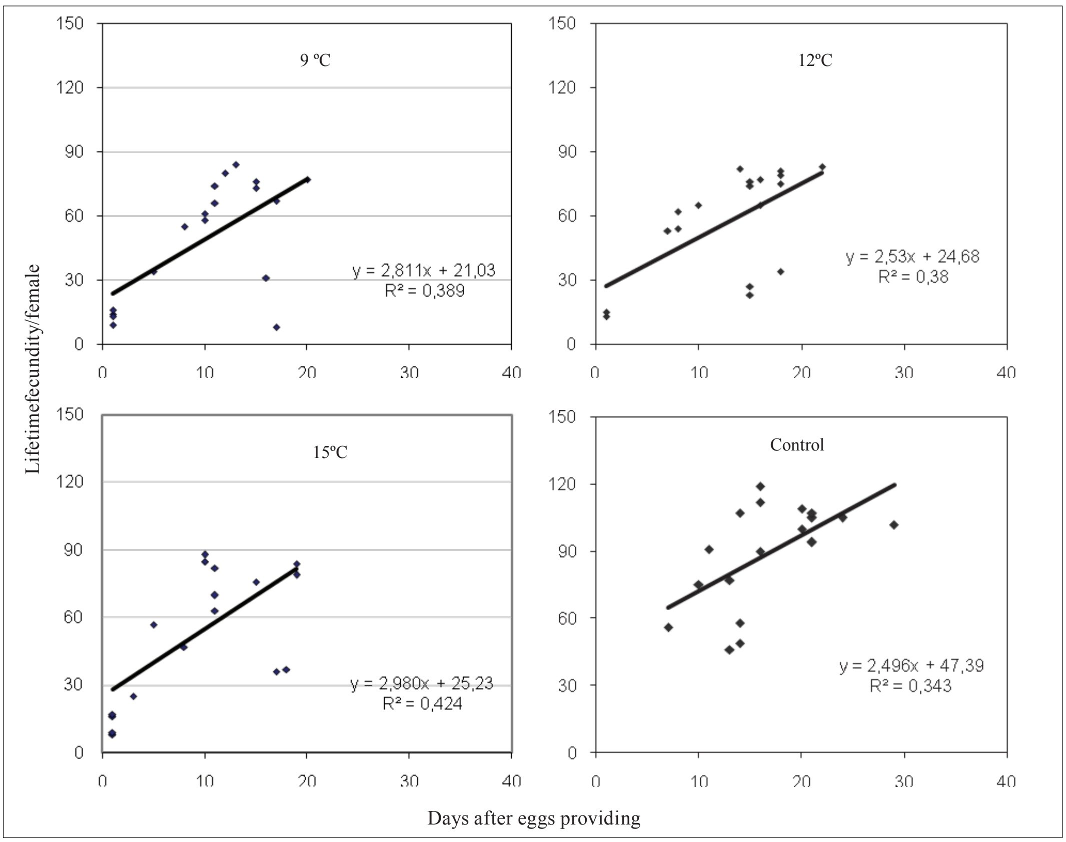

Fig. 3. Relationship between the lifetime fecundity and longevity of *Trissolcus semistriatus* stored at different temperatures

eggs was recorded; the regression analysis on survival of the fed specimens evidenced a liner trend for both females and males.

Conclusions

These results, adult individuals of *T. semistriatus* can store at various temperature and time periods for 6 months in laboratory conditions. Therefore, there is no need the continuing of *T. semistriatus* culture in laboratory conditions during the summer. Although longevity and fecundity of stored parasitoid adults decrease at low temperature, they can use for starting mass- culture in laboratory as agent of biological control of SP. Regarding reproductive biology of *T. semistriatus* adults stored in different temperatures, will be useful to mass culture and supplementary release the parasitoid for the biological control of the SP for future research.

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