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Development of *Trissolcus semistriatus* (Nees) (Hymenoptera: Scelionidae) in Cold-Stored Host

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Abstract— *Trissolcus (Hymenoptera: Scelionidae) species are important egg parasitoids of many pentatomid species. In the present study, investigated whether Trissolcus semistriatus (Nees) (Hymenoptera: Scelionidae) can be mass-reared in the eggs of Graphosoma lineatum L. (Hemiptera: Pentatomidae) stored at -80 °C for two years. To improve the mass production of T. semistriatus, the biological parameters of the parasitoid—such as parasitization rate, adult emergence rate, development time, and sex ratio and adult viabiliy —were determined after storing one-day-old eggs of G. lineatum at -80°C for two years. To ensure continuity of mass rearing, adult female parasitoids that emerged from two-year-old stored eggs were given two-year-old stored host eggs again, and the same biological parameters were examined. Unstored G. lineatum eggs were used as controls. The parasitism rate was greater than 92% in all experiments and the emergence rate of the progeny was greater than 95%. There was no difference in parasitism of T. semistriatus between stored and non-stored G. lineatum eggs, but there was a difference in the emergence of progeny. The sex ratio was generally more in favour of females. The development time of male and female parasitoids was influenced by the storage of host eggs. In the current study, it was found that the egg parasitoid T. semistriatus could be mass reared for 2 years in eggs of G. lineatum stored at -80 °C.*

Keywords— *Biological control, Mass-rearing techniques, Pentatomidae, Storage, Trissolcus spp.*

I. INTRODUCTION

Egg parasitoids are an important group in studies on biological control. *Trissolcus* (Hymenoptera: Scelionidae) species form an important group of egg parasitoids, generally prefer eggs of Pentatomidae as hosts [1, 2, 3]. Among the pentatomids it prefers are important pest species such as *Halyomorpha halys* (Stål), *Nezara viridula* (L.), *Piezodorus guildinii* (Westwood), *Euschistus heros* (F.), *Dolycoris baccarum* L., *Aelia rostrata* Boh., *Podisus nigrispinus* (Dallas), *Eurydema ornata* (L.), *Holcostethus vernalis* (Wolff) (Hemiptera: Pentatomidae) etc. The mass production of *Trissolcus* spp. is limited by the absence of pentatomids year-round. Therefore, it is necessary to develop alternative mass rearing methods that hosts are not vailable during these periods. One of these methods is to expose the host eggs to cold. For this purpose, different methods of cold storage are generally used to maintain the viability of the host eggs. In developing biological control programs, the storage of parasitoids and their hosts is often used to maximize the number of parasitoids available [4]. The temperature range used can range from freezing to simple refrigeration [5]. Host eggs can be stored in cold storage for short or long periods of time. Mass rearing of parasitoids in host eggs stored for a certain period of time ensures the continuity of production of these parasitoids and their easy use when released in the field. Cold tolerance is a trait that is influenced by a number of biotic and abiotic factors depending on cold exposure. These factors influence the morphology, behaviour and physiology of natural enemies as well as parameters such as development, longevity, fecundity, parasitism and sex ratio [6, 7, 8, 9].

Releasing egg parasitoids during the period when they are most effective against their hosts significantly impacts the success of biological control [10, 11]. To ensure this period is not missed, it is essential to have an adequate supply of parasitoids available for release. The availability of abundant hosts for the production of egg parasitoids will facilitate parasitoid production. The ability to store host eggs in cold conditions without losing their properties will positively affect the production of egg parasitoids and enable their simultaneous release into fields during critical stages of pest outbreaks. Production of parasitoids is important for their use in release studies. One of the most important issues in the mass rearing of parasitoids in the laboratory is the easy availability of their hosts. Parasitoids can be produced by collecting adult hosts in the wild and then obtaining their eggs in the laboratory. However, the use of different methods, such as cultivation in the laboratory, storage, etc., makes it easier to obtain the desired number of hosts at the desired time. Pentatomidae species can be bred in the laboratory, but there may be problems with continuity of production after a few generations [12]. *Trissolcus* species have been produced using a variety of hosts and methods. Cold storage of host eggs is one of these methods [13, 14, 15, 16, 17, 18]. Several studies have been conducted on the storage of eggs collected in the wild and their use for parasitoid production when the host species of *Trissolcus* species are abundant. According to these studies, the biological data of the parasitoid are influenced by differences in egg storage, storage temperature and storage techniques [19, 20, 21, 22, 23]. In the laboratory mass rearing of *Trissolcus* species, eggs of various Pentatomid species were stored at low temperatures, and it was determined that *Graphosoma lineatum* (Hemiptera: Pentatomidae) eggs were the most suitable host [16, 18]. According to one study, the eggs of *G. lineatum* can be stored in liquid nitrogen (-196 °C) for five years for use in *Trissolcus* production [13].

In the present study, we determined biological data such as the parasitism rate of *T. semistriatus*, emergence rate, development time, sex ratio and adult life span on eggs of *G. lineatum* kept at -80°C for two years. To investigate whether

the continuity of mass rearing of the parasitoid can be achieved in stored eggs, the adults hatched from eggs stored at -80°C for two years were resupplied with *G. lineatum* eggs stored at -80°C for two years, and the biological data such as parasitism rate, emergence rate, development time and sex ratio were examined. In addition, unstored *G. lineatum* eggs were administered to the parasitoids that emerged from the last experiment to determine whether the biological data of the parasitoids in these eggs had changed.

II. Materials and Metods

2.1 Rearing of Graphosoma lineatum Adults in the Laboratory

The colony of *G. lineatum* was formed from individuals collected from plants of *Heracleum platytaenium* Boiss. and *Myrtus communis* L. and *Conium maculatum* L. (Apiales Apiaceae) in natural areas in Ankara, Türkiye. Coordinates (latitude and longitude) of the area where the insects were collected: 40°11'N 31°54'E, and the altitude is 675m. The adults were placed in 20x30 cm plastic cages and fed *H. platyphylla* seeds. To meet the water requirements of the adults, small tubes filled with water and sealed with cotton were placed at the bottom of the container. The adults were kept in climate chambers under standard conditions of 26 ± 1 °C, 65 ± 5% relative humidity, and a 16:8 L:D. The seeds used for food were replaced every three days.

2.2 Stock Culture of Trissolcus semistriatus

The egg parasitoid *T. semistriatus* used in the study was obtained from the Directorate of Plant Protection Central Research Institute. *T. semistriatus* was mass-reared in a glass tube (2x18 cm) from *G. lineatum* eggs in the laboratory. One-day-old *G. lineatum* eggs, as well as male and female *T. semistriatus* individuals, were placed in the tube. The parasitoids were fed thin strips of honey. The tube was placed in the climate chamber under the following conditions (26 ± 1 °C, 65 ± 5% relative humidity, and a 16:8 light-dark cycle).

2.3 Storage of Graphosoma lineatum eggs at -80°C

The freshly laid eggs (0-24 h after oviposition) of *G. lineatum* were collected with daily controls. The eggs were wrapped in aluminum foil and placed in an Eppendorf tube [19]. The tube was then stored in a freezer at -80°C for two years.

2.4 Evaluation of the Biology of T. semistriatus in G. lineatum Eggs Stored at -80°C for Two Years (1st Experiment)

The *G. lineatum* eggs, which had been stored for two years in an aluminum foil-wrapped Eppendorf tube at -80°C, were removed from the freezer and thawed by immediate immersion in 30°C water for five seconds [19]. The eggs were taken out of the aluminum foil and placed on blotting paper to dry and subjected to parasitism by *T. semistriatus.* One *G. lineatum* egg cluster (average 14 eggs) was placed in each tube (2x18 cm). One-day-old adult males and females of *T. semistriatus* were placed in the same tube and the eggs subjected to parasitism by the parazitoid for two days. The parasitoids were fed with thin honey strips. The tubes kept in climate chamber, under controlled conditions of temperature (26±1°C), relative humidity (65±5%) and photophase (16:8), until emergence the parasitoids. The parasited eggs were later counted and the number of male and females emerged from each egg mass recorded in daily controls. Upon emergence parasitoids were identified to sex and counted. The experiment was carried out with 20 replicates. Comparison with parasitism in *G. lineatum* fresh eggs was used as a control.

In this study determined the parasitism rate (The rate of the total number of eggs to those that have turned black and those with parasitoid development), the parasitoid emergence rate (All the adults which emerged, plus those which had some perforation in the parasited egg chorion), the sex ratio (females /males + females) and the development time (the period from the day the parasitoid parasitises the egg to the day the adult parasitoid hatches). To verify the results, all eggs without adult emergence were dissected.

Additionally, to assess the viability of individuals emerging from stored eggs, male and female parasitoids emerging on the same day from this experiment were placed in a tube and fed honey. The viability of adults was determined through daily checks. The experiment was conducted with 57 male and 197 female individuals.

2.5 The Biology of T. Semistriatus Obtained From The First Experiment was Analysed in G. Lineatum Eggs Stored at -80°C For Two Years (2nd Experiment)

For this experiment, *G. lineat* eggs stored in a deep freezer at -80°C for two years were placed in a 2x18 cm tubes after the method described in the first experiment were applied. One egg clutch (14 eggs on average) was placed in each tube. One-day-old one female and one male adult of *T. semistriatus* obtained from the first experiment (F1) were placed in the same tube. The parasitoids were fed with thin honey strips. The parasitoids were removed from the tube after two days. The parasitism rate, emergence rate, sex ratio and development times of the parasitoids were determined by daily checks. The experiment was carried out under controlled conditions of temperature (26 ± 1 °C), relative humidity (65 ± 5%), and photoperiod (16-8 L:D) until the parasitoids emerged. The experiment was carried out with 20 replicates.

2.6 The Biology of T. semistriatus Obtained From the second experiment was Evaluated in one-day-old Eggs of G. lineatum (3rd Experiment)

In this experiment, the performance of parasitoids obtained from the second experiment was evaluated in One-day-old eggs of *G. lineatum*. For this purpose, one-day-old one females and one male individual from the second experiment were transferred to a tube and provided with one-day-old 2-3 *G. lineatum* eggs clusters (28-36 unstored *G. lineatum* eggs). The parasitoids were fed with thin honey strips. The parasitoids were removed from the tube after two days. The parasitism rate, emergence rate, sex ratio and development time of the parasitoids were determined with daily controls. The experiment was carried out under controlled conditions of temperature (26 ± 1 °C), relative humidity (65 ± 5%), and

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photoperiod (16-8 L:D) until the parasitoids emerged. The experiment was carried out with 20 replicates.

2.7 Statistical Analysis

The experiment layout of the experiments was a factorial completely randomized design, with twenty replications. If variance analysis revealed differences between the characters, the Tukey test was used to rank these differences according to their significance. The Pearson chi-square test was applied in the examination of the distribution of sexes in the groups. Duncan's test was used as a multiple comparison test. Statistical analyses were conducted using SPSS 25.0 software. The statistical significance level was determined as 0.05.

III. RESULTS

3.1 The Parasitism rate

The parasitism rate of *T. semistriatus* in *G. lineatum* eggs stored at -80°C for two years (first experiment) was 92.23%, while this rate was 93.92% on non-stored eggs (the control experiment) (Table 3.1). The parasitism rate of host eggs did not differ between eggs that had been stored for two years and eggs that had not been stored (df=3; F=0.01; p=0.001). The parasitism rate of *T. semistriatus* females obtained from the first experiment, in *G. lineatum* eggs stored at -80°C for two years (second experiment), and non-stored *G. lineatum* eggs (third experiment) was 93.57% and 92.97%, respectively.

T. semistriatus females obtained from the first experiment in *G. lineatum* eggs stored at -80°C for two years (second experiment) and in non-stored *G. lineatum* eggs (third experiment), the parasitism rate of the eggs was 93.57% and 92.97%, respectively. Both were statistically in the same group and did not differ from the control group (Table 3.1). The lowest value was recorded for the first experiment. The results showed that storing host eggs did not affect the parasitism of *T. semistriatus*. Parasitoids emerging from stored eggs parasitized over 92% of both stored (second experiment) and unstored (third experiment) eggs.

TABLE 3.1 Parasitism and emergence rate of *Trissolcus semistriatus* in *Graphosoma lineatum* eggs stored in deep freezer (-80 °C) and non-stored

Experiment *	Parasitism rate (%) (Mean± Std. Error)	Emergence rate (%) (Mean± Std. Error)
1	92.23±0.02	95.63±0.03b**
2	93.57±0.04	100.00±0.00a
3	92.97±0.02	94.64±0.02b
Control	93.92±0.05	90.00±0.05 ab

* Experiment (1): *Trissolcus semistriatus* were given *Graphosoma lineatum* eggs stored at -80 C for two years
Experiment (2): *Trissolcus semistriatus* obtained from first experiment were given *Graphosoma lineatum* eggs stored at -80 °C for two years.
Experiment (3): *Trissolcus semistriatus* obtained from second experiment were given *Graphosoma lineatum* non-stored eggs (one day)
Control: *Trissolcus semistriatus* were given *Graphosoma lineatum* eggs non-stored eggs (one day)
**Numbers in each column followed by a different letter are significantly different (p< 0.05) for contingency table

3.2 The emergence rate

The emergence rate of *T. semistriatus* from *G. lineatum* eggs stored at -80°C for two years (first experiment) was found to be 95.63%, while it was 90.00% in the non-stored eggs (control) (Table 3.1). The difference between the stored eggs and the control group was significant (df=3; F=2.11; p=0.001). The emergence rate from the host eggs of second and third experiment were 100% and 94.64%, respectively, and there was a difference between them and the control group (Table 3.1). The emergence rate of adults from stored and unstored eggs was over 90%, indicating that long-term storage did not affect the emergence of parasitoids.

3.3 The sex ratio

The sex ratio of *T. semistriatus* on *G. lineatum eggs* stored at -80°C for two years (first experiment) was 0.77 and the number of females was higher and there was a difference between them compared to the control (0.88) (df=3; F=10.77; p=0.001) (Table 3.2). The sex ratio was 0.87 for the second experiment and 0.59 for the third experiment. There was a difference between them and the control group (0.88) (Table 2). In this study, the second experiment was in favour of the females, and they were in the same group as the control group with as statistically no difference between them (Table 3.2).

3.4 Development time (days)

The mean development time of the females in the control was determined to be 10.57±0.03 days. The mean development time of 1st and 2nd generation females developing in eggs stored for two years was found to be 11.83±0.09 and 11.66±0.05 days, respectively, 1 day longer than in the control. On the other hand, the development time of female individuals in fresh host eggs given to 2nd generation individuals (3rd experiment) emerged from stored eggs was found to be 8.75±0.05 days, even shorter than the control (Table 3.3). In experiment 1, 2, 3 and the control, the development time of the males was shorter than that of the females, and the shortest development time of the males was achieved in experiment 3 (Table 3.3).

TABLE 3.2 Sex ratio of *Trissolcus semistriatus* in *Graphosoma lineatum* eggs stored in deep freezer (-80 °C) and non-stored

Experiment *	Female Number	Male Number	*P value*****	Sex Ratio** (Mean±Standard Error)
1	197	57	*<0.001*	0.77±0.62b***
2	229	34		0.87±0.21c
3	312	212		0.59±0.24a
Control	220	29		0.88±0.08c

* Experiment (1): *Trissolcus semistriatus* were given *Graphosoma lineatum* eggs stored at -80 C for two years
Experiment (2): *Trissolcus semistriatus* obtained from first experiment were given *Graphosoma lineatum* eggs stored at -80 °C for two years
Experiment (3): *Trissolcus semistriatus* obtained from second experiment were given *Graphosoma lineatum* non-stored eggs (one day)
Control: *Trissolcus semistriatus* were given *Graphosoma lineatum* eggs non-stored eggs (one day)
** Sex Ratio=Females /Males + Females
*** Numbers in each column followed by a different letter are significantly different (p< 0.05) for contingency table
**** Pearson Chi-Square,

The experiments revealed that there was an interaction between male and female individuals in terms of developmental time (df=3; F=5.484; p=0.001). In all experiments, there was a difference between male and female individuals in terms of development time compared to the

control. In both sexes, the shortest development time was achieved in the third experiment and the longest in the first experiment. While the difference between the development times of the female and male individuals in the experiments was about 1.5 days, this period was found to be 2 days in the control (Table 3.3).

TABLE 3.3 *Trissolcus semistriatus* female and male development time (days) in *Graphosoma lineatum* eggs stored and not stored in deep freezer (-80 oC)

Experiments*	N	Female Mean±Std. Error (Min-Max)	N	Male Mean±Std. Error (Min-Max)
1	197	11.83±0.09 a** (10.00-19.00) A	57	10.52±0.35 a (8.00-19.00) B
2	229	11.66±0.05 b (10.00-14.00) A	34	9.59±0.25 b (8.00-14.00) B
3	312	8.75±0.05 d (7.00-11.00) A	212	7.29±0.03 d (7.00-10.00) B
Control	220	10.57±0.03 c (10.00-12.00) A	29	8.55±0.09 c (8.00-9.00) B

* Experiment (1): *Trissolcus semistriatus* were given *Graphosoma lineatum* eggs stored at -80 C for two years
Experiment (2): *Trissolcus semistriatus* obtained from first experiment were given *Graphosoma lineatum* eggs stored at -80 ºC for two years.
Experiment (3): *Trissolcus semistriatus* obtained from second experiment were given *Graphosoma lineatum* non-stored eggs (one day)
Control: *Trissolcus semistriatus* were given *Graphosoma lineatum* eggs non-stored eggs (one day)
** Numbers in each column followed by a different letter are significantly different (p< 0.05) for contingency table

It was found that there was a difference between the development times of the female individuals depending on the storage times of the eggs and the female individuals in each experiment were in statistically different groups. The same situation was also observed in the male parasitoids (Table 3.3).

In this study conducted to evaluate the survival times of adult parasitoids emerged from eggs stored for two years (obtained from the first treatment), it was determined that females lived an average of 22.49±0.48 (7-32) days and males had a shorter survival time than females, 17.25±0.80 (3-31) days.

IV. Discussion

In experiments 1, 2, and 3, parasitism rate of the egg parasitoid *T. semistriatus* on host *G. lineatum* eggs stored at -80°C for two years was not different from the control group and was above 92%. Therefore, it was concluded that storing *G. lineatum* eggs at -80°C for two years did not affect the parasitism rate of *T. semistriatus*. When females emerged from stored eggs were given eggs stored for another two years (second experiment) and unstored eggs (third experiment), no difference in parasitism was observed (Table 3.1). Based on the results of the study, it was concluded that storing host eggs for a long period of two years does not affect the parasitism rate of the parasitoid. This means that individuals emerged from stored eggs can parasitize both stored and non-stored eggs at the same rate. These results suggest that eggs stored for extended periods can be used in *T. semistriatus* mass rearing studies. Our results are consistent with the 95.4% parasitism rate reported by Correia-Ferreira and Oliveira (1998) for *N. viridula* (Hemiptera: Pentatomidae) eggs wrapped in aluminum foil and stored at -196°C for one year using their method. Another study using the same approach found that the parasitism rate of *G. lineatum* eggs stored at -80°C for one to twelve months ranged from 30.4% to 96.9% [18]. Additionally, Gennadiev and Khlistovskii [13] found that the parasitism rate of *G. lineatum* eggs stored at -196°C for five years exceeded 50%. McIntosh et al. (2019) [22] found that the parasitism rate of *Trissolcus japonicus* (Ashmead) (Hymenoptera: Scelionidae) in 0-3 day old eggs of *Halyomorpha halys* (Stal, 1855) (Hemiptera: Pentatomidae) stored at -80°C for 77-1352 days is 27%, decreasing by 1.2% per month. In the same study, researchers reported that the parasitism rate in eggs stored (older than one day) for 3 to 490 days was 53% and decreased by 2.6% each month compared to the storage period. These findings, which contradict our results, suggest that the discrepancy may be due to differences in our storage techniques and the ages of the stored host eggs. Many researchers have studied the storage of Pentatomidae and Scutelleridae eggs at different temperatures. Among these researchers, Popov [24] reported that the parasitism rate of *Dolycoris baccarum* L. (Hemiptera: Pentatomidae) eggs were between 94 and 54% after 6 months of storage at -20°C; İslamoğlu and Kornoşor [25] reported that *Trissolcus festivae* Victorov (Hymenoptera: Scelionidae) parasitised 80-86% of sunn pest eggs after 2-4 months of storage at -21°C; Kodan and Gürkan [17] reported an average parasitism rate of over 50% by *Trissolcus grandis* Thomson (Hymenoptera: Scelionidae) when *D. baccarum* eggs were stored at -18 °C for between 5 and 245 days; Corrêa-Ferreira and Moscardi [15] reported that the storage of eggs of *Nezara viridula* L. (Hemiptera Pentatomidae) at -15 °C for 0-360 days caused parasitisation of *Trissolcus basalis* Wollaston (Hymenoptera: Scelionidae) in an average ratio of 63.75%; Kıvan and Kılıç [16]reported that storage of eggs of *G. lineatum eggs* at -20°C for 1-5 months caused parasitisation of *T. semistriatus* at a ratio of 88.4-49.5%; Mahmoud and Lim [20] reported that when *D. baccarum* eggs were stored at -18°C for 8 days, the parasitism rate by *Trissolcus nigripedius* Nakagawa decreased to 44%. Most of these studies emphasise that the parasitism rate of eggs decreases with increasing storage time. These studies appear to differ from our results. However, it should be noted that these experiments were generally carried out at temperatures between -15 and -20°C, and results may vary depending on temperature, host type, egg storage method, and parasitoid species.

In the study, the emergence rates of parasitoids recorded values above 90% in all applications. Although there was a statistical difference in the emergence rates of parasitoids compared to the control group, the difference was not too great. The best emergence rate was obtained in the second trial. This result indicates that storage had no influence on the emergence rate. Corrêa-Ferreira and Oliveria [19] stored *N. viridula* eggs in liquid nitrogen (-196 °C) using different methods and obtained an emergence rate of 97.4% with eggs wrapped in aluminium foil (the method we used). Emergence rates of *T. basalis* from three different Pentatomidae eggs stored in liquid nitrogen for six months have been reported to be between 61.8and 87 % [21]. Mahmoud and Lim [20] found no difference in emergence rates of parasitoids from non-

stored and stored eggs, which is consistent with our results. Emergence rates of *T. semistriatus* ranged from 98.2 % to 100 % after *G. lineatum* eggs were stored at -20°C for 1-5 months [16]. Mcintosh et al. [22] found a low emergence rate of the parasitoid in frozen eggs and could not explain whether this was due to the number of eggs used for the experiment or the parasitoid. Wong et al. [23] reported that the emergence rate of *T. japonicus* from *H. halys* eggs was 58.80% and 58.24% in non-stored and refrigerated eggs kept in a petri dish at 8 °C for 14 days, respectively. It is hypothesised that the difference between the researchers' results and our results is due to the fact that the methods and materials used are not the same.

Although there was a difference in the sex ratio of the parasitoids in the first and third experiments compared to the control, the sex ratio was in favour of the females in all experiments. The proportion of females was lower in the third experiment compared to the other experiments, but emergence was still in favour of females. In the host eggs that were stored for two years, the number of females was higher in the sex ratio, indicating that storage did not affect the sex ratio. The studies emphasise that storage does not affect the sex ratio and the proportion of females is high [20, 21, 22, 23]. In our study, the number of males and females in the third experiment was close to each other because the number of eggs given to the same number of parasitoids in this experiment was higher than in the other experiments. Referring to Wilson (1961), Colazza and Wajnberg [26], in their study on the relationship between egg clutch size and sex, found that females of *T. basalis* normally mate only once. The researchers reported that *T. basalis* females initially lay their eggs with male eggs and then, after laying a certain number of female eggs, start laying an increasing number of male eggs at a certain time. It is hypothesised that the conditions mentioned in these studies are the cause of the increase in the number of males in our third experiment.

In the present study, male parasitoids completed their developmental period faster than females, emerging 1-2 days earlier. As studies with *Trissolcus* species have shown, the males emerge before the females and wait on the egg pack to mate with them [27, 28, 29, 30, 31, 32, 33]. In our study, the development time of the parasitoids was longer with the first and second experiments using stored eggs than with the control group using non-stored eggs. This period is not very long and has been determined to be 1-2 days. According to various studies, the development times of *Trissolcus* species are longer in host eggs that are stored at different temperatures than in those that are not stored [15, 20, 18, 22, 23]. In the study, the development time of the parasitoids in the non-stored eggs (one day old) parasitised by the parasitoids from the third experiment was even shorter than in the control; the reason for this could not be explained.

In this study it was found that female parasitoids emerging from eggs stored for two years live longer than male parasitoids. A difference of 5 days was found between the viability of females and males. Mahmoud and Lim [20] reported that storage had no effect on the longevity of adults. In the studies conducted, the viability of females of T. semistriatus reared on non-stored eggs of Eurygaster maura L. (Hemiptera: Scutelleridae) was 34.80 days; this value was 38.70 days for T. grandis and the viability of male parasitoids was found to be 10.40 days and 10.80 days, respectively [34, 35]. Similarly, Tarla [36], T. semistriatus females survive 16.2 days and males 11.7 days in Eurygaster integriceps Puton (Hemiptera: Scutelleridae) eggs at 26 °C. Considering that Trissolcus species show the highest level of parasitism in the first ten days of their life (Kodan 2007), it is assumed that the life span of the individuals hatched from the stored eggs will be sufficient for parasitism according to the test results. However, it is not known how long individuals hatching from stored eggs will survive in the field.

The easy mass rearing of parasitoids will increase the application possibilities of biological control. Having sufficient hosts is also very important for mass rearing parasitoids. This study was conducted to produce *T. semistriatus* in eggs of *G. lineatum* stored at -80 °C for 2 years. As a result of the study, it was demonstrated that the parasitoid can develop in the host eggs stored for two years. In the study, the biological data of the parasitoids in the stored host eggs were similar to those in the non-stored eggs. It has been shown that the stored host eggs can be successfully utilised when the parasitoid is needed. The results of our study suggest that they will shed light on the production of parasitoids belonging to the *Trissolcus* genus in the laboratory and facilitate the production of parasitoids used in biological control of pests. The performance of these produced parasitoids needs to be evaluated by release studies.

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