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Effect of gradually decreased temperature on adult longevity and fecundity of *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)

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ABSTRACT

In this study, effect of gradually decreased temperature on larval stage of endoparasitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) reared on pupae of *Galleria mellonella* L. (Lepidoptera: Pyralidae) in laboratory conditions were investigated. Parasitized host pupae were acclimated gradually decreasing temperature; for 3 days at 25, 20, 15 and 10°C, then 2, 3, 4 and 5 days at 4°C, respectively. Duration of adult emergence after parasitization, adult longevity and fecundity were determined after each acclimation. With the increasing of duration time at 4°C, we found these results; adult longevity and fecundity decreased.

Keywords: Adult longevity, fecundity, gradually decreased temperature, Hymenoptera, *Pimpla turionellae*.

Tedrici Azalan Sıcaklığın *Pimpla turionellae* (Hymenoptera: Ichneumonidae)' nın Ömür Uzunluğuna ve Yumurta Verimine Etkileri

ÖZET

Bu çalışmada, laboratuvar şartlarında *Galleria mellonella* L. (Lepidoptera: Pyralidae) puplarında yetiştirilen endoparazitoid *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae)' nın larval evresine, konak içinde uygulanan tedrici azalan sıcaklığın etkileri araştırılmıştır. Parazitlenen konak pupları sırasıyla 25, 20, 15 ve 10°C' lerde 3' er gün, 4°C' de 2, 3, 4 ve 5' er gün bekletilmiştir. Uygulamalar sonucunda çıkan bireylerin ergin ömür uzunlukları ve dişilerin yumurta verimi araştırılmıştır. Elde edilen sonuçlara göre, 4°C' de bekletme süresinin artması ile ömür uzunluğu ve yumurta verimi azalmıştır.

Anahtar Kelimeler**:** Ergin ömür uzunluğu, yumurta verimi, tedrici azalan sıcaklık, Hymenoptera, *Pimpla turionellae*.

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1. INTRODUCTION

Pimpla turionellae L. is a solitary endoparasitoid hymenopter species. The adult parasitoid female lay her eggs in the host after paralyzing it and the larvae hatching from this egg feed on the host material. The parasitoids completing their larval development leave the host as adults after completing the prepupa and pupa stages which are the sequential development stages [1].

Endoparasitoid species are used as biological control agents within the biological control studies which recently became very important [2,3].

In order for using a species of a parasitoid within biological control studies, species should be reared in masses in laboratory environment [4,5]. For gathering the mass culture of the parasitoids in laboratory environment to be used, the method of keeping the convenient stage of the host under low temperature is applied and thus it is ensured that the host and the parasitoid to be reared concurrently. For the convenience of this method, researches should be performed over significant issues such as; to what extent do these insects kept in low temperature got affected by this method, how their physiological and biochemical adaptation take place, how these adaptations affect the development and the fertility and their effects on future generations.

Because of their resistance to low temperature, it is possible to benefit from low temperature as a method of keeping the insects [6-9]. But since various physiological variations may occur within these insects depending upon the low temperature and this affects the development and the fecundity of the insects [10] (de Kort 1990), the optimum keeping period should be determined [11,12].
In consequence of previous studies where the parasitoid species were exposed to low temperature, it was determined that the ratio of mature, sexual ratio, adult longevity and fecundity got affected significantly [2,11, 13-15]. It is projected that several negative effects emerged on the insects after low temperature application may be relieved through gradually decreasing temperature application. Similar to the nature, applying the temperature decrease gradually in the laboratory environment is important for the adaptation of the insect to the low temperature. The effects of gradually decreasing temperature over the fecundity of *P. turionellae* in pupa were researched and it was determined that the extension of storage time in low temperature (4ºC) affects positively, that the adult hatching percentage increased and the fecundity is higher [2]. The positive effect of gradually decreasing temperature was also revealed within the Koveos's study [16] over *Bactocera olea* (Diptera) adults. The 5% of the adult individuals maintain their longevity when taken from 24ºC to -6,5ºC for 2 hours, on the other hand the survival ratio of the ones stored between 0 and 10ºC for 2 hours before being taken to -6,5ºC increased up to 80% and 92%.

Keeping *P. turionellae* in low temperatures for the purpose of using in biological control studies is possible merely by completely understanding the resistance of this species to low temperature. Therefore determining the effects of gradually decreasing temperature over the development of the parasitoid is important. In this study, the effects of gradually decreasing temperature applied in the host during the larval phase over the *P. turionellae' s* adult longevity and the fecundity of the females.

2. METHODS

The culture of *G. mellonella* which is a host species of *P. turionellae,* was raised in semi-synthetic food prepared by profiting Bronskill [17], in glass jars the brim of which was covered by muslin. Each jar containing food was added approximately 50 *G. mellonella* eggs and left for development under 29±1ºC, %60±5 relative humidity and darkness conditions. The last phase larvae raised in the jars were taken and placed into beakers containing white tissue paper and the brims of the beakers were covered with muslin and under same conditions they were enabled to proceed to the pupa phase. The acquired host pupae were placed in to cages housing *P. turionellae* adults and the parasitization was ensured. After some 16- 20 days the adult parasitoids hatching from the host pupae were used in the continuity of the stock culture and the establishment of the experimental groups.

P. turionellae stock culture was raised in cages, under 25±1°C, % 60±5 relative humidity and natural photo-period conditions by being fed with 50% honey solution and with host pupa every third day. Some part of the female parasitoids acquired through the parasitized host pupae were placed in beakers after copulation for establishing the experimental groups. As from the seventh day, they were allowed to lay eggs to check whether they were fertile or not. The parasitized host pupae were opened, the eggs were counted and the hatching of the eggs were checked. Thus, the females confirmed to be fertile were taken into separate beakers and used in the experiments. Cotton pieces immersed into 50% honey solution were placed in the beakers containing

these females in each day at the same time and one hour nourishment was enabled. Also a host pupa was placed into each of these beakers in every third day as a food. The female individuals reaching the age of 20 days were not used for the experiments.
The same sized host pupae were separated and placed into beakers containing females as 2 pieces of pupae for each female and the pupae were provided to be parasitized by the females. The parasitization was made every other day.

The parasitized pupae were placed into paper cups and were kept in Sanyo brand incubator respectively; 3 days at 25, 20, 15 and 10°C, 2, 3, 4 and 5 days at 4°C. The parasitized pupae exposed to gradually decreasing temperature, were taken out from the incubator by the end of the application and placed at 25°C and kept till the formation of young. Each of the young individuals was placed into separate beakers. The young life length of hatched individuals and the egg fertility of the females was ascertained. As from the hatching day, these adults were nourished with 50% honey solution and host pupa. The copulated female adults, beginning from the 7th day of pubescence, every other day, were given host pupa to ensure the parasitization and this process was maintained during their entire adult life. After 24 hours, the parasitized pupae were dissected in petri dishes including 0.8% NaCl solution and the laid eggs were counted. Thus, total number of longevity laid eggs by the females acquired by the low temperature application is confirmed and the fertility is ascertained.

The same processes also made for the control group by storing the pupae at 25°C beginning from parasitization till adult hatching without gradually decreasing temperature application. The experimental groups and the control group were conducted in four separate recurrence consisted of 60 individuals.

The emerging changes depending upon the gradually decreasing temperature were compared through One Way Variance Analysis SPSS 15.0 [18]. The arcsine square-roots of the values calculated in percentages were taken before the variance analyses [19]. The differences between the averages were determined by Tukey honesty significant difference (Tukey HSD) tests. Within the evaluations, the significance level was taken as a base a α=0.05.

3. RESULTS

3.1. Longevity of Adult Female and Male Individuals

Longevity of female and male parasitoids emerged due to gradually decreasing heating procedure are given at Table 1.

Table 1: Longevity of female and male parasitoids emerged due to heating procedure having decreasing gradual (day)

Duration (day)[z]	Sex of Parasitoid[xy]	
	♀	♂
0	35,80±1,63a	13,93±0,56a
2	26,05±1,89b	12,18±0,66ab
3	28,28±2,23ab	9,72±0,43bc
4	30,11±2,19ab	10,82±0,80b
5	29,35±1,79ab	9,42±0,40c

[x] Measures are average of 4 repeats each consists 60 persons.

[y] Goups (a-c) at the same column having the same letter are indifferent.(P>0,05)

[z] "0" control group (kept at 25ºC' permanently), 2-3-4-5; time declares detention periods at 4ºC of pupae at experimental groups after holding 3 days at 25ºC, 3 days at 20ºC, 3 days at 15ºC, 3 days at 10ºC (day).

(ANOVA) Tukey' s test which is one of the paired comparison was applied.

As result of comparison among control and experimental group, longevity was detected on female parasitoids in all groups. It is detected that females, coming out pupae which are hold at 4°C during 2 days, have shorter life than either control group and other application groups. There is no statistical difference in respect to female lifespan. There is contraction at male longevity through extending holding period at 4°C.

3.2. Fecundity of Female Parasitoid

Fecundity of female parasitoids which gradually decreasing heating procedure are given at Table 2.

Table 2: Fecundity of female parasitoids which gradually decreasing heating procedure

Duration (day)[z]	Number of Egg- laying[xy]
0	18,58±0,99a
2	9,85±0,97b
3	11,19±1,03b
4	13,31±1,36b

5	12,53±1,16b

[x] Measures are average of 4 repeats each consists 60 persons.

[y] Goups (a-b) at the same column having the same letter are indifferent.(P>0,05)

[z] "0" control group (hold at 25ºC permanently), 2-3-4-5; time declares detention periods at 4ºC of pupae at experimental groups after holding 3 days at 25ºC, 3 days at 20ºC, 3 days at 15ºC, 3 days at 10ºC (day). (ANOVA) Tukey' s test which is one of the paired comparison was applied.

According to data acquired as results of tests, decreasing of fecundity of female parasitoids in all groups was detected regarding to low heating application when comparing to control group. It is detected that there is no statistical difference regarding to number of egg left among groups.

4. DISCUSSION

At biological control applications, knowledge of major longevity of parasitoid to be used and factors affecting efficiency are crucial for mass production of them and reaching success at biological control [20]. There must be suitable storage system and biology and physiology of species must be known well due to mass production at short term is quite difficult.

Being equal of parasitoid and host culture at the same time is not always possible at laboratory applications. That is the reason, if there is not enough female parasitoids when gathering many guest insects then keeping suitable stage of host insect at low temperature aiming at detention of parasite may be a suitable method. Uphill struggle may be applied by keeping at low temperature for a while to gather parasitoids in adequate number through taking advantage of resisting feature of insects to low temperature.

Longevity of insects is a feature to be affected by low temperature. Low temperature reduces longevity of insects. Matadha et. al. [21], *Encarsia citrina* (Hymenoptera: Aphelinidae) have researched effect of low temperature and detected that decreasing heat reduces longevity of insects. Effects of low temperature (+4 and 10°C) to improvement *P. turionella* in host were observed and increasing application period was detected that reduces longevity of adult individuals [22]. *Trissolcus basalis* (Hymenoptera: Scelionidae) and *Telenomus podisi* (Hymenoptera: Scelionidae) was kept till initial of pupa dwells at 18ºC and was exposed to 12 and 15ºC during 120-210 days, no pupa maturing which is kept at 12ºC, and shorter longevity of individual at 15ºC was detected [23]. Similar results are detected at this application. Longevity of female and male parasitoids are shortened when comparing control groups to experimental groups which are exposed to gradually decreasing temperature application.

Efficiency of parasitoid is crucial for biological control [24]. Parasitoid species used for biological control are expected to be efficient for egg- laying of adult individuals and are expected to have higher ratio of hatching after keeping at low temperature [2]. Efficiency for egg- laying of mature female are decreased as period of exposing to cold extends [2,14,25-28]. Effect of temperature at fecundity of *Aprostocetus vaquitarum* (Hymenoptera: Eulophidae) was observed and lower egg- laying capacity was detected at lower temperatures [27]. It is detected at low temperature application carried out with *Agasicles hygrophila* Selman& Vogt (Coleoptera: Chrysomelidae) that fecundity decreases as period of exposing to cold extends [29]. It is detected at this application that fecundity efficiency of female parasitoits decreases when comparing control groups to all experimental groups. But there is no statistical difference in respect to number of eggs left among groups. Positive effect of extending detention period at (4°C) and higher efficiency of fecundity are detected at different application aiming at researching effect of gradually decreasing temperature applied during pupa stage on fecundity efficiency of *P. Turionellae* [30]. The result indicates that larval period of *P. Turionellae* is effected by low temperature more than others when comparing to this application.

As a result, gathering parasitoids through keeping them at low temperature to make biological contention rising in importance in today's world able to use is suitable method due to chemical control to harmful pests has negative effects on human and environment.

Results of the application indicates that keeping at low temperature is suitable storage system to make mass production and to produce sufficient number aiming at using *P. Turionellae* for biological control and specially gradually decreasing low temperature must be preferred. Gradual low temperature application give more positive results in terms of similarity with natural conditions and making pests accustom to cold.

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