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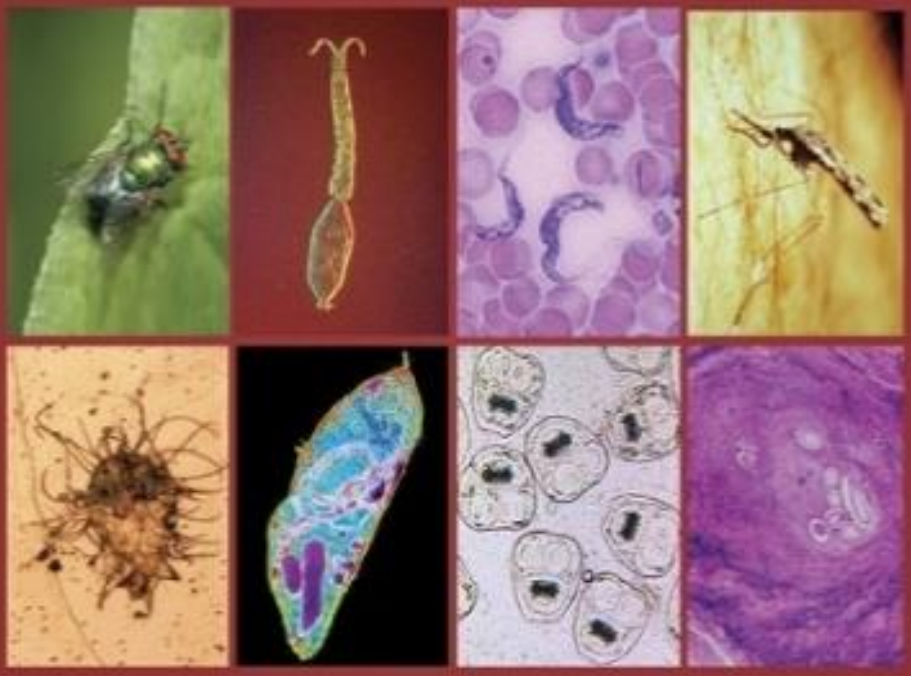

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Effect of Exposure of Internal Phase of *Apanteles angaleti* to Low Temperature (15˚C) on its Survival and its Effect on the Efficacy of Produced Adults

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ABSTRACT

This study was conducted to find the feasibility of prolonging the storage period of the internal phase of the larvae of *Apanteles angaleti* parasite at 15˚C temperature for various periods of time: 0, 72, 113, 126, 140, 182, 184 days to figure out the impact of cooled storage on the efficacy of the produced females post storing period. It has been shown in the results that the periods of growth of both the parasite and the host under 27˚C after various storing periods at 15˚C varies statistically of growth for both host and parasite not exposed to cooling from the periods' regime. Prolonging the period of storing under 15˚C temperature for up to 184 days might affect the percentage of development of both the host and the parasite. Furthermore, it has also been noticed that the sex ratio and the ages of the first generation were affected.

INTRODUCTION

When using parasites in biological control programs, it is necessary to find a suitable low temperature to store various stages of the parasites in order to maintain the laboratory colony and measure the periods of the adult parasites to be released in the field when a pest is detected. The storage procedure is done via testing low convenient temperatures between 0 to 15˚C for various periods so that it will not affect the parasite (Legner,1976; Al-Izzi, *et al.*,1999; Colinet and Boivin,2011). It has been shown that the various growth stages of the parasite can help to endure low temperatures. Therefore, the studies that included various storing periods for various growth phases have shown that it has an effect in regards to the growth and development of the stages under study (Juand and Morrison,1980; Thompson,1985; Mohamed and El-Heneidy, 2020), as well as finding the late effect on the capabilities of the adult parasite that are a result of various storage phases at low temperatures and its ability of parasitism (Legner, 1976; Guzman and Peterson, 1986; Gardner, *et al.*,2012; Peverieri, *et al.*, 2015). The parasite *Apanteles angaleti* is the main parasite for the *Ectomyelois ceratoniae* insect. It can affect the early larval stages of the insect and it is pass through two phases during its development i.e. the internal phase, which is the egg and larval phases within the hosts' larva, and the external phase, which includes the emergence of the larva from the host and its development into pupae (Al-Maliky and Al-Izzi,1986).

The goal of this investigation concentrated on the effect of low temperature (15˚C) on the internal phase of the parasite stored at various ages and various periods of time, and to show the effect of this cold storage (15ºC) on the growth and sustainability of the parasite then, the capabilities of the produced adults shall be determined.

MATERIALS AND METHODS

The internal phase was obtained from a laboratory colony of the *Apanteles angaleti* parasite, which was adapted to the laboratory conditions for several years to achieve this study.

Effect of Cold Storing on the Growth and Development of the Parasite:

The temperature of 15˚C was chosen due to the fact that it affects stimulating the internal phase in order to enter the diapause phase in which the development into a pupa does not occur. A number of previous studies have shown that raising temperatures above 15˚C will result in the insect development into an adult within two months (Al-Izzi, *et al.*,1999; Gardner, *et al.*,2012).

The parasitism on the hosts' larva was conducted at 1-3 days of age in organic glass containers with diameters of 20 x 20 x 20 cm^3 at ideal circumstances (27˚C and 50-60% Humidity (Al-Maliky, *et al.*,1989). The larvae were removed the next day after parasitism (both in tested and non-infested/control larva) and were divided into three groups:

The first group of the larvae were maintained in an incubator at 15˚C and were estimated to be 0-24 hours old, and stored for various periods of time ranging as follows: 0.0, 72, 91, 113, 126 and 184 days.

The second group of the larvae was left in the incubator at 27˚C for 48-72 hours then transferred to 15˚C and stored for 140 and 182 days.

The third group of the larvae was left in the incubator at 27˚C for a period of 144 and168 hours then transferred to 15˚C.

The storing of the larvae was done in glass tubes measuring 7 cm in length and a diameter of 2.5 cm. in every treatment, 300-350 tubes were used containing the artificial diet to feed the host larva (5 larvae per tube). The tubes were sealed by using mull-coated cotton. The rate of the development of the parasites larvae and the host (not infested) were measured as well as the percentage of the emergence of adults after transferring to 27˚C for all treatments. The growth and development of the parasite were monitored during the storing periods which was done via dissecting host larvae.

Effect of Cold Storing on the Adults Activities:

In order to find the capability of the produced adults under early storing of the internal phase of the parasite at 15˚C. Which, completed its growth at 27˚C after cold storage. The adult males and females were extracted from both groups (storage period), each pair of adults were placed in an organic glass box measuring 15x15x15 cm^3 and were host 50 larvae from the host aging 1-3 days were introduced in a plate containing an artificial diet. The host larvae were introduced daily up to the end of the growth period of the parasite. The adult parasite was provided with a 10% sugar solution for feeding. 10 boxes were used for each group, the host's larvae were then transferred after being exposed to the parasite for 24 hours to glass tubes containing the nutritional diet of the host and stored at 27˚C for growth and development. The ages of the produced adults, numbers of developed pupae of the infected larvae as well as the percentage of the number of females and the effect of parasitism were calculated. Duncan method was used to analyze the findings (SAS,2009).

RESULTS AND DISCUSSION

Effect of Cold Storage on the Periods of Growth and Development of the Parasite and the Host

It can be seen from the results of the larval development that the parasite and its

host larvae have evolved into pupae then into adults at the cold storage conditions for different periods of time after transferring them to 27˚C (Fig. 1). The period needed to complete the internal phase of the parasite into the pupae phase in the first group is different from the period it takes the larvae that have not been exposed to cold storage, where it ranges from 6.4 days at 184 days storage to 7.3 days at 72 days in comparison with the period needed to evolve the internal phase of the parasite at 27˚C which is 9.3 days.

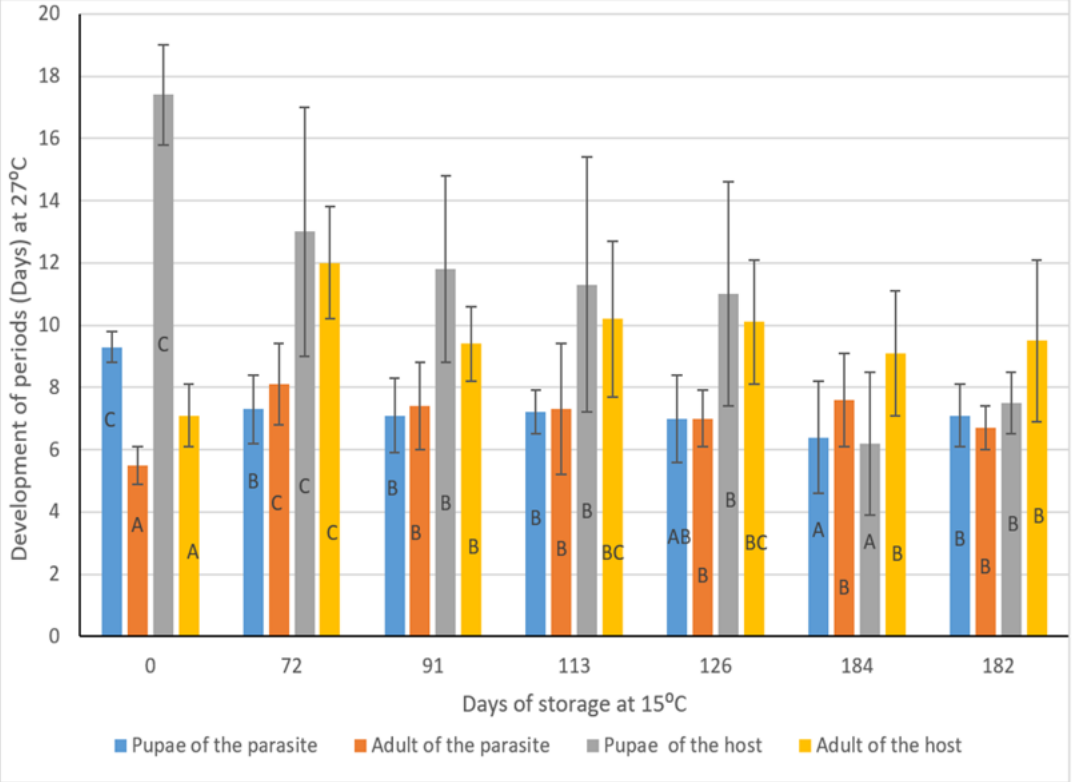

Fig. 1: Effect of storing periods at 15ºC on the development of the internal phase of the parasite *Aponteles angaleti* and the larvae of its host *(Ectomyelois ceratonia)* exposed either at age 0-24 hrs. (Periods 0.0, 72, 91,113,126 and 184 day) or at age 48-72 hrs. (Period 182 days).

Upon these results, one could argue that this parasite does not go through the obligatory Diapause phase, but rather it can pass through the facultative Diapause phase that resumes as soon as the unnatural growing conditions are removed. The decrease of the development period to 6.4 days indicates that there is a slow growth during the storing process for the internal phase, which is evident by dissecting some stored samples (larvae of the host) and examining them under the microscope where it can be seen that after this long period of storage, the parasite is still in the final larva phase according to phase descriptions conducted previously on another species parasites (Cardon and Oatman,1971; Queirez, *et al.*, 2017), while they have been stored at the age of 0-24 hours i.e. in the egg phase, according to the same above references, the growth or inability to grow during the cold storage varies in accordance to the insects' species and storage temperatures (Friesen, *et al.*,1979; Peverieri, *et al.*,2015). Cold storage also affected the period of growth to the pupae phase and the emergence of the adults after transferring them to 27˚C, where it took 5.5 days for non-treated pupae (control) while it prolonged the period of development for the pupae that its larvae exposed to cold storage with notable statistical differences. The storage effect can be seen not only for the parasite but also

for the host. In addition to the age during the storage period which is 3-4 days, it can be seen that the development of the larva to the pupae phase was significantly less than the controlled treatment which took 17.4 days, whilst the period was 6.2 days for the 184 days treatment with an intangible increase in other treatments with fewer storage periods. As for the pupae phase, it was affected too and the period it needs for the completion of its growth was increased in comparison with the controlled treatment which was 7.2 days, whilst it needed 12 days in the 72 days treatment. This period is decreased with the increasing storage periods.

Figure 1, also illustrates that storing the 48-72 hours old larvae of the infested host with the internal phase of the parasite for 182 days had the same effect on the growth and development of the parasite as well as the host, where the needed time for transformation into pupae then adult was 7.1 and 6.7 days for the parasite and 7.5 and 9.5 days for the larvae and pupae of the host respectively.

As for the storage for 144-164 days old larvae which was the final larval phase for the parasite that was about to emerge outside of the host, 15˚C did not help the parasite in going through a long diapause phase, where it exited the body of the host and transformed to pupae within 9.5 days. When transferring these pupae to 27˚C, it managed to complete its growth and become an adult within 7.7 days.

The effect of cold storage on the percentage of development of the larvae and the pupae of the parasite and the host at 27˚C after early storage in 15˚C for various periods can be seen in Figure 2. The development rate of the parasite and the host larvae were between 74.5 to 88% in comparison with the controlled treatment which was 96.1%. The percentage of development for the pupae of the parasite and the host respectively to an adult were also affected by the prolonged storage periods (184 days) where it reached 56% and 55% consecutively while the percentage was higher in other treatments. Cold storage effect on the survival of the parasite of other insects can vary too and depend on the storage temperature and its periods (Legner, 1976; Colinet and Boivin, 2011; Gardner, *et al.*, 2012).

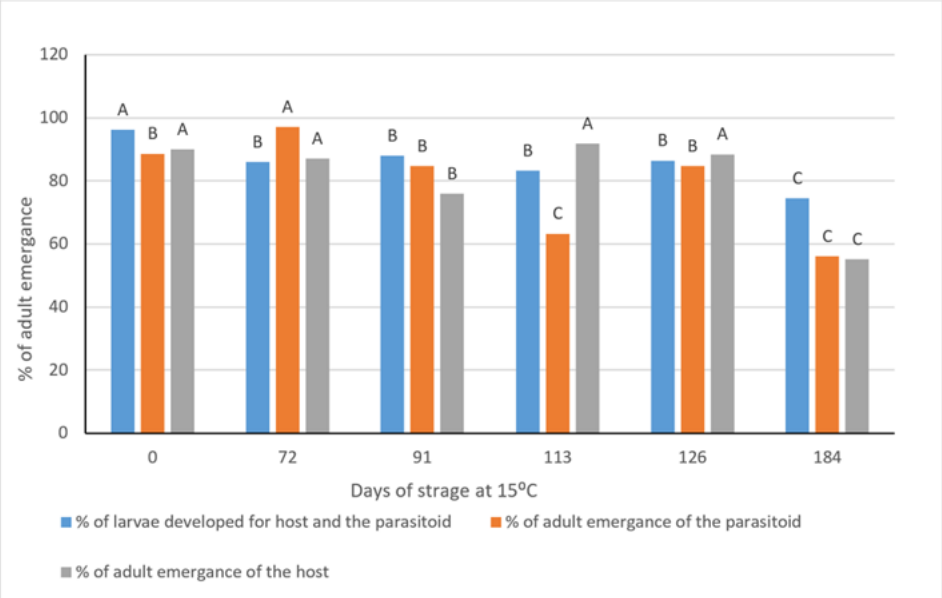

Fig 2: Percentage of development of the parasitoid *Apanteles agaleti* and its host *Ectomyelois ceratoniac* at 27^0C after stored at 15^0C for different periods of time.

Effects of Cold Storage on Adults Activities:

Many measurements were taken in order to find the effect of cold storage at

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15˚C for various periods of exposure on the adults' activities, these include the number of produced pupae for each female, number of females in the first generation, parasitism percentage and the ages of adults for both stored groups as shown in Table 1 and Figure 3. It can be noticed from these results that male ages were not affected and there are no tangible differences between them and the control group that was not subjected to the same storage period. As for the females, the stored samples for 184 days have shown a noticeable increase from those that have been stored for 126 days. The increases in ages of the adults and ineffectiveness by storage was also found in other parasites (Legner, 1976; Farid, *et al.*,2001; Tezze and Botto,2004; Favetti, *et al.*, 2014; Mohamed and El-Heneidy, 2020).

Table 1: Efficacy of adult of the parasitoid *Apanteles angaleti* at 27ºC after storing its internal stage at a different time (days) at 15ºC either at 0-24 hrs. old for the following periods (0.0,72, 91, 126 and 184) or at 48-72hrs. old for the following periods (0.0, 140 and 182).

Days of storage at 15ºC	Number of parasitoid pupae produced by female (Mean ± SD)	% Of female	% of parasitism (Mean ± SD)
0.0	93.9±31.3ab	43.0	38.1±9.5ab
72	103.9±21.5b	37.3	41.2±6.9ab
91	92.3±46.4ab	24.3	52.0±14.8b
126	55.7±45.7a	13.4	34.3±16.6ab
184	65.2±38.5ab	5.0	28.2±11.2a
0.0	93.9±31.3a	43.0	38.1±9.5a
140	84.7±36.7a	25.0	27.8±10.4a
182	106.3±51.8a	29.2	45.1±8.5a

Means in the same column followed by the same letter are not significantly different at P≤ 0.05

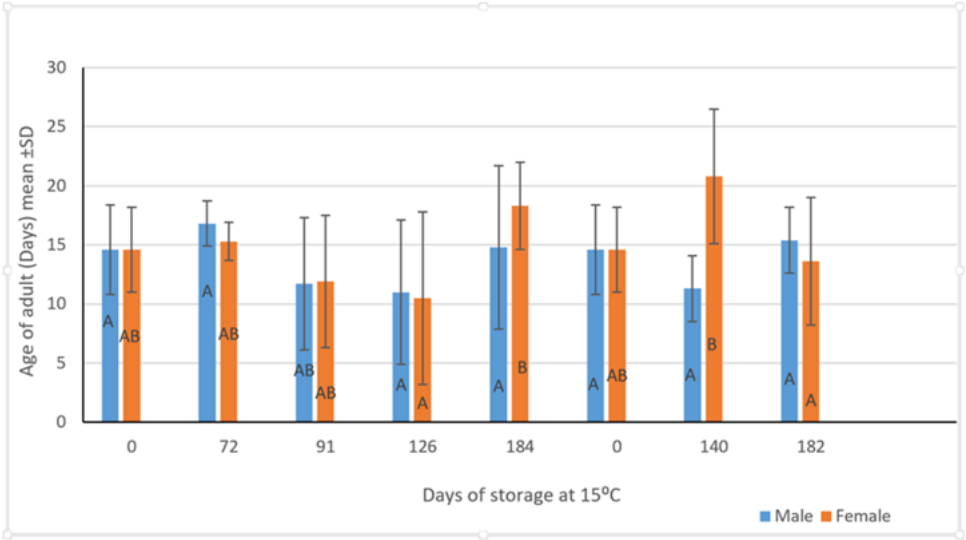

Fig. 3: Age of the parasitoid *Apanteles angaleti* emerged at 27^{0}Cafter storage in its internal stage at 15^{0}C at either 0-24 hrs. old for the following periods (0.0, 72, 91,126 and 184 days) or at 48-72 hrs. old for the following periods (0.0, 140, and 182 days).

Cold storage for various periods did not have any clear negative effect on the number of produced pupae for each female in spite of the difference of averages except for the apparent decrease in the 126 days of storage which resulted in 55.7 pupae per female against the 72 days which resulted in 103.9 pupae. In spite of that, the production

of the female of the controlled treatment was 93.9 pupae per female. This variation between increase and decrease in production was also noticed in other parasites and predators of different insects (De Bach 1943; Legner, 1976; Tezze and Botto,2004; Colinet and Boivin, 2011).

The effect of cold storage on the number of females in the first generation decreased with the increases of the storage period, where the percentage in the treatments were as follows 37.3, 24.3, 13.4, 5.0 for the periods of the storage 27, 91,126,184 days respectively. While the percentage in non-treated insects is 43%. It is worth mentioning that a number of females produced males only in both treatments (126 and 184 days) in spite of monitoring the copulation process. The females of this parasite multiply via parthenogenesis and produced males only while both sexes are produced upon completion of copulation. The effect of surrounding conditions on the parents was studied by (Flanders, 1939; Sanower, *et al.*, 2018), who stated that low temperatures occasionally lead to cause partial or full infertility for males due to its negative impact on the activity of their sperm.

Parasitism rate was not affected in most treatments except for 184 days, where the percentage reached 28.2% which is statistically different from that of 91 days in spite of the fact that it did not differ from the controlled treatment which was 38.1%. To know the extent of the effect of cold storage on the activity of the adults after having stored older phases (48 and 72 hrs.). This effect can be seen in Figure 3 that the ages of adult males were not affected, but there is an evident increase in the ages of females in the storage period of 140 days where it reached 20.8 days in spite of that it is not statistically different from the controlled treatment, however, it differs from the 182 days storage period which reached 13.6 days. As for the number of produced pupae per female (Table 1), it increased in both treatments and does not differ statistically from the controlled treatment. As for the number of females in the first generation, it decreased from normal rates but less severely than the first group treatment of 0-24 hours. The cause of either sex for this decrease of numbers of produced females due to cold storage is unknown for this parasite.

The sex ratio was affected too due to the cold storage it is not a general trait on the rest of the parasites, because this percentage has been found not to get affected when storing some houseflies' parasites, a period of 180 days at 10˚C (Legner,1976; Silva, *et al.*, 2019). One could argue that the cold storage effect differs with different insects and ages as well as the temperature and the periods of storage.

In conclusion, there is a possibility of storing various phases of this parasite for various periods. For prolonging the periods of storage for several months, it is preferable to take place during the internal phase of the parasite which includes, as previously mentioned, the egg and larva stages. The age of 48-72 hours for the larva is the best period for storage. Moreover, the 15˚C did not have a major effect on the produced females and on the growth and development of the parasite. These are critical principles when choosing storage temperatures in spite that there is an effect on the sex ratio, this effect differs with different ages for storage. Further studies in regards to which of the sexes is responsible for this effect are needed.

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