

## Storage the Pupae of *Diaeretiella rapae* (M'Intosh) (Hym.: Aphididae) inside Mummies of Three Aphid Species; *Brevicoryne brassicae* (L.), *Aphis nerri* Boyer De Fonscolombe and *Aphis gossypii* (Glov.) (Hom.: Aphididae)

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### ABSTRACT

Storage of the pupae of *Diaeretiella rapae* in the mummies of three aphid species; *Brevicoryne brassicae* L., *Aphis nerri* B. and *Aphis gossypii* G. under the laboratory conditions of  $15\pm 1^\circ\text{C}$  and  $77.0\pm 2\text{ R.H.}$  was studied. Highest percentages of emergence (96, 64 and 48%) were obtained when the mummies were kept at  $6^\circ\text{C}$  for 6 hours for one week before storage in the mummies of *B. brassicae*, *A. nerri* and *A. gossypii*, respectively. Emergence rate was always higher in the mummies after three and four weeks, than after six and eight weeks. Obtained results showed successful storage of freshly formed mummies of *D. rapae*, for two months at  $6^\circ\text{C}$  in the three aphid species.

**Key Words:** Storage, Mummies, *Diaeretiella rapae*, *Brevicoryne brassicae*, *Aphis nerri*, *Aphis gossypii*.

### INTRODUCTION

Aphids are able to build up high populations before parasitoids become sufficiently active. This is one of the main problems which worries those concerned with biological control of these pests. To assure large numbers of parasitoids at the proper time for release, it may be helpful to store these agents needed for future releases. Storage and release of the adult parasitoids is also important when males are lacking. In this case, it is possible to hold virgin females after they have deposited male eggs and subsequently, mate with their own male progeny as mentioned by Debach (1964) and Ibrahim (1987). Storage of parasitoids and effect of low temperature on hymenopteran parasitoids represent a field of study by many workers, e.g. Anderson (1935), Ibrahim (1987) and Saleh (2004). Hanna (1935) observed high mortality, undesirable sex ratio, and poor fertility from stored adult parasitoids. Debach (1964), reported three causes of mortality among stored adults, (1) cold (2) starvation and (3) desiccation. Wiackowski (1962) found that a long cold storage could damage the sperm in males of *Aphidius smithi*, Lyon (1968) in France and Saleh (2004) in Egypt stored *D. rapae*, freshly formed mummies for more than two months at  $5^\circ\text{C}$ . On the other hand, Ragab *et al.* (2002) and Saleh (2004) in Egypt showed that *D. rapae* parasitized and developed to adult in *B. brassicae*, *Aphis craccivora*, *A. nerri*, *Myzus persicae*, *Sitobion avenae* and *A. gossypii*.

Aim of the present study is to shed light on storage of the parasitoid *D. rapae* in mummies of three aphid species.

### MATERIALS AND METHODS

*B. brassicae*, *A. nerri* and *A. gossypii* were reared on three host plants cabbage, duranta and hibiscus, respectively. The aphid species were reared on caged young seedlings of their hosts or reared on either cut flowering shoots in a caged pot or on detached young leaves set flat on the bottom of clear plastic jar. A laboratory culture of the adult parasitoid, *D. rapae* was obtained from field parasitized cabbage aphid *B. brassicae* (mummies). Mummified aphids were placed singly in small glass tubes until emergence of adult parasitoids which were fed on 10% sugar solution. Adult parasitoids emerged after three to four days and at least for three generations were reared on appropriate host aphid species.

To study effect of storage on the parasitoid, *D. rapae* parasitized three aphid species, aphid colonies were observed daily and newly formed mummies were isolated individually in a small glass tube ( $9 \times 2\text{ cm}$ ). The mummies were divided into three groups according to the pre-storage period which lasted 6, 12 and 18 hours at average temperature of  $10\pm 0.5^\circ\text{C}$ . After these periods, mummies were directly stored in the refrigerator (at  $6^\circ\text{C}$ ) for one, two, three, four, six and eight weeks, for the three aphid species. At the end of the storage period, the mummies were kept in the laboratory at  $15\pm 1^\circ\text{C}$  and  $77.0\pm 2\% \text{ R.H.}$  and were examined three times daily until emergence of adults. Non-emerged mummies were dissected and examined under a binocular microscope and dead stages were recorded. Percentages of adult emergence and mortality rates were estimated.

## RESULTS AND DISCUSSION

Results presented in (Tables 1, 2 and 3), show that the maximum percentage of emergence (96, 64 and 48%) were obtained from mummies of *B. brassicae*, *A. nerii* and *A. gossypii*, respectively, when the mummies were kept at 6°C for 6 hours for one week before storage, while the maximum percentage of mortality (68, 52 and 12%) occurred when the mummies were kept in the refrigerator for 18 hours before storage in *A. gossypii*, *A. nerii* and *B. brassicae* mummies, respectively. The total number of mummies kept at different periods before storage (6, 12 and 18 hours) and then stored for one week, recorded 92, 56 and 40% emergence from the mummies of *B. brassicae*, *A. nerii* and *A. gossypii* and 60, 44 and 8% mortality were reported on *A. gossypii*, *A. nerii* and *B. brassicae*, respectively, but the mummies kept for the same pre-storage periods and then stored for two weeks recorded 78.67, 46.67 and 32.0% emergence in the mummies of *B. brassicae*, *A. nerii* and *A. gossypii* and 68, 53.33 and 21.33% mortality in *A. gossypii*, *A. nerii* and *B. brassicae*, respectively. The highest total percentage of emergence for three, four, six and eight weeks storage period were (73.33, 37.33 and 30.67 %), (60, 33.33 and 28%), (54.67, 29.33 and 22.67%) and (46.67, 21.33 and 17.33%) in the mummies of *B. brassicae*, *A. nerii* and *A. gossypii*, respectively. Meanwhile, the highest total percentage of mortality in the same periods were (69.33, 62.67 and 26.67%), (72.0, 66.67 and 40.0%), (77.33, 70.67 and 45.33) and (82.67, 78.67 and 53.33%) in the mummies of *A. gossypii*, *A. nerii* and *B. brassicae*, respectively. Emergence rate of *D. rapae* was always higher after three and four weeks, than after six and eight weeks of storage. In addition, after three weeks, the first emergence during storage took place in the mummies of *B. brassicae*, and after four weeks in the mummies of *A. nerii* and *A. gossypii*. Archer *et al.* (1973) and Hofavang and Hagvar (1977) observed that the mummies of aphidiids had a high rate of emergence if when kept newly formed at low temperature, therefore the age in which the parasitoids had been stored seemed to be the best. Similar conclusion was reported by Ibrahim (1987) in Egypt who stored freshly formed mummies of *A. uzbekistanicus* for one month at 3° C after being kept in the refrigerator at 8±0.5° C for periods of 6, 12 and 15 hours. Different results were obtained in *Aphidius matricariae* Hal. two and four weeks when been tested (Shalaby and Rabasse, 1979 and Scopes *et al.* 1973). These results agree with the work of Lyon (1968) in France and Saleh (2004) in Egypt who stored freshly formed mummies of *D. rapae* on the aphid species *B. brassicae*, for more than two months at 5° C after being kept in the refrigerator at 8±0.4° C for 6, 12, 9, 12 and 15 hours.

Table (1): Percentage of emergence and mortality of *Diaeretiella rapae* from mummies of *Brevicoryne brassicae*, after storage at 6 °C.

| Period of storage at 6°C                | One week |     |     |       | Two weeks |     |     |       | Three weeks |       |       |       | Four weeks |       |      |       | Six weeks |      |      |       | Eight weeks |      |      |       |    |
|-----------------------------------------|----------|-----|-----|-------|-----------|-----|-----|-------|-------------|-------|-------|-------|------------|-------|------|-------|-----------|------|------|-------|-------------|------|------|-------|----|
|                                         | 6h       | 12h | 18h | Total | 6h        | 12h | 18h | Total | 6h          | 12h   | 18h   | Total | 6h         | 12h   | 18h  | Total | 6h        | 12h  | 18h  | Total | 6h          | 12h  | 18h  | Total |    |
| Period before storage 10±0.5            | 6h       | 12h | 18h | Total | 6h        | 12h | 18h | Total | 6h          | 12h   | 18h   | Total | 6h         | 12h   | 18h  | Total | 6h        | 12h  | 18h  | Total | 6h          | 12h  | 18h  | Total |    |
| Total no. of mummies                    | 30       | 30  | 30  | 90    | 30        | 30  | 30  | 90    | 30          | 30    | 30    | 90    | 30         | 30    | 30   | 90    | 30        | 30   | 30   | 90    | 30          | 30   | 30   | 90    |    |
| No. of mummies dissected before storage | 5        | 5   | 5   | 15    | 5         | 5   | 5   | 15    | 5           | 5     | 5     | 15    | 5          | 5     | 5    | 15    | 5         | 5    | 5    | 15    | 5           | 5    | 5    | 15    |    |
| Larva                                   | 3        | 4   | 3   | 10    | 5         | 3   | 4   | 12    | 3           | 3     | 2     | 8     | 2          | 2     | 3    | 7     | 3         | 2    | 3    | 8     | 2           | 2    | 4    | 8     |    |
| Pupa                                    | 2        | 1   | 1   | 4     | 0         | 2   | 1   | 3     | 2           | 1     | 2     | 5     | 1          | 2     | 1    | 4     | 0         | 1    | 2    | 3     | 2           | 1    | 0    | 3     |    |
| Adult                                   | 0        | 0   | 1   | 1     | 0         | 0   | 0   | 0     | 0           | 1     | 1     | 2     | 2          | 1     | 1    | 4     | 2         | 2    | 0    | 4     | 1           | 2    | 1    | 4     |    |
| No. of mummies stored                   | 25       | 25  | 25  | 75    | 25        | 25  | 25  | 75    | 25          | 25    | 25    | 75    | 25         | 25    | 25   | 75    | 25        | 25   | 25   | 75    | 25          | 25   | 25   | 75    |    |
| Emerged                                 | Adults   | 24  | 23  | 22    | 69        | 21  | 20  | 18    | 59          | 19    | 19    | 17    | 55         | 17    | 15   | 13    | 45        | 14   | 15   | 12    | 41          | 13   | 12   | 10    | 35 |
|                                         | Males    | 11  | 12  | 10    | 33        | 10  | 9   | 8     | 27          | 9(2)  | 8(1)  | 8     | 25         | 7(2)  | 7(2) | 6(1)  | 20        | 6(2) | 6(1) | 5(2)  | 17          | 6(2) | 6(2) | 5(1)  | 17 |
|                                         | Female   | 13  | 11  | 12    | 36        | 11  | 11  | 10    | 32          | 10(3) | 11(2) | 9(1)  | 30         | 10(4) | 8(3) | 7(2)  | 25        | 8(3) | 9(2) | 7(3)  | 24          | 7(3) | 6(2) | 5(2)  | 18 |
| No emerged mummies                      | 1        | 2   | 3   | 6     | 4         | 5   | 7   | 16    | 6           | 6     | 8     | 20    | 8          | 10    | 12   | 30    | 11        | 10   | 13   | 34    | 12          | 13   | 15   | 40    |    |
| Dead adult                              | 1        | 2   | 2   | 5     | 3         | 4   | 5   | 12    | 3           | 2     | 4     | 9     | 3          | 6     | 8    | 17    | 6         | 5    | 7    | 18    | 7           | 6    | 7    | 20    |    |
| Dead pupa                               | 0        | 0   | 1   | 1     | 1         | 0   | 1   | 2     | 2           | 3     | 3     | 8     | 2          | 3     | 2    | 7     | 3         | 2    | 6    | 11    | 5           | 5    | 5    | 15    |    |
| Dead larva                              | 0        | 0   | 0   | 0     | 0         | 1   | 1   | 2     | 1           | 1     | 1     | 3     | 3          | 1     | 2    | 6     | 2         | 3    | 0    | 5     | 0           | 2    | 3    | 5     |    |
| % emergence                             | 96       | 92  | 88  | 92.0  | 84        | 80  | 72  | 78.67 | 76          | 76    | 68    | 73.33 | 68         | 60    | 52   | 60    | 56        | 60   | 48   | 54.67 | 52          | 48   | 40   | 46.67 |    |
| % mortality                             | 4        | 8   | 12  | 8.0   | 16        | 20  | 18  | 21.33 | 24          | 24    | 32    | 26.67 | 32         | 40    | 48   | 40    | 44        | 40   | 52   | 45.33 | 48          | 52   | 60   | 53.33 |    |

H: hour

( ): adults emerged during storage

Table (2): Percentage of emergence and mortality of *Diaeretiella rapae* from mummies of *Aphis nerii*, after storage at 6 °C.

| Period of storage at 6°C                | One week |     |     |       | Two weeks |     |    |       | Three weeks |       |    |       | Four weeks |      |      |       | Six weeks |      |      |       | Eight weeks |       |      |       |    |     |     |       |  |
|-----------------------------------------|----------|-----|-----|-------|-----------|-----|----|-------|-------------|-------|----|-------|------------|------|------|-------|-----------|------|------|-------|-------------|-------|------|-------|----|-----|-----|-------|--|
|                                         | 6h       | 12h | 18h | Total | 6h        | 12h | 6h | 12h   | 18h         | Total | 6h | 12h   | 6h         | 12h  | 18h  | Total | 6h        | 12h  | 6h   | 12h   | 18h         | Total | 6h   | 12h   | 6h | 12h | 18h | Total |  |
| Total no. of mummies                    | 30       | 30  | 30  | 90    | 30        | 30  | 30 | 90    | 30          | 30    | 30 | 90    | 30         | 30   | 30   | 90    | 30        | 30   | 30   | 90    | 30          | 30    | 30   | 90    | 30 | 30  | 30  | 90    |  |
| No. of mummies dissected before storage | 5        | 5   | 5   | 15    | 5         | 5   | 5  | 15    | 5           | 5     | 5  | 15    | 5          | 5    | 5    | 15    | 5         | 5    | 5    | 15    | 5           | 5     | 5    | 15    | 5  | 5   | 5   | 15    |  |
| Larva                                   | 4        | 5   | 3   | 12    | 3         | 2   | 4  | 9     | 4           | 3     | 2  | 9     | 3          | 2    | 2    | 7     | 2         | 4    | 3    | 9     | 2           | 2     | 3    | 7     |    |     |     |       |  |
| Pupa                                    | 1        | 0   | 2   | 3     | 2         | 2   | 1  | 5     | 0           | 1     | 2  | 3     | 1          | 2    | 3    | 6     | 1         | 1    | 1    | 3     | 2           | 1     | 1    | 4     |    |     |     |       |  |
| Adult                                   | 0        | 0   | 0   | 0     | 0         | 1   | 0  | 1     | 1           | 1     | 1  | 3     | 1          | 1    | 0    | 2     | 2         | 0    | 1    | 3     | 1           | 2     | 1    | 4     |    |     |     |       |  |
| No. of mummies stored                   | 25       | 25  | 25  | 75    | 25        | 25  | 25 | 75    | 25          | 25    | 25 | 75    | 25         | 25   | 25   | 75    | 25        | 25   | 25   | 75    | 25          | 25    | 25   | 75    |    |     |     |       |  |
| Emerg ed                                | Adults   | 16  | 14  | 12    | 42        | 14  | 11 | 10    | 35          | 11    | 9  | 8     | 28         | 10   | 8    | 7     | 25        | 9    | 7    | 6     | 22          | 7     | 5    | 4     | 16 |     |     |       |  |
|                                         | Males    | 7   | 7   | 5     | 19        | 7   | 5  | 5     | 17          | 5     | 4  | 4     | 13         | 5(1) | 3(1) | 3(1)  | 11        | 4(1) | 3    | 3(1)  | 10          | 3(1)  | 2    | 2(1)  | 7  |     |     |       |  |
|                                         | Female   | 9   | 7   | 7     | 23        | 7   | 6  | 5     | 18          | 6     | 5  | 4     | 15         | 5(2) | 5(1) | 4(1)  | 14        | 5(2) | 4(1) | 3     | 12          | 4(2)  | 3(1) | 2(1)  | 9  |     |     |       |  |
| No emerged mummies                      | 9        | 11  | 13  | 33    | 11        | 14  | 15 | 40    | 14          | 16    | 17 | 47    | 15         | 17   | 18   | 50    | 16        | 18   | 19   | 53    | 18          | 20    | 21   | 59    |    |     |     |       |  |
| Dead adult                              | 4        | 4   | 5   | 13    | 4         | 5   | 6  | 15    | 6           | 7     | 6  | 19    | 5          | 6    | 7    | 18    | 5         | 7    | 8    | 20    | 5           | 6     | 9    | 20    |    |     |     |       |  |
| Dead pupa                               | 3        | 4   | 4   | 11    | 3         | 5   | 6  | 14    | 5           | 4     | 5  | 14    | 6          | 6    | 5    | 17    | 6         | 7    | 6    | 19    | 7           | 8     | 7    | 22    |    |     |     |       |  |
| Dead larva                              | 2        | 3   | 4   | 9     | 4         | 4   | 3  | 11    | 3           | 5     | 6  | 14    | 4          | 5    | 6    | 15    | 5         | 4    | 5    | 14    | 6           | 6     | 5    | 17    |    |     |     |       |  |
| % emergence                             | 64       | 56  | 48  | 56    | 56        | 44  | 40 | 46.67 | 44          | 36    | 32 | 37.33 | 40         | 32   | 28   | 33.33 | 36        | 28   | 24   | 29.33 | 28          | 20    | 16   | 21.33 |    |     |     |       |  |
| % mortality                             | 36       | 44  | 52  | 44    | 44        | 56  | 60 | 53.33 | 56          | 64    | 68 | 62.67 | 60         | 68   | 72   | 66.67 | 64        | 72   | 66   | 70.67 | 72          | 80    | 84   | 78.67 |    |     |     |       |  |

H: hour

(): adults emerged during storage

Table (3): Percentage of emergence and mortality of *Diaeretiella rapae* from mummies of *Aphis gossypii*, after storage at 6°C.

| Period of storage at 6°C                | One week |     |     |       | Two weeks |     |    |     | Three weeks |       |    |       | Four weeks |      |      |       | Six weeks |      |      |       | Eight weeks |       |      |       |    |     |     |       |  |
|-----------------------------------------|----------|-----|-----|-------|-----------|-----|----|-----|-------------|-------|----|-------|------------|------|------|-------|-----------|------|------|-------|-------------|-------|------|-------|----|-----|-----|-------|--|
|                                         | 6h       | 12h | 18h | Total | 6h        | 12h | 6h | 12h | 18h         | Total | 6h | 12h   | 6h         | 12h  | 18h  | Total | 6h        | 12h  | 6h   | 12h   | 18h         | Total | 6h   | 12h   | 6h | 12h | 18h | Total |  |
| Total no. of mummies                    | 30       | 30  | 30  | 90    | 30        | 30  | 30 | 90  | 30          | 30    | 30 | 90    | 30         | 30   | 30   | 90    | 30        | 30   | 30   | 90    | 30          | 30    | 30   | 90    | 30 | 30  | 30  | 90    |  |
| No. of mummies dissected before storage | 5        | 5   | 5   | 15    | 5         | 5   | 5  | 15  | 5           | 5     | 5  | 15    | 5          | 5    | 5    | 15    | 5         | 5    | 5    | 15    | 5           | 5     | 5    | 15    | 5  | 5   | 5   | 15    |  |
| Larva                                   | 3        | 4   | 2   | 9     | 3         | 4   | 3  | 10  | 2           | 3     | 4  | 9     | 3          | 2    | 4    | 9     | 2         | 3    | 2    | 7     | 2           | 3     | 2    | 7     |    |     |     |       |  |
| Pupa                                    | 2        | 1   | 3   | 6     | 2         | 1   | 2  | 5   | 2           | 2     | 1  | 5     | 2          | 3    | 1    | 6     | 2         | 1    | 1    | 4     | 2           | 1     | 1    | 4     |    |     |     |       |  |
| Adult                                   | 0        | 0   | 0   | 0     | 0         | 0   | 0  | 0   | 1           | 0     | 0  | 1     | 0          | 0    | 0    | 0     | 1         | 1    | 2    | 4     | 1           | 1     | 2    | 4     |    |     |     |       |  |
| No. of mummies stored                   | 25       | 25  | 25  | 75    | 25        | 25  | 25 | 75  | 25          | 25    | 25 | 75    | 25         | 25   | 25   | 75    | 25        | 25   | 25   | 75    | 25          | 25    | 25   | 75    |    |     |     |       |  |
| Emerg ed                                | Adults   | 12  | 10  | 8     | 30        | 11  | 7  | 6   | 24          | 10    | 7  | 6     | 23         | 9    | 7    | 5     | 21        | 8    | 5    | 4     | 17          | 6     | 4    | 3     | 13 |     |     |       |  |
|                                         | Males    | 5   | 4   | 3     | 12        | 5   | 3  | 3   | 11          | 5     | 3  | 2     | 10         | 4(1) | 4(1) | 2     | 10        | 3(1) | 2    | 2(1)  | 7           | 2     | 2(1) | 1     | 5  |     |     |       |  |
|                                         | Female   | 7   | 6   | 5     | 18        | 6   | 4  | 3   | 13          | 5     | 4  | 4     | 13         | 5(2) | 3(1) | 3(1)  | 11        | 5(2) | 3(1) | 2     | 10          | 4(1)  | 2    | 2(1)  | 8  |     |     |       |  |
| No emerged mummies                      | 13       | 15  | 17  | 45    | 14        | 18  | 19 | 51  | 15          | 18    | 19 | 52    | 16         | 18   | 20   | 54    | 17        | 20   | 21   | 58    | 19          | 21    | 22   | 62    |    |     |     |       |  |
| Dead adult                              | 2        | 4   | 5   | 11    | 4         | 6   | 5  | 15  | 5           | 7     | 6  | 18    | 6          | 5    | 6    | 17    | 8         | 4    | 6    | 18    | 6           | 8     | 9    | 23    |    |     |     |       |  |
| Dead pupa                               | 6        | 7   | 8   | 21    | 7         | 8   | 11 | 26  | 7           | 6     | 9  | 22    | 7          | 11   | 12   | 30    | 7         | 13   | 10   | 30    | 9           | 10    | 9    | 28    |    |     |     |       |  |
| Dead larva                              | 5        | 4   | 4   | 13    | 3         | 4   | 3  | 10  | 3           | 5     | 4  | 12    | 3          | 2    | 2    | 7     | 2         | 3    | 5    | 10    | 4           | 3     | 4    | 11    |    |     |     |       |  |
| % emergence                             | 48       | 40  | 32  | 40    | 44        | 28  | 24 | 32  | 40          | 32    | 24 | 30.67 | 36         | 28   | 20   | 28    | 32        | 20   | 16   | 22.67 | 24          | 16    | 12   | 17.33 |    |     |     |       |  |
| % mortality                             | 52       | 60  | 68  | 60    | 56        | 72  | 66 | 68  | 60          | 68    | 66 | 69.33 | 64         | 72   | 80   | 72    | 68        | 80   | 84   | 77.33 | 66          | 84    | 88   | 82.67 |    |     |     |       |  |

H: hour

(): adults emerged during storage

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## المخلص العربي

تخزين عذارى طفيل *Diaeretiella rapae* (M'Intosh) داخل موميوات ثلاثة أنواع من المَن وهي مَن الكرنب *Aphis gossypii* (Glov.) ومَن القطن *Aphis nerii* ومَن التفلة *Brevicoryne brassicae* (L.)

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أظهرت الدراسة الحالية إمكانية تخزين طفيل *Diaeretiella rapae* على ثلاثة أنواع من المَن وهي مَن الكرنب *B. brassicae* ومَن التفلة *A. nerii* ومَن القطن *A. gossypii* تحت الظروف المعملية حرارة  $1 \pm 10$  درجة مئوية ورطوبة نسبية  $77 \pm 2$  وكانت أعلى نسبة خروج للطفيل هي (٩٦، ٦٤ و ٤٨%) عندما تم حفظ الموميوات على درجة حرارة ٦ درجة مئوية لمدة أسبوع على مَن الكرنب ومَن التفلة ومَن القطن على التوالي. وكان معدل خروج الطفيل عالي من الموميوات بعد ثلاثة وأربعة أسابيع بالمقارنة بستة وثمانية أسابيع من التخزين. كما أظهرت النتائج نجاح تخزين الموميوات حديثة التكوين للطفيل *D. rapae* لمدة ثمانية أسابيع على درجة حرارة ٦ درجة مئوية على ثلاثة أنواع المَن.