

BIOLOGICAL PARAMETERS FOR MASS-REARING OF THE APHID PARASITOID SPECIES, *APHELINUS ALBIPODUS* HAYAT & FATIMA (HYMENOPTERA: APHELINIDAE).

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Abstract

Aphids are among the serious insect pests attacking several plants. Among their most important aphid parasitoids' species is, *Aphelinus albipodus* Hayat & Fatima (Hymenoptera: Aphelinidae). Basic biological parameters of *A. Albipodus*, i.e. longevity, fecundity, host preference and storage of mummies, as basic contributions to the information needed for its mass rearing were studied under laboratory conditions. All produced adults of *A. albipodus* were females, mean longevity (days) of adult stage, with and without feeding on honey was estimated as 10.85 ± 0.82 days, when fed, and 1.44 ± 0.12 days, when starved. Total number of eggs/ female was 67.9 ± 7.21 (30-110) (egg/ female) on *Rhopalosiphum padi* L. Obtained results considered for mass rearing suggested that the aphid species, *Sitibion avenae* was the favorite aphid species for rearing *A. albipodus* based on both total number of mummies/cage and percentage of emergence. Highest emergence rates (94.49%) were estimated at 15 °C in mummies stored for one week. Lowest number of mummies/ female (19.6 ± 2.89 mummies) was recorded at 12 °C, in mummies stored for four weeks. *A. albipodus* can be used in winter crops as a biocontrol agent against the aphid species, *Aphis craccivora* Koch, *S. avenae* and *R. padi*.

Key Words: *Aphelinus albipodus*, longevity, fecundity, host preference, storage, mass rearing.

INTRODUCTION

Aphids attack a wide range of plants, including herbs, trees and even some mosses. Although many species form dense colonies are on the aerial parts of plants, other attack roots. Aphids damage the plants roughly through loss of sap by sucking, reaction of plant tissues stimulated by aphid saliva, excreting viscous honeydew on it. Sooty-moulds usually develop and finally transmission of viral diseases to plants. A complex of parasitoids (Aphidiidae and Aphelinidae) and predators (Coccinellidae, Syrphidae, Cecidomyiidae and Chrysopidae) have been reported attacking aphids (Christine *et al.*, 1999).

The first step in a mass-rearing program is the trial to rear the natural enemy on natural hosts in an economical way. Most of the natural enemies are reared in this way. For biological control, mass rearing of insects involves several constraints, among them are insectary's conditions, temperature, relative humidity and day light, in addition to fecundity and host preference of the natural enemy species as well efficiency of its storage before use.

For many beneficials, short-term storage methods have been developed and consist usually of placing the natural enemies as immatures at temperatures between 4 and 15 degree °C. Normally storage periods only last several weeks but even then reduction in fitness is the role. Problems related to a good planning of production and difficulty to predict demands make it necessary to have storage methods and facilities available.

Storage is often needed to ensure the production of a large number of individuals for release at the right time, i.e., when climatic conditions are favorable or when the target pest is present. According to Stary (1970a), mummies seemed more tolerant to cold storage than adults. Reports have shown that female fecundity after storage may increase (Hofsvang and Hagvar 1977), remain unchanged (Archer and Eikenbary 1973), or decrease (Polgar 1986). Rabasse and Ibrahim (1987) considered an 80% emergence of adults after cold storage as a threshold of efficiency for biological control programs.

One potential agent, the aphid parasitoid species, *Aphelinus albipodus* Hayat & Fatima (Hymenoptera: Aphelinidae) was collected from Russian wheat aphid, *Diuraphis noxia* (Mordwilko) hosts in the vicinity of Tahcheng, People's Republic of China and imported to the USA in 1992 for biological control of the latter pest species then exported to Egypt in 2001 for the biological control of cereal aphids particularly, *Rhopalosiphum padi* L. Specimens of the native *Aphelinus* sp. emerged from cereal aphid species, collected from Egyptian wheat fields, were sent to Dr. M. Hayat (the author of the species), Department of Zoology, Aligarh Muslim University, Aligarh, India for identification. The result showed that both the native and the exotic parasitoid species were the same, *A. albipodus*.

Since life table and thermal requirements of *A. albipodus* were studied by Adly *et al.*, (2006), respectively, the present study concerned with the other biological parameters, fecundity, longevity, host preference and storage of mummies under laboratory conditions as basic contributions to the information needed for the mass rearing of the parasitoid.

MATERIALS AND METHODS

A. albipodus is a unisex (adults are all females). It was reared on *R. padi* maintained on wheat (*Triticum aestivum*) seedlings. Colonies of both *A. albipodus* and *R. padi* were kept in the laboratory under controlled conditions ($23\pm 1^{\circ}\text{C}$, 60-70% R.H. and photoperiod L: D 16: 8). All biological studies were experimented under the abovementioned laboratory conditions.

Longevity: Formed mummies of the parasitoids were placed individually in small glass vials until adult emergence. Two groups each of newly emerged adults were left to complete their longevities, the 1st group was left unfed and the 2nd was fed on droplets of honey. Longevity of adults in each group was estimated (30 replicates / treatment).

Fecundity: Ten aphid parasitoid females, each was provided daily with 100 nymphs (2nd and 3rd instars of *R. padi*) in Petri-dishes containing droplets of honey and wheat leaves, their ends were dipped in moistened paper towels until death, following the technique of Michels *et al.* (1987). Parasitized aphids were dissected daily to determine the number of eggs laid / female per day and the total number of eggs laid by each female (10 replicates / treatment).

Host Preference: *A. albipodus* was provided with adequate number of nymphs of each of the aphid species (*Aphis craccivora* Koch., *Sitibion avenae* and *R. padi*) for 24hr. Replicates (10 replicates / treatment) consisted of 100 2nd and 3rd individuals from each species individually when one species of the aphids was exposed, 50 nymphs from each species, when two species of the aphids were exposed, and 33 nymphal from each species when the three species of the aphids were exposed. Caged plants were placed in a growth chamber and maintained at L: D 16:8 photoperiods and $23 \pm 1^{\circ}\text{C}$. Aphids were fed on wheat and/or faba bean plants until forming mummies. Mummies were collected and kept in small glass vials. Proportion of parasitized aphids within 24hr. was estimated.

Mass Rearing: Mass rearing of *A. albipodus* had five phases: (1) preparation of plant material (the basic materials included wheat, barley and faba bean seeds, sawdust, water and containers for plant growing), (2) rearing of aphids, (3) rearing of parasitoid, (4) storing of parasitoid and (5) removal of old plant materials. Activities connected with each phase were carried out in a separate room to prevent contamination.

The parasitoid, *A. albipodus* culture was mass reared in cloth cages by releasing 15 mummies of the parasitoid on wheat, barley and faba bean seedlings infested with *R. padi*, *S. avenae* and *A. craccivora* individually, respectively. After 8-9 days, the plants were checked for mummies. The sites and number of the mummies

were recorded on whole plant (leaf, stem and root) and on the sawdust. The mummies were collected from the plants and kept in vials until emergence of parasitoid adults. The number of mummified aphids produced per cage and percentage of emergence were recorded (20 replicates / treatment).

Storage of Mummies: Newly formed mummies of *R. padi* (five replicates, 15 mummies/replicate) from the laboratory cultures of the parasitoid *A. albipodus* were stored in incubators at each of 10, 12 and 15 $\pm 0.5^{\circ}\text{C}$) for this study. Mummies were stored at pupal stage. Mummies were stored for one month. Every week, five replicates (100 mummies) for each treatment, were taken out of the incubator for evaluating emergence rate and fecundity. The five replicates were provided with adequate number of aphids in a growth chamber at $23 \pm 1^{\circ}\text{C}$ until their death. Number of formed mummies was counted to estimate the effect of storage on reproduction. Emergence rate was estimated through the number of emerged adults.

Obtained data were recorded and statistically analyzed using ANOVA statistical method.

RESULTS AND DISCUSSION

Longevity: (days) of adult stage, with and without feeding on honey, was estimated as 10.85 ± 0.82 (4-15) days, when fed, and 1.44 ± 0.12 (1-2) days, when unfed. Statistical analysis showed that there was a significant difference between the longevity of fed and unfed parasitoid females.

These results agree with those Cloutier *et al.* (1981) who reported that in aphidiidae, the longevity was variable. Minimum survival occurred without water and food. Shalaby and Rabasse (1979) found that aphid parasites which fed on honeydew showed longer life-span than those fed on bee-honey. Julio *et al.* (1997) estimated the longevity of *A. albipodus* from China at the three temperatures 10, 21.1 and 26.6°C , 50-70% R.H. and L: D 14:10 on Russian wheat aphid, *D. noxia*. The longevity of both males and females was longest at 10°C , differences in longevity were not significant between 21.1 and 26.7°C in both sexes. Harley *et al.* (1971) reported that the longevity of adults' parasite *A. asychis* was approximately 18 days at 23.9°C and 9 days at 32.2°C .

Fecundity: is measured by the number of eggs laid by a parasitoid female (daily and throughout its longevity). Ovipositional periods at $23 \pm 1^{\circ}\text{C}$ were divided into pre-ovipositional, ovipositional, and post-ovipositional periods. Ovipositional periods (days) and total number of eggs/ female were 0.05 ± 0.22 (0-1), 7.4 ± 0.05 (4-13) and

0.75±0.14 (0-2) days, 67.9±7.21 (30-110) (egg/ female) on *R. padi*. All adults of *A. albipodus* were females (thelytokous) (Adly *et al.* 2006).

From laboratory observations the followings could be concluded:

- Female of the aphid parasitoid *A. albipodus* was able to distinguish the already parasitized aphids (always one egg in the parasitized aphid was found through dissection), thus avoiding super-parasitism.
- It didn't been noticed that the parasitoid fed on the aphids (predatory behavior) as known about *A. albipodus*.
- *A. albipodus* used to assess its host quality with its ovipositor before oviposition.

Stary (1970 b) and Cloutier *et al.* (1981) reported that in Aphidiidae, the ovipositing-female was able to distinguish the already parasitized aphids to a certain extent, thus avoiding super-parasitism which might occur in the laboratory and in the field. Philip and Thomas (2002) reported that a single *A. albipodus* female after 1h produced a maximum of six progeny from 40 aphids on the wheat plants. The mean ovipositional time for *A. albipodus* was 119s compared. The time between attacks was much great for *A. albipodus* at all prey densities, partially a result of this parasitoid feeding on aphids. *A. albipodus* had many fewer eggs ready for oviposition *A. albipodus* remained for extended periods on wheat plants with many aphids, allowing development of additional eggs and parasitization of more aphids. Farias *et al.* (1999) reported that *A. asychis* appeared to assess host quality with its ovipositor after insertion and *A. matricariae* appeared to assess host quality by antennal palpation before ovipositor insertion. Although adult *A. asychis* uses some hosts for nutrition rather than oviposition and *A. matricariae* does not, *A. matricariae* killed more hosts per unit time than *A. asychis*. Although some *D. noxia* defended them when attacked, aphid defense did not reduce parasitism by either wasp species.

Host Preference: percentage of adults' emergence of the aphid parasitoid, *A. albipodus* in the three experiments decreased when more than one aphid species was exposed. Highest mean numbers of mummies were on *A. craccivora* (19.1 ± 1.1) followed by *S. avenae* (5.8 ± 0.4) and then *R. padi* (3.6 ± 0.3), although the lowest percentage of emergence rate was on *A. craccivora* in all cases (Table 1). Therefore, *A. albipodus* can be used in winter crops as a biological control agent against the three studied aphid species. Statistical analysis showed that there were significant differences among *R. padi*, *S. avenae* and *A. craccivora* in the mean numbers of the mummies / female in all experiments.

Sary (1970 b) reported that the host selection process of aphidiids is in agreement with the general scheme for parasitic Hymenoptera. Host finding activities start with the selection of a suitable habitat. The food plants of the host aphids play an important role, as the parasitoids seem to be attracted to them (allomones). The attractiveness to the host aphids by the parasitoids is apparently due to the perception of the host kairomones, which seem to be present in honeydew. Host and parasitoid population densities are also important in conditioning the searching activity of the parasitoid. Vision, tactile responses of the antennae and aphid movement may play a significant role.

Results are in agreement with Elliot *et al.* (1999) who found that four strains of the parasitoid, *A. albipodus* had identical mummy formation ratings for seven aphid species (*A. helianthi*, *S. avenae*, *S. graminum*, *M. persicae*, *D. noxia*, *Lipaphis erysimis* and *D. tritici*). *A. albipodus* seems to be polyphagous, so that could be effective as biological control agent because of its ability to parasitize several common aphids, a trait could contribute to development of large numbers of parasitoids to colonize aphids infesting winter wheat fields. , Recently, Tatsumi and Takada (2005) assessed the potentials of indigenous parasitoid, *A. albipodus* as biological control agent against three pest aphids, *A. gossypii*, *M. persicae* and *M. euphorbiae*. *A. albipodus* could be an effective biological control agent against *M. persicae* and *M. euphorbiae*.

Mass Rearing of *A. albipodus*: Data concerning mummies' site were taken in consideration to determine which particular site on the plant was preferred by each aphid species for mummification. Preferred sites for mummification were arranged according to the plant part (leaf, stem and root). Numbers of mummies on plant/ cage were 101.29±5.6, 165±7.34 and 182.5±16.2 on *R. padi*, *S. avenae* and *A. craccivora*, respectively, compared with the respective numbers on sawdust/cage 82±7.1, 107.4±12.76 and 129.7±13 (Table 2).

From the observations made in the laboratory and the results of the data, it seems that the parasitized aphid normally moves to any extent of the plant before it starts mummification. Mummies found on or in the sawdust probably because the plant becomes overloaded with aphids or begins to dry then the aphids migrate from it, some of the fallen aphids would probably form mummies in sawdust

Results cleared that total numbers of mummies/cage on *A. craccivora* were higher than on *S. avenae* and *R. padi*, they were 288.07±25, 272.4±11.58 and 164.25 ±9.02, respectively. On the other hand, the lowest percentage of emergence rate was 67.07% for *A. craccivora*. *R. padi* and *S. avenae* percentages of emergence rate were almost similar, 84.24 and 84.94%, respectively (Table 2).

Table 1. Mean numbers (\pm S.E and range) of mummies / female and percentages of emergence of the parasitoid, *Aphelinus albipodus* when three aphid species were exposed in three different experiments, at 23 °C, 60-70% R.H., and L:D 16:8.

No. of aphid exposed	Mean no. of mummies/ female			Percentage of emergence rate		
	<i>R. padi</i>	<i>S. avenae</i>	<i>A. craccivora</i>	<i>R. padi</i>	<i>S. avenae</i>	<i>A. craccivora</i>
One aphid species	10.2 \pm 1.1 (5 - 15)	14.1 \pm 0.8 (10 - 17)	20.3 \pm 1.6 (15 - 27)	85.98	88.4	79.1
Two aphid species	6.2 \pm 0.4 (4 - 8)	15 \pm 0.9 (10 - 18)		82.2	79.9	
	5.3 \pm 0.4 (4 - 8)		23.3 \pm 0.9 (20 - 27)	81.2		75.3
		7.4 \pm 0.8 (4 - 11)	18.5 \pm 1.2 (14 - 25)		80	77.8
Three aphid species	3.6 \pm 0.3 (2 - 5)	5.8 \pm 0.4 (4 - 7)	19.1 \pm 1.1 (14 - 25)	80.6	84.1	70.9

N=10

Results showed that total numbers of mummies/cage on *A. craccivora* were higher than on *S. avenae* and *R. padi*, showing 288.07 \pm 25, 272.4 \pm 11.58 and 164.25 \pm 9.02, respectively. On the other hand, the lowest percentage of emergence was 67.07% for *A. craccivora*. *R. padi* and *S. avenae* percentages of emergence were almost similar, 84.24 and 84.94%, respectively (Table 2).

Statistical analysis showed that there were significant differences among *R. padi*, *S. avenae* and *A. craccivora* for the total numbers of mummies / cage obtained.

Obtained results suggested that the *S. avenae* was the favorite aphid species for rearing the aphid parasitoid, *A. albipodus* based on both total number of mummies/ cage and percentages of emergence.

Stary (1970 b) found that the broad bean seedlings were the most suitable host plant for mass-rearing of the pea aphid. Adult parasitoids of *A. smithi* mass collection equipment has been developed (comprising a vacuum cleaner), older plants with mummified aphids should be removed from the rearing, cut and transferred into separate rearing cages, where the emerged parasitoids are later collected in masses.

Mummies have been found to be the most useful stage for cold storage. The gradual accumulation of mummies enables a relatively high production by even a small rearing laboratory.

Table 2. Mass rearing of *Aphelinus albipodus* on *Rhopalosiphum padi*, *Sitobion avenae* and *Aphis craccivora* under the laboratory conditions of $23\pm 1^{\circ}\text{C}$, photoperiod L: D 16:8 and 60-70% R.H.

Aphid species	<i>R. padi</i>	<i>S. avenae</i>	<i>A. craccivora</i>
No. of mummies on plant/cage	101.29 \pm 5.6 (60-134)	165 \pm 7.34 (100-200)	182.5 \pm 16.2 (76-275)
No. of mummies on sawdust/cage	82 \pm 7.1 (53-155)	107.4 \pm 12.76 (47-163)	129.7 \pm 13 (75-218)
Total no. of mummies/cage	164.25 \pm 9.02 (101-220)	272.4 \pm 11.58 (147-313)	288.07 \pm 25 (116-474)
% of emergence	84.24%	84.94%	67.07%

Obtained data agree with the findings of Cate *et al.* (1973) who reported that the preferred site for mummification of parasitized aphid, *S. graminum* exposed to *A. asychis* was leaves (1967 mummies). Next was the stem with 1200 mummies. There were little differences in the whorl, sand and container with 121, 172 and 114 mummies, respectively.

Storage of Mummies: Data presented in table (3) summarize mean emergence rates and the mean numbers of mummies/ female of the emerged adults' out came from the stored mummies at 10, 12 and 15 $\pm 0.5^{\circ}\text{C}$, for one month.

Among all the storage temperatures, the lowest emergence rates, (5.33%) were estimated when the mummies were stored at 10 $^{\circ}\text{C}$ for four weeks. On the contrary, highest numbers of mummies/ female (154.2 \pm 8.63) were recorded at 15 $^{\circ}\text{C}$ for the storage for one week. Highest emergence rates (94.49%) were estimated at 15 $^{\circ}\text{C}$ on the mummies stored for one week. Lowest number of mummies/ female (19.6 \pm 2.89 mummies) was recorded at 12 $^{\circ}\text{C}$, in the mummies stored for four weeks. Accordingly, the storage period had a little effect on the emergence rate than its effect on the number of mummies / female. After storage at 10 $^{\circ}\text{C}$ for four weeks, a few parasitoid adults emerged 5.33% (about 0-2 parasitoids / replicates therefore, the parasitoids weren't exposed to aphids. During the fourth week, some adult parasitoids emerged from the stored mummies inside the incubator at 15 $^{\circ}\text{C}$.

Statistical analysis showed that there were significant differences among the temperatures 10, 12, and 15 $^{\circ}\text{C}$, in the emergence rates and total numbers of mummies/ female in all cases. Statistical analysis also showed that there were no significant differences between the temperatures 12 and 15 $^{\circ}\text{C}$ in the emergence rates

and total numbers of mummies/ female, in all cases, except in total number of mummies/ female in the third week. But there were significant differences between them in total numbers of mummies/ female in all cases.

These results agree with those of Marcella *et al.* (1997), who measured the effect of storage for 0, 2, 4, 7, and, 14 days at 10°C on adult emergence from mummies in *A. albipodus*. Adult emergence decreased significantly when mummies were stored for 14 days compared to 0-7 days. Emergence rates of the stored mummies for one and two weeks averaged 76.5, and 67%, respectively.

In conclusion, obtained results cleared that for mass rearing of *A. Albipodus*, *S. avenae* was the favorite aphid species based on both total number of mummies and percentages of emergence. Highest emergence rates (94.49%) were estimated at 15 °C on mummies stored for one week while the lowest number of mummies/ female (19.6 ± 2.89 mummies) was recorded at 12 °C, in mummies stored for four weeks. *A. albipodus* can be used in winter crops, as a biocontrol agent against the aphid species, *A. craccivora*, *S. avenae* and *R. padi*.

Table 3. Mean percentage of adult emergence and numbers of mummies/ female (\pm S.E and range) of *Aphelinus albipodus* emerged from stored mummies at 10, 12 and 15 \pm 0.5°C, for one month.

Week(s)	Temperatures					
	10 °C		12 °C		15 °C	
	% Adult emergence	No. of mummies/ female	% Adult emergence	No. of mummies/ female	% Adult emergence	No. of mummies/ female
One	75.64	118.6 \pm 8.14 (99 - 137)	91.4	148.2 \pm 11.1 (113-175)	94.49	154.2 \pm 8.63 (133-185)
Two	43.33	76.8 \pm 4.33 (66 - 86)	85.53	79.2 \pm 2.44 (72 - 85)	89.46	89.2 \pm 2.82 (84-99)
Three	25.7	21 \pm 1.64 (18 - 27)	75.58	33.2 \pm 3.47 (23 - 41)	88.18	54.6 \pm 4.02 (44-64)
Four	5.33		52.6	19.6 \pm 2.89 (11 - 27)		

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الخصائص البيولوجية الهامة فى التربية الكمية لطفيل المن النوع

***APHELINUS ALBIPODUS* HAYAT & FATIMA
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تعتبر آفة المن بين الآفات الحشرية الخطيرة التي تهاجم أنواع نباتية عديدة. ومن أهم طفيليات المن النوع *Aphelinus albipodus* Hayat & Fatima. درست الخصائص البيولوجية الأساسية للطفيل مثل طول العمر، الكفاءة التناسلية، التفضيل العوائلى، وتخزين الموميوات، كمساهمات أساسية للمعلومات اللازمة للتربية الكمية تحت الظروف المعملية. كانت جميع الحشرات الكاملة للطفيل من الإناث، قدر متوسط طول العمر (بالأيام) للطور الكامل بالتغذية على العسل وبدون تغذية 10.85 ± 0.82 يوما عند التغذية، و 1.44 ± 0.12 يوما عند التجويع. بلغ مجموع عدد البيض/ الأنثى 67.9 ± 7.21 (٣٠-١١٠) (بيضة/أنثى) على نوع من الشوفان *Rhopalosiphum padi*. أظهرت النتائج المتحصل عليها والتي تؤخذ في الاعتبار عند التربية الكمية للطفيل، أن نوع المن *Sitibion avenae* كان النوع المفضل لتربية الطفيل بناء على كل من العدد الإجمالى للموميوات/ قفص والنسبة المئوية لخروج الحشرات الكاملة منها. قدر أعلى معدل للخروج (٩٤,٤٩%) على درجة ١٥ مئوية من موميوات تم تخزينها لمدة أسبوع واحد. بينما سجل أدنى عدد من الموميوات/ أنثى (19.6 ± 2.89 موميوات) على درجة ١٢ مئوية من موميوات تم تخزينها لمدة أربعة أسابيع. يوصى باستخدام الطفيل *A. albipodus* فى الزراعات الشتوية كعامل مكافحة بيولوجية ضد أنواع المن *Aphis R. padi* & *S. avenae* & *craccivora* Koch.

كلمات مفتاحية: *Aphelinus albipodus*، طول العمر، الكفاءة التناسلية، التفضيل العوائلى، التخزين، التربية الكمية.