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Preliminary Experiments for Rearing and Use of the Predatory Aphid Midge, *Aphidoletes aphidimyza* (Rondani) (Diptera: Cecidomyiidae) for Controlling the Cotton aphid, *Aphis gossypii* on Cucumber

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ABSTRACT

Rearing of the predator *Aphidoletes aphidimyza* and its prey, *Rhopalosiphum padi* was carried out in two controlled condition rooms of 25±1°C, 70% R.H. and 16 L: 8 D photoperiod. Results showed that cotton wool and peat as pupation substrates gave higher adult emergence rates than sand. Peat keeps the humidity for a longer time. In addition, it is possible to be reused several times. Thus, peat is cheaper than cotton wool in rearing of the predator under investigation. It could be recommended that, to save on staff, time and cost, production should be carried over a 4-day period where the cumulative production was 91% of deposited eggs and 90.9% adults were obtained while total eggs and adults (100%) were obtained in 7 days. In a base unit of 500 individuals of the predator, the total cost comprising; basic cost and peat was 3.077 L.E. where this cost doesn't include the cages and boxes cost. Three releasing rates (1 pupa: 3 aphids, 1: 10 and 1: 20) were tested in cages to control the cotton aphid, *A. gossypii* on cucumber by the predator. The present findings suggest further experiments to test this predatory midge for controlling aphids in greenhouses by one release at a ratio of 1 pupa: 10 aphids.

Key words: *Aphidoletes aphidimyza*, Cecidomyiidae, rearing, biological control, *Aphis gossypii*, cucumber.

INTRODUCTION

The predatory midge, *Aphidoletes aphidimyza* is a general aphid predator, attacking different species of aphids on different host plants (Wood-Baker, 1965; Harris, 1973 and Nijveldt, 1988). Mass production of this predator was undertaken on various aphid species on various host plants with sand, peat or cotton wool as pupation substrates (Bondarenko and Asyakin, 1975; Rimpiläinen, 1980; Lieburg and Ramakers, 1984; Popov and Belousov, 1987; Belousov and Popov, 1989). Since the beginning of the 1970's, *A. aphidimyza* has been used for biological aphid control, mainly in Finland, USSR, Denmark, Germany and Netherlands on commercial vegetables and roses growing in greenhouses and outdoors (Harris, 1982; Krivan and Havelka, 2000). Research on *A. aphidimyza* has increased concerning its mass production and its effectiveness to control aphids on various plants, especially in greenhouses (Gilkeson, 1990; Bennison and Corless, 1993; Quentin *et al.*, 1995 and Mulder *et al.*, 1999). The present work aims to undertake rearing of this predator and investigate its efficacy against aphids under Egyptian environmental conditions. The cost of the predator production is also considered.

MATERIALS AND METHODS

Mass Rearing Trial

A. aphidimyza and the cereal aphid, *R. padi* as natural prey were reared in two separated rooms in Chrysopa-Mass Production Project at Faculty of Agriculture, Cairo University at conditions of 25±2°C, 70-80 % R.H. and 16:8 h L:D photoperiod.

A. The Cereal Aphid *R. padi*

Wheat seeds were immersed in water for 24 hrs. The agricultural perlite was also immersed in water for 2-3 h. Two cm high of perlite (=75 g) was placed in metallic dishes (20 cm diameter and 3 cm high). 40 g wheat seeds were dispersed and fixed thoroughly to the agricultural perlite. Dishes were covered by plastic cover to preserve humidity needed for seed germination. These dishes were kept in a transparent hard plastic cage of 2 m high, 3 m long and 70 cm wide. This cage has 4 shelves; 2 shelves for wheat seedlings and the other two for infested ones. Three days after plantation, when plants became 1 cm high, the plants were infested with the aphid, *R. padi* at a level of 1 infested dish / 5 uninfested ones. Five days later, the infested plants were harvested for re-infestation or to be used as prey source for predator larvae.

B. *A. aphidimyza*

Adult Rearing and Egg Collection

A rearing unit for *A. aphidimyza* was designed measuring 100 cm wide, 60 cm long and 200 cm high and was divided into 10 sub-units of 50x60x40 cm each. Each sub-unit has two openings of 20x20 cm covered by muslin cloth for aeration. One hundred pupae of *A. aphidimyza* were provided to each sub-unit together with a pot containing 10 bean seedlings (20 cm high) infested with 200 aphids, *Aphis craccivora*, drops of nutritional solution (1 bee honey: 1 yeast) were provided on the inner surface of the cage as food for adults of the predator. A small Petri dish of water, filled with horticultural perlite was added to avoid drowning of insects (Gilkeson, 1987). The bean pot had to be changed daily (egg collection) until all adults of the predator died. The total eggs were counted on each plant.

Rearing of Larvae

A plastic box of 15x20x10 cm size bottomed by a sheet of thin plastic was filled with 2 cm high of moistened peat. The bean plants (with eggs) were placed on this peat layer together with individuals of the aphid, *R. padi* as preys for the predator larvae. The top of these boxes has an opening area of 12 x 17 cm covered with muslin cloth that facilitates aeration inside each box. All boxes were kept inside the same room of the rearing unit.

C- Selection of Pupation Substrate

Three different substrates for larval pupation of *A. aphidimyza* were tested *i.e.*, peat, sand and cotton wool, to indicate their effect on pupal duration and rate of adult emergence. Each treatment was replicated 5 times. Glass boxes of 5 cm high and 7 cm diameter were used in this experiment; the bottom of each box was covered with a layer of 2 cm by one of the above-mentioned substrates and moistened daily with water. Twenty mature larvae of the predator were introduced into each box on the pupation substrate.

D. Reuse of Peat as Pupation Substrate

In this experiment, three larval rearing plastic boxes were used as replicates to investigate this effect. Each box was provided with 300 mature larvae of the predator and sufficient number of aphid, *R. padi* placed on a layer of 3 cm thick moistened peat that was sterilized at 80°C for 1 hour to prevent the contamination by fungi and other microorganisms. After emergence of adults, the same peat was used 10 successive times and adult emergence rate was recorded each time.

E. Selection of Host Plant as Ovipositional Site

Four host leguminous plants (20 cm high) each infested by 50 individuals of *A. craccivora* were tested as oviposition sites for the predator under investigation. These plants were faba bean (*Vicia faba*), kidney bean (*Phaseolus vulgaris*), cowpea (*Vigna unguiculata*) and peas (*Pisum sativum*). The four tested plants were kept in the same cage of 50X50X50 cm in size with five mated females (2 days old) of *A. aphidimyza*. The experiment was repeated 5 times. After 24 h, the number of eggs on each plant was counted.

Releasing Procedure

Sixteen iron-frame cages (60 cm wide and long by 150 cm high) covered with muslin; each contained 1 cucumber plant, *Cucumis sativus* in a pot (25 cm diameter). Plants were infested by 100 aphid individuals of *Aphis gossypii* per plant. Cages were placed in open field beside the project facility. Four replicates of three release rates (1 predator pupa: 3 aphid, 1: 10 and 1: 20) and control were used in a complete randomized design (CRD). *A. aphidimyza* mature larvae were placed in rearing boxes with moistened peat (on October 1). When pupae were formed, the boxes were placed inside the cages (on October 9). The sex of adults was determined in the laboratory from other boxes contained larvae collected from the same culture and they were released in a ratio of 1 male: 1 female. A second release of adults was made 9 days later. Leaves from each plant were examined every day beginning with the first release date; all aphids and *A. aphidimyza* larvae were counted by 4 X lens.

Statistical Analysis

Data for all experiments were statistically analyzed using classification one-way ANOVA using a software package "MSTAT version 4, 1987". Significant differences among these means were carried out by Duncan's multiple range test ($P \leq 0.05$).

RESULTS AND DISSCUSION

Production of the Cereal Aphid, *R. padi*

As shown in Table (1), the obtained results showed that each rearing dish produced a weight of 1268.6±38.9 mg cereal aphids. One-gram from this aphid contains 4510 aphid individuals. Thus, each rearing dish produced an average of 5721.4 aphid individuals.

A rearing room contains four shelves for aphid production each has 45 rearing dishes (20 cm diameter). Consequently, 180 dishes/5 days or 1080 rearing dishes are produced monthly. This production technique could produce 1.37 kg aphids per month.

Table (1): Some parameters related to mass rearing of *A. aphidimyza*.

Paramters		Mean ± SD (range)
Aphid production/dish/mg		1268.8±38.9 (1179-1383)
% of deposited eggs of *A. aphidimyza* on various ovipositional substrates (plants)	Faba bean	21.1±1.6 (30.6-33.2)
	Kidney bean	34.7±2.7 (31.1-37.9)
	Cowpea	14.7±1.0 (14.1-15.7)
	Peas	18.3±1.9 (16.3-19.9)
Emergence rate (%) of *A. aphidimyza* with reuse of peat		89.7±0.86 (86.5-92.2)

Rearing of the Predatory Midge, *A. aphidimyza*

Prior to the rearing of the predator under investigation, three various pupation substrates *i.e.* sand, peat and cotton wool were tested to evaluate their effect on pupal duration and rate of adult emergence. The presented results in Table (2) show that the aforementioned substrates did not affect the pupal duration, where pupae lasted 7.25, 7.1, 6.75 days in the respective substrates sand, peat and cotton wool. A similar finding was recorded by Markkula *et al.* (1979b). On the contrary, in the present investigation, the tested substrates affected the adult emergence rate where it reached 74% with sand, and 89% for both peat and cotton wool. Markkula *et al.* (1979b) recorded the same rate for sand and peat while perlite gave a low emergence rate of 20%. They mentioned that the optimal emergence rates from peat and sand were 60-80% and 70-80%, respectively.

Reuse of Peat as Pupation Substrate

As for the possibility of continuous usage of peat as pupation substrate, the obtained results showed no significant differences among adult emergence rates from peat reused 10 times as pupation substrate. These rates ranged from 86.5% to 92.2%, with a final adult emergence rate of 89.7±0.86% (Table 1). Moreover, peat keeps the humidity for a longer time than in both sand and cotton wool. Therefore, peat was used as economical pupation substrate in rearing, *A. aphidimyza* in the present investigation.

Table (2): Effect of different substrates on pupal development and adult emergence rate of *A. aphidimuyza*.

Substrate	% Adult emergence (Mean±SD)	Pupal duration in days (Mean±SD)
Sand	74.00±4.30 b (60-85)	7.25±0.23 a (6-10)
Peat	89.00±5.40 a (70-100)	7.10±0.18 a (6-9)
Cotton wool	89.00±2.90 (80-95) a	6.75±0.23 a (6-9)

Mean in the same column followed by the same letter are not significantly different ($P \leq 0.05$).

Selection of Host Plant as Ovipositional Substrate

It appears that both faba bean and kidney bean plants attracted the females of *A. aphidimyza* for oviposition more than both cowpea and peas plants where the mean percentages were 32.1, 34.7, 14.7 and 18.3%, respectively (Table 1). In this respect, Chalnder (1966) mentioned that the aphidophagous insects select plants by visual stimuli. Mansour (1975) stated that the *Aphidoletes* females could discriminate between the different species and varieties of plants. Seeds of faba bean are cheaper in comparison with other leguminous seeds, and it is easy in handling and quick in growing. Thus, faba bean plants infested with *A. craccivora* were used as ovipositional substrate in rearing of the predator.

Production of *A. aphidimyza*

Total egg production from one sub-unit of the predator-rearing unit is presented in Table (3). It shows that adults emerged from 100 pupae lived 9 days at maximum (2 days preoviposition period and 7 days oviposition period for females). Oviposition increased gradually during the first three days. Thereafter, the daily number of deposited eggs decreased as the number of females decreased gradually. The total number of deposited eggs reached 3203 and the number of individuals that completed their development were 2031 adults. During the seven days, the ratio of adults produced from deposited eggs fluctuated from 61.4 to 68.7% with a mean ratio of 63.4%. The produced adults (2031 individuals) consumed a yield of 16 aphid-rearing dishes during their larval stage whereas 1846.6 adults needed 14 aphid dishes for oviposition during the first four days.

Regarding the cumulative egg production, 91% were deposited during the first four days of oviposition, and produced 90.92% of the total adults produced. Consequently, it could be recommended that, to save staff, time and cost, production should be carried over 4 days period instead of 7 days. Therefore, in one sub-unit, a rearing cycle of 7 days (1 day to change the contents and clean the rearing unit, 2 days for preoviposition and 4 days for oviposition period) produced 1846.6 adults of the predator with a sex ratio approximately 1:1. The rearing unit has 10 sub-units, and the room contains 2 rearing units.

Consequently, total production of the predator's adults per month could be calculated as 1846.6 adults x 10 sub-units x 2 rearing units x 4 rearing cycle = 147728 adults / month.

Estimation of the Production Cost of *A. aphidimyza* over a Monthly Basis

For this purpose all the accountable sources of expenses were determined and are listed hereunder:

- Actually, every five days, 180 rearing dishes of the cereal aphid *R. padi* are produced. Thus, 1080 rearing dishes are produced monthly. This value of aphid production is required for production of *A. aphidimyza* in the two rearing units monthly. The estimated cost doesn't include the price of rearing cages and boxes.
- Working team about 500 L.E, water and electricity (= 50 L.E.), equipments for sterilization and maintenance (= 20 L.E.). For *R. padi* rearing over a monthly period, wheat and perlite, 1080 dishes x [0.15 L.E. (75 gm perlite) + 0.04 L.E. (40 gm wheat seeds)] = 205.20 L.E.
- For *A. aphidimyza* production over a monthly period: 2 rearing units can produce actually 147 680 adults monthly, bean seeds (4 kg x 2.5 L.E. = 10 L.E.) and bees honey and yeast (= 20 L.E.). Thus, the basic monthly total is about 805.5 L.E.
- In a base unit of 500 individuals of the predator, one box contains 200 g of peat (= 0.35 L.E.) and the basic cost is about 2.727 L.E. Thus, total = 3.077 L.E.
- Consequently, the production cost of one adult of the predator, *A. aphidimyza* is 0.0062 L.E.
- The present price (= 0.0062 L.E./ insect) is about 33 fold less than that reported by Cranshaw *et al.* (1996) who mentioned that the prices quoted by suppliers showed a three fold range of $ 0.027 to $ 0.075 / insect (mean = $ 0.0443).

Preliminary Releasing Test

Three releasing rates (1 pupa: 3 aphids, 1: 10, 1: 20) were tested for control of the cotton aphid, *A. gossypii* on cucumber plants by the predatory midge, *A. aphidimyza* in the previously described cages. Results of this experiment are presented in Fig. (1), to show the population fluctuations of aphids and larvae of the predator.

Larvae of the predator appeared on the fourth day after release on cucumber plants in all cages by the rates of 13, 14 and 4.5 individuals / plant at release rates of 1 pupa: 3 aphids, 1: 10 and 1: 20, respectively. The larval count tended to increase gradually reaching the rate of 47, 43 and 20 individuals/plant on the seventh day for the respective release rates. This increase in numbers of larvae was followed by a decrease in aphid population/plant. Then, numbers of larvae of the predator decreased gradually. At the same time, the population of aphids decreased to be zero at release rates of 1: 3 and 1: 10 on the 10*th* and 11*th* day post release, respectively. No larvae were noticed on day 12 in the release rates of 1: 3 and 1: 10 while 2.5 larvae/plant were found in the rate of 1: 20; the population of aphids reached 2650 individuals/plant on the same day on control plants.

When the second release was carried out 10 days after the first release (at the rate of 1: 20), the number of larvae increased gradually from the 13*th* to the 15*th* days. Despite this increase among predator larvae, the population

Table (3): Egg and adult production of *A. aphidimyza* from a sub-unit of the rearing unit.

Suc. days	Egg pro.	Cu. egg pro.	Cu. egg pro. (%)	Adult pro.	Cu. adult pro.	Cu. adult pro. (%)	Dev. rate (%)	No. of used Aphid dishes
1	533.3 ±65.7	533.3	16.65	365.3 ±42.5	365.3	18	68.7±2.0	3
2	849.3 ±31.2	1383	43.17	527.3 ±11.2	892.6	44	62.1±1.2	4
3	928.3 ±103.0	2310	72.14	562 ±52.1	1454.6	71.6	61.4±5.8	4
4	603.7 ±57.7	2914	91.00	392.0 ±44.1	1846.6	90.9	64.9±3.9	3
5	213.3 ±34.2	3127	97.60	136.3 ±27.2	1983.0	97.6	63.1±2.7	1
6	63.0 ±26.5	3190	99.60	39.0 ±15.9	2022.0	99.5	62.3±1.1	0.5
7	14.0 ±14.0	3204	100	9.0 ±9.3	2031.0	100	66.4±22.2	0.5
Total	3204.0 ±178.0	3204	100	2031.0 ±93.9	2031.0	100	63.4±2.0	16

Suc.= Successive, pro.= Produced, Cu.= Cumulative, Dev.= Developed adults

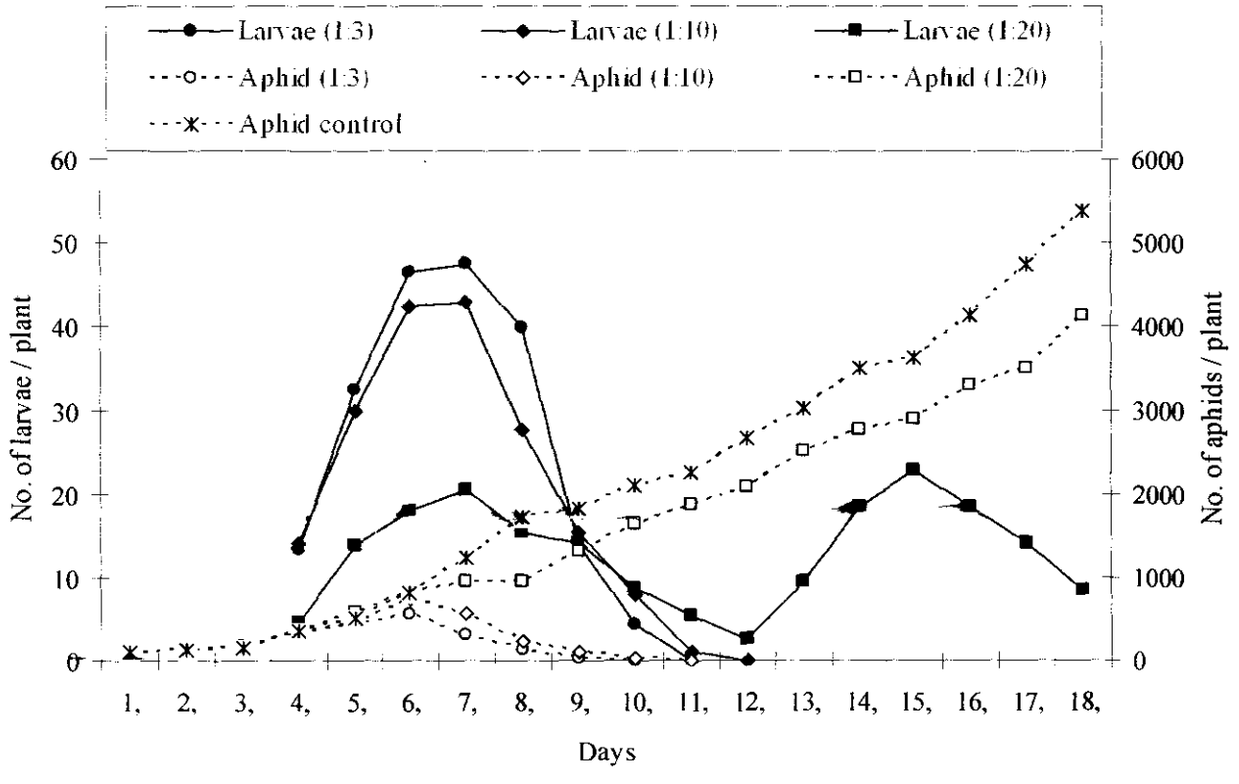

Fig.(1): Population of aphids and *Aphidoletes aphidimyza* larvae in releasing rates experiment to control *Aphis gossypii* on cucumber plants in the test cages.

of aphids increased reaching 4125 individuals/plant at the end of the experiment while aphids population on control plants reached 5375 individuals/plant. It was noticed that results of 1:3 and 1: 10 releases were similar for controlling aphids in the present investigation. Many authors showed that the release rate of 1 pupa: 3 aphids were suitable for controlling aphids in greenhouses (Markkula *et al.*, 1979a; Markkula and Tiittanen, 1982; Gilkeson and Hill, 1987). Other authors, *e.g.*, Bondarenko (1987) and Gilkeson and Hill (1987) stated that release rate of 1: 9 or 1: 10 can be successful in controlling aphids in controlled greenhouses. It appeared in the present investigation that the lowest releasing rate of 1:20 was inadequate for controlling aphids in the test cages on cucumber even after the second release. On the contrary, Cheng *et al.* (1992) recorded that the release rate of 1: 20 when the aphid population reached 200/plant reduced the aphid population by 75.1-91.8% in the greenhouses. Moreover, Lenteren (1987) mentioned that one release of the gall midge in the ratio of 1:50 prevented pest reproduction for a month. The present findings suggest further investigation to use the predatory midge for controlling aphid in greenhouses in Egypt at a ratio of 1 pupa: 10 aphids.

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