Pure Appl. Biol., 9(1): 609-617, March, 2020
http://dx.doi.org/10.19045/bspab.2020.90066

Research Article

Laboratory test of the *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae) against the pupae of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

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Citation
Muhammad Awais, Niaz Hussain Khuhro, Muhammad Hamayoon Khan, Raza Muhammad Memon and Muhammad Usman Asif. Laboratory test of the *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae) against the pupae of *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae). Pure and Applied Biology. Vol. 9, Issue 1, pp609-617. http://dx.doi.org/10.19045/bspab.2020.90066

Received: 03/08/2019 Revised: 25/10/2019 Accepted: 18/11/2019 Online First: 21/12/2019

Abstract

Fruit flies being serious pest of fruits and vegetables are responsible for huge economic losses in the world. Pupal parasitoid, *Dirhinus giffardii* is one of the most significant biological control agents that has been used effectively in controlling the fruit flies. Laboratory studies were conducted to investigate the parasitism, emergence and post emergence sex ratio of *D. giffardii* through different exposure times and parasitoid density against the pupae of melon fruit fly, *Bactrocera cucurbitae*. The studies manifested that the exposure time and parasitoid density had a significant effect on the parasitism and emergence ratio of parasitoids. Results revealed that mean rate of parasitism (45.66) was the highest in case of five pair of parasitoids and mean rate of emergence (42.66) of *D. giffardii* was also the highest on exposure period of six days. Further, it was observed that, exposure time and parasitoid density had no significant effect on the post emergence sex ratio of male and female parasitoids. The mean per female parasitism was increasing with the increase in number of pairs of *D. giffardii* and reached to its peak after exposure time of six days. These findings suggest that *D. giffardii* has a great parasitizing potential against the pupae of *B. cucurbitae* and can effectively suppress its populations.

Keywords: *Bactrocera cucurbitae*; *Dirhinus giffardii*; Emergence; Parasitism; Sex ratio

Introduction

The melon fruit fly, *Bactrocera cucurbitae* (Coquillett) is the most damaging pest of vegetables. It is distributed all over the world but mostly cause significant damage in Asian countries like China, Pakistan, India, New Guinea, Nepal, Hawaiian Islands and Philippines [1]. According to a survey *B. cucurbitae* harms over eighty one host plants, however it is the main pest of cucurbitaceous

vegetables [2]. In general, the fruit flies cause massive losses to fruits and vegetables in the entire world and are known as key insect pest of the horticultural fruit plants. Approximately, they have 4,000 species which are well identified in all over the world [3]. In Pakistan, the *B. cucurbitae* causes 50-90% losses in fruits like melon, guava, citrus, mango and cucurbits [4]. Melon fruit fly can also injure non-cucurbit host plants by damaging their stem, flowers and root tissues [5]. The adult melon fruit fly lay 300-1000 eggs, during egg laying process ovipositor pierces the skin of fruits, after the eggs hatch, maggots begin to start feeding on the pulp of fruits by making tunnels inside the fruits [6]. During feeding, maggots consume food to store energy for the upcoming pupal stage which cause post-harvest losses and due to this reason market value of fruits become reduced [7].

In general, farmers use enormous amount of pesticides for the suppression of melon fruit fly which is declared as a quarantine pest. Agrochemicals and insecticides are notorious elements that become the prominent fragment of worldwide agriculture systems from the last century. Pesticidal leftovers did scatter in the environment, causing shocking defacement of land ecosystems and contaminating the human foods [8, 9]. The human health is on real threat and facing certain difficult problems because in our agro-ecosystem, agrochemicals deposits are present at every time [10]. In order to control pests, growers adopt the habit of frequent and unjudicious application of pesticides as a result, insect pests develop resistance that make vulnerable their competence and also depreciate the environment [11]. Different human fitness related concerns are correlated with pesticides, such as cancers, nausea, headaches, birth defects, endocrine disruption and infertility [12]. Particularly due to constant exposure of pesticides children health is at more risk [13]. Therefore, reduce fruit fly losses by using non-chemical methods will be implemented. There are some substitutes to pesticides that do not cause the same environmental problems. One of these replacements include biological control methods which provide one of the most effective, obviously safe, and feasible mechanisms against insect pests [14]. By using biological control tactic, living organisim such as parasite, predator and disease causing organism is being introduced in to the environment of pests to reduce their population. Parasitoid, *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae) is a useful biological control agent which can be used as a surrogate of pesticides hazards to lessening the population of fruit flies [15]. *D. giffardii* is a pupal parasitoid that attacks a wide range of fruit-infesting tephritids, including *B. cucurbitae*, *B. tryoni*, *B. dorsalis*, and *B. oleae* [16]. Original host of this parasitoid is *Ceratitis capitate*, it is native of West Africa and have the ability to parasitize dipterous houseflies [16, 17]. It has been used for the first time in West Africa to control Black Soldier Fly [18].

Pupal parasitoid, *D. giffardii* pierces the puparial skin of the fruit fly pupa and oviposits inside the puparium. This parasitoid can produce healthy and bigger size offspring when its females parasitize to larger pupae of host species [19]. The fecundity rate of *D. giffardii* starts to decline as its age increases, for better mass rearing, it has been recommended that the parasitoids would be discarded after 15 days [20]. The biological control with the help of *D. giffardii* is a strong management platform of the tephretids and has quarantine importance [21]. Previous, studies were performed to determine the efficiency of *D. giffardii* on various aged pupa, but very few researches were conducted on the latent and the parasitism enactment of this parasitoid against *B. cucurbitae*. Furthermore, the present study

was planned to evaluate the performance of *D. giffardii* in controlling melon fruit fly.

Materials and methods

In order to evaluate the parasitism of *D. giffardii* against pupae of *B. cucurbitae*, experiments were performed at Bio-Control Research Laboratory of Fruit Fly, Plant Protection Division, Nuclear Institute of Agriculture (NIA), Tandojam, Sindh during December, 2018. Laboratory reared culture of biological control agent *D. giffardii* (12 days old) and melon fruit fly pupae (2 day old) were used as a stock culture during the experiments following five replications, under laboratory conditions (28 ± 2°C and 65 ± 5% RH). Parasitoids were released in pair form (1 pair, 2 pair, 3 pair, 4 pair and 5 pair) on each 100 pupae of melon fruit fly for different exposure periods (1 day, 2 day, 3 day, 4 day, 5 day & 6 day).

Rearing of *Bactrocera cucurbitae*

The fruit flies, *B. cucurbitae* were mass reared on pumpkin and artificial diet containing wheat bran (26%), sugar (12%), dried troula yeast (3.6%), Sodium benzoate (0.1%), Methyl-p-hydroxybenzoate (0.1%) and water (58%) **[22]**. Eggs of fruit flies were placed directly on the diet trays having artificial diet. These eggs were collected in plastic glasses having 0.5 mm holes around them smeared internally with guava juice and put in adult fruit fly cages. The hatched larvae feed on the diet till complete maturation. After the full fed larvae jumped out of the trays and fell on the substrate (sand/saw dust) for pupation, the pupae were collected through sieving and used for maintaining the culture and experiments. The adult fruit flies were provided protein hydrolysate and sugar.

Rearing of *Dirhinus giffardii*

The colony of parasitoids *D. giffardii* being well maintained at (NIA, Tando jam) bio-control agents rearing lab from the last several years. Parasitoids were reared in glass cages on pupae of *B. cucurbitae* and artificial diet, a fresh diet solution (30% honey and 70% water) were offered to the parasitoids through soaked cotton wigs which were impregnated with honey and water.

Data analysis

After mentioned exposure periods rate of parasitism, sex ratio and parasitoids emergence was recorded and results were analyzed by using the software statistics 8.1. One-way analysis of variance (ANOVA) and Tukey's honestly significant difference (HSD) test were used for comparisons of means among different treatments.

Results

The investigations on pupae of melon fruit fly (*B. cucurbitae*) were conducted by using various exposure periods and parasitoid pairs in order to check the parasitism, emergence and post emergence sex ratio of *D. giffardii*. The results obtained from the experiments showed that after the exposure time of 24 hour, the maximum mean parasitism of 24.33±1.20 was recorded by using 5 pairs of *D. giffardii* followed by 17.67±1.76, 14.67±1.20, 8.33±2.03 and 4.33±0.88 on 4>3>2 and 1 pair, respectively. The maximum emergence of *D. giffardii* from parasitized pupae was recorded 21.00±2.00 while the minimum emergence was 3.33±0.88. Analysis of variance of the present results indicated that there is a significant difference in the various treatments *(P<0.05)*. Sex ratio of emerged parasitoids showed that maximum male (59.45±3.68) were recorded by 2 pairs of *D. giffardii* while maximum females (47.57±0.25) were recorded after 1 day exposure period (Table 1).

After 48 h exposure time, results depicted that mean parasitization by 1, 2, 3, 4 and 5 pairs of *D. giffardii* were 7.66±1.76, 10.33±1.46, 15.00±1.73, 19.33±2.02 and 25.33±1.45, respectively. The same sequence was also true for mean parasitoid emergence where maximum mean emergence of *D. giffardii* was 21.67±1.46 by five pairs while the minimum emergence was recorded

5.00±1.15 by 1 pair. Post emergence sex ratio of parasitoids revealed that highest male and female percentage of *D. giffardii* was 61.27±2.82 and 49.12±0.87, respectively. Percentage of male and female sex ratio did not differ significantly among all the treatments (Table 2). The results after 3 days exposure period showed that the maximum parasitism (28.33±2.03) and maximum emergence (26.00±1.73) was recorded from five pairs of *D. giffardii.* The maximum male adult emergence (54.40±2.21) was observed by four pairs and maximum female adult emergence of 48.48±1.51 was recorded from 1 pair of *D. giffardii* after the exposure time of 3 days. It was recorded that parasitoid pairs and exposure time have significant effects on the parasitism and emergence rate of *D. giffardii*. Maximum male and female sex ratio after the exposure time of four days were 54.40±2.21 and 48.48±1.51 by four pairs and 1 pair of *D. giffardii,* respectively (Table 3).

Results after 96 hours exposure time revealed that maximum mean parasitism was 32.33±2.61 and minimum mean parasitism was 10.00±2.30 by 5 and 1 pair of parasitoids, respectively. The highest rate of emergence of parasitoids was 30.33±2.90 and lowest was 8.00±2.30 on five pairs and 1 pair of *D. giffardii,* respectively. Maximum post emergence sex ratio of male *D. giffardii* was 54.93±2.53 and for female was 49.60.±2.53 after the four days exposure time (Table 4). Similar trend of parasitism and emergence was observed after 120 hours exposure time, which showed that maximum mean parasitism of 35.33±4.66 and maximum mean emergence of 33.66±4.40 were observed by 5 pairs of *D. giffardii.* Maximum male sex ratio of emerged *D. giffardii* was 55.18±2.89 and lowest was 48.50±0.78 after the five days exposure time (Table 5). Results of 144 hours exposure time revealed that maximum mean parasitism and mean emergence was 45.66±2.60 and 42.66±2.33, respectively whereas minimum mean parasitism and mean emergence was 13.00±2.30 and 11.33±2.60, respectively. Lowest female percentage (46.10±2.08) while the highest male percentage (53.89±2.08) were recorded from single pair of *D. giffardii* (Table 6). Maximum per female mean parasitism by *D. giffardii* against the pupae of *B. cucurbitae* was 13.00±2.30 and this was observed after the exposure period of 144 h, while minimum mean per female parasitism by *D. giffardii* was 4.16±1.01 at the exposure period of 24 h (Fig. 1).

Table 1. Pupae of *Bactrocera cucurbitae* parasitized by *Dirhinus giffardii*, emergence and sex ratio of parasitoids after 1 day exposure period

No. of *D. giffardii* Released on Host (Pupae)	No. of Parasitized Pupae	No. of Emerged *D. giffardii*	Sex Ratio of *D. giffardii*	
			Male (%)	Female (%)
1 pair	4.33 ± 0.88 c	3.33 ± 0.88 d	58.88 ± 4.84 a	41.11 ± 4.84 a
2 pair	8.33 ± 2.03 c	7.00 ± 2.31 cd	59.45 ± 3.68 a	40.54 ± 3.68 a
3 pair	14.67 ± 1.20 b	11.67 ± 1.45 bc	54.63 ± 2.44 a	45.37 ± 2.44 a
4 pair	17.67 ± 1.76 b	13.67 ± 1.46 b	53.89 ± 2.08 a	46.10 ± 2.08 a
5 pair	24.33 ± 1.20 a	21.00 ± 2.00 a	52.43 ± 0.25 a	47.57 ± 0.25 a

Means sharing similar letters in columns are not significantly different at $p<0.05$

Table 2. Pupae of *Bactrocera cucurbitae* parasitized by *Dirhinus giffardii*, emergence and sex ratio of parasitoids after 2 days exposure period

No. of *D. giffardii* Released on Host (Pupae)	No. of Parasitized Pupae	No. of Emerged *D. giffardii*	Sex Ratio of *D. giffardii*	
			Male (%)	Female (%)
1 pair	7.66 ± 1.76 d	5.00 ± 1.15 d	61.27 ± 2.82 a	38.73 ± 2.82 b
2 pair	10.33 ± 1.46 cd	8.33 ± 2.02 cd	53.33 ± 3.33 ab	46.66 ± 3.33 ab
3 pair	15.00 ± 1.73 bc	13.33 ± 1.45 bc	52.79 ± 1.41 ab	47.20 ± 1.41 ab
4 pair	19.33 ± 2.02 ab	17.00 ± 1.73 ab	57.70 ± 4.16 ab	42.29 ± 4.16 ab
5 pair	25.33 ± 1.45 a	21.67 ± 1.46 a	50.87 ± 0.87 b	49.12 ± 0.87 a

Means sharing similar letters in columns are not significantly different at $p<0.05$

Table 3. Pupae of *Bactrocera cucurbitae* parasitized by *Dirhinus giffardii*, emergence and sex ratio of parasitoids after 3 days exposure period

No. of *D. giffardii* Released on Host (Pupae)	No. of Parasitized Pupae	No. of Emerged *D. giffardii*	Sex Ratio of *D. giffardii*	
			Male (%)	Female (%)
1 pair	8.33 ± 2.02 d	7.00 ± 2.08 d	51.51 ± 1.51 a	48.48 ± 1.51 a
2 pair	13.00 ± 2.30 cd	11.33 ± 2.60 cd	53.89 ± 2.08 a	46.10 ± 2.08 a
3 pair	18.00 ± 2.30 bc	16.00 ± 2.30 bc	51.66 ± 1.66 a	48.33 ± 1.66 a
4 pair	23.00 ± 2.31 ab	21.00 ± 2.31 ab	54.40 ± 2.21 a	45.59 ± 2.21 a
5 pair	28.33 ± 2.03 a	26.00 ± 1.73 a	52.74 ± 1.95 a	47.25 ± 1.95 a

Means sharing similar letters in columns are not significantly different at $p<0.05$

Table 4. Pupae of *Bactrocera cucurbitae* parasitized by *Dirhinus giffardii*, emergence and sex ratio of parasitoids after 4 days exposure period

No. of *D. giffardii* Released on Host (Pupae)	No. of Parasitized Pupae	No. of Emerged *D. giffardii*	Sex Ratio of *D. giffardii*	
			Male (%)	Female (%)
1 pair	10.00 ± 2.30 d	8.00 ± 2.30 d	52.00 ± 1.41 a	48.00 ± 1.41 a
2 pair	15.00 ± 2.30 cd	13.33 ± 2.02 cd	54.22 ± 2.55 a	45.77 ± 2.55 a
3 pair	21.00 ± 2.31 bc	19.00 ± 2.30 bc	54.93 ± 2.53 a	45.06 ± 2.53 a
4 pair	26.33 ± 2.60 ab	24.33 ± 2.02 ab	50.39 ± 1.72 a	49.60 ± 2.53 a
5 pair	32.33 ± 2.61 a	30.33 ± 2.90 a	51.68 ± 0.168 a	48.32 ± 0.16 a

Means sharing similar letters in columns are not significantly different at $p<0.05$

Table 5. Pupae of *Bactrocera cucurbitae* parasitized by *Dirhinus giffardii*, emergence and their sex ratio of parasitoids after 5 days exposure period

No. of *D. giffardii* Released on Host (Pupae)	No. of Parasitized Pupae	No. of Emerged *D. giffardii*	Sex Ratio of *D. giffardii*	
			Male (%)	Female (%)
1 pair	11.00 ± 2.30 c	9.33 ± 2.60 c	55.18 ± 2.89 a	44.81 ± 2.89 b
2 pair	18.33 ± 2.60 bc	16.66 ± 2.33 bc	52.07 ± 1.120 ab	47.92 ± 1.12 ab
3 pair	26.33 ± 2.60 ab	24.00 ± 2.30 ab	54.00 ± 2.52 b	46.00 ± 2.49 b
4 pair	34.66 ± 3.17 a	32.00 ± 3.17 a	51.49 ± 0.78 ab	48.50 ± 0.78 a
5 pair	35.33 ± 4.66 a	33.66 ± 4.40 a	52.22 ± 0.81 ab	47.77 ± 0.81 ab

Means sharing similar letters in columns are not significantly different at $p<0.05$

Table 6. Pupae of *Bactrocera cucurbitae* parasitized by *Dirhinus giffardii*, emergence and sex ratio of parasitoids after 6 days exposure period

No. of *D. giffardii* Released on Host (Pupae)	No. of Parasitized Pupae	No. of Emerged *D. giffardii*	Sex Ratio of *D. giffardii*	
			Male (%)	Female (%)
1 pair	13.00 ± 2.30 d	11.33 ± 2.60 d	53.89 ± 2.08 a	46.10 ± 2.08 a
2 pair	22.00 ± 2.30 c	19.33 ± 2.40 c	51.38 ± 1.38 a	48.61 ± 1.38 a
3 pair	27.66 ± 2.90 bc	24.66 ± 2.90 bc	52.77 ± 1.46 a	47.22 ± 1.46 a
4 pair	35.00 ± 2.30 b	31.66 ± 2.33 b	51.72 ± 1.03 a	48.27 ± 1.03 a
5 pair	45.66 ± 2.60 a	42.66 ± 2.33 a	51.57 ± 0.40 a	48.42 ± 0.40 a

Means sharing similar letters in columns are not significantly different at $p<0.05$

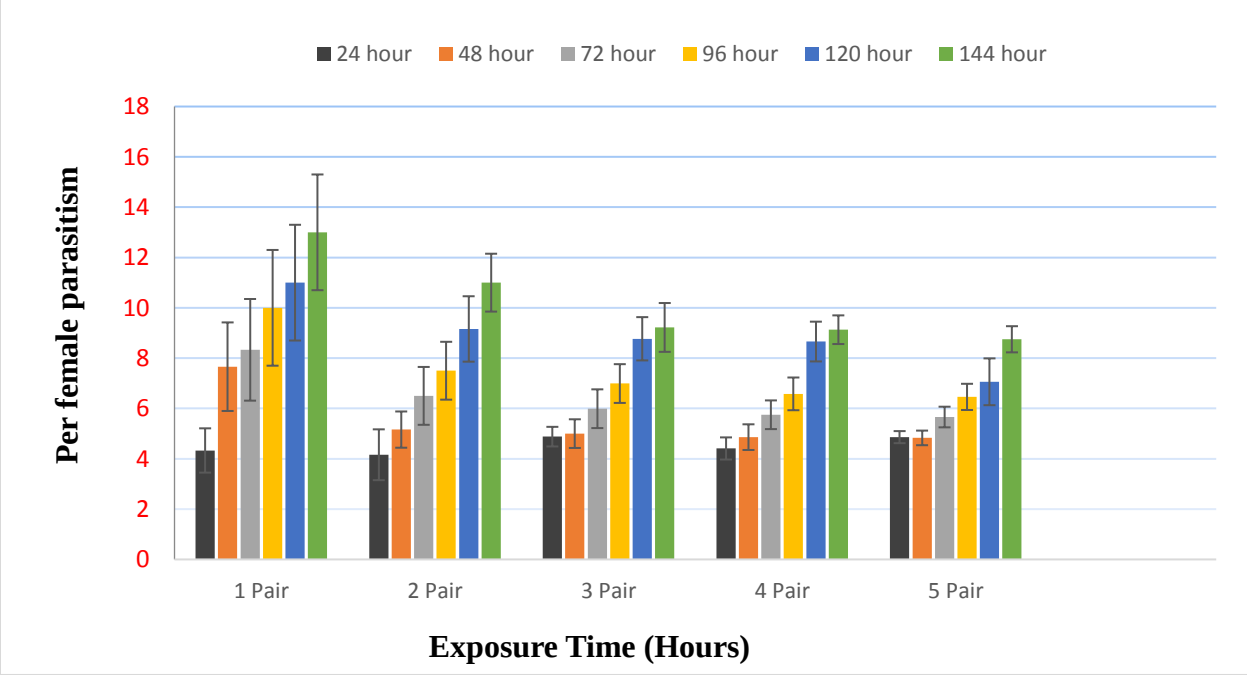

Figure 1. The per female parasitism rate of *Dirhinus giffardii* against the pupae of *Bactrocera cucurbitae* on different exposure periods (P ≤ 0.05)

Discussion

The parasitism, emergence and post emergence sex ratio of *D. giffardii* through different exposure times and parasitoid pairs were tested using laboratory experiments. A pronounced effect of parasitoids and exposure time on parasitism rate of *D. giffardii* was noticed. Results showed that number of parasitoids and exposure time have significant effects on the parasitism rate of *D. giffardii* against pupae of melon fruit fly. Maximum mean parasitization was observed at the 144 h exposure time and lowest at the 24 h. The present study confirmed that the pupal parasitoid *D. giffardii* is highly effective to parasitize the melon fruit fly pupae **[21]**. The trend of parasitization showed that increasing parasitoid pairs have significant effects on the rate of parasitism. It was observed that, as the number of parasitoid pairs on the pupae of melon fruit fly increased, meanwhile rate of parasitized pupae also increased, due to increase in per female parasitization and exposure time. Results showed that pupal parasitoid *D. giffardii* have high level of parasitism against the pupae of *B. cucurbitae.* Biological control by using *D. giffardii* could be an effective and safe approach for controlling tephritid flies. The method was successful and worth applying in the control of these pests **[23-26]**.

The relationship between parasitization and emergence ratio of parasitoids strongly supports the phenomena that as we increase the parasitoid pairs, the number of emerged parasitoids also increase. It was observed that maximum mean emergence was in case of five pair of parasitoids while exposure time also had significant effect on the emergence of *D. giffardii.* Results revealed that based on exposure periods, the rate of mean parasitoid emergence trend was observed in following order 6 day>5 day>4 day>3 day>2 day> and 1 day, respectively.

The trend of post emergence sex ratio depicted that male and female emerged parasitoids were not in equal numbers and exposure time and number of parasitoids have no significant effect on them. Some factors are known to influence the sex ratio of parasitoid progeny, such as parental sex ratio, host size, and host age, while parasitoids (*Itoplectis naranyae* and *Pimpla nipponica*) could change the sex ratio of their offspring in response to host age **[27-29]**. In contrast **[30]** reported that the age of the host had no effect on the progeny sex ratio of the wasp *Brachymeria lasus*. In our study, we found that the male and female emerged parasitoids were almost in different proportion but in some observations males were dominant regarding sex ratio **[31]**. Further, it was concluded that number of parasitoids, exposure time and per female parasitism significantly interlinked with each other (Fig. 1).

Conclusion

In conclusion, parasitism rate and emergence percentage of the pupal parasitoid, *D. giffardii* was satisfactory on the host (Melon fruit fly pupae). The results suggest that *D. giffardii* could be a suitable candidate for the biological control of *B. cucurbitae.* The present study will also be helpful in reduction of the fruit flies population through mass production and release of pupal parasitoid *D. giffardii.*

Authors' contributions

Conceived and designed the experiments: M Awais & NH Khuhro, Performed the experiments: M Awais, MH Khan & MU Asif, Analyzed the data: M Awais & RM Memon, Contributed reagents/ materials/ analysis tools: M Awais, MH Khan, RM Memon & MU Asif, Wrote the paper: M Awais & NH Khuhro.

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