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Exposure Time and Age Links Parasitism, Emergence and Development of Pupal Parasitoid *Dirhinus giffardii* Against *Bactrocera zonata*

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Abstract

The parasitism, emergence and development of pupal parasitoid, *Dirhinus giffardii* (Silvestri) was assessed against the pupae of the fruit fly, *Bactrocera zonata* (Saunders), under laboratory conditions. The fruit fly and *D. giffardii* were reared in glass cages on the artificial diet, and a known number of different 1-hour (fresh), 1-day, 2-day, 3-day and 4-day old pupae were offered to the respective parasitoids for a period of 6, 12, 18 and 24 hours. It was noted that the parasitism was increased gradually with an increase in pupal age and exposure time. The highest parasitism occurred on 3-day old pupae followed by 4-day, 2-day, 1-day and 1 hour (fresh) old pupae. The studies also manifested that exposure time and host age have a significant effect on the oviposition, per female parasitism, percent parasitism, emergence and development of pupal parasitoid, *D. giffardii*. The average developmental time of parasitoid was recorded significantly longer in 1-hour (fresh) old pupae than in the older pupae. The study revealed that *D. giffardii* is a virtuous candidate for the biological control of *B. zonata* and the pupae of *B. zonata* might be the perfect host for laboratory rearing of this parasitoid.



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Introduction

The peach fruit fly, *Bactrocera zonata* is considered one of the most economic key pests for numerous kinds of fruits of temperate, tropical and subtropical regions [1, 2]. The fruit flies (Diptera: *Tephritidae*) have almost 4,000 species that have been spread all over the world [3-5]. They are highly polyphagous species that attack several vegetables and fruits like citrus, guava, mango, peach, tomatoes and cucurbits [6]. Adult fruit flies are pale yellow to blackish-brown, which are similar more or less about the size of a housefly. Their bodies are posteriorly cone-shaped and have a sharp ovipositor at the tip of the abdomen [7]. Female fruit flies with the help of ovipositor lay eggs underneath the skin of fruits and vegetables. The eggs grow into larvae that nourish the decaying skin of the fruit. Throughout the feeding practice, larvae make tunnels inside the fruit pulp and in the tunnels, maggots start infestation [8]. After the infestation, the rotting of vegetables and fruits starts rapidly and they turn uneatable or fall to the ground. Fruit and vegetable growers of Pakistan have been facing many problems due to fruit flies. The market value, yield, quarantine security and international trade of vegetables and fruits are decreasing at the world-wide level [9].

Farmers generally use a huge amount of pesticides for the suppression of *B. zonata*, but they are unable to control this quarantine pest. Pakistan can save 30 million US \$ annually if the infestation of this pest is reduced [10]. Insecticides have been used in all the agriculture systems in the previous century. At the same time, it was diagnosed that agrochemical remains disseminate in the environment. They are generating dreadful despoilment of land ecosystems and are also involved in contaminating the human foods that is a real threat to human health [11, 12]. Growers adopt the habit of frequent application of pesticides to control the pests, which causes different human fitness-related apprehensions, such as cancers, nausea, headaches, birth defects, endocrine disruption, infertility and children's health [13, 14]. Chemical control methods carriage numerous environmental hazards and they are also involved in affecting the biotic and abiotic elements of the environment [15]. To escape insecticides risks, biological control is found to be a virtuous substitute to setback the insect pest population in fruits and vegetable crops. Biological control techniques offer one of the most proficient,

naturally rigorous, and sustainable mechanisms against pests [16]. Biological control is comparatively stable, non-toxic, efficient and environmentally friendly. It can be well-defined as the achievement of parasites, parasitoids, predators and pathogens to retain the pest populations at the lowest level of economic damage and in this way non-target species also stay safe. Effective natural enemies are the best biological tools for the suppression of insect pests [17].

The biological control with the support of *Dirhinus giffardii* (Hymenoptera: Chalcididae) is a durable insect pest management platform of the tephritids and has quarantine prominence [18]. Parasitoid, *D. giffardii* is an efficient pupal parasitoid of fruit-infesting tephritids, that is inhabitant of West Africa and local host of this parasitoid is *Ceratitis capitata*. It has been used for the first time in West Africa to control Black Soldier Fly and dipterous house flies. *D. giffardii* is reported as a strong biological control agent against fruit flies and has quarantine importance [18, 19, 20]. Fruit growers can get benefits from this pupal parasitoid by proper application of this beneficial insect at a suitable time and stage on their orchards, that have the ability of controlling the fruit flies successfully [16]. *D. giffardii* female lay eggs inside the pupae by rupturing the puparial wall of the fruit fly pupae. After hatching from the egg, larvae of *D. giffardii* remain inside the puparium, consume the flesh of the host and accomplish his life from egg to pupa inside the host pupae [21]. Keeping in view, the present study was designed to appraise the parasitism potency, per female parasitism, number of emerged parasitoids and developmental period of *D. giffardii* against different age pupae of *B. zonata* at different time intervals.

Materials and Methods

To evaluate the parasitism of *D. giffardii* against the pupae of *B. zonata*, the experiment was performed at Bio-Control Research Laboratory of Fruit Fly, Plant Protection Division, Nuclear Institute of Agriculture (NIA), Tandojam, Sindh, Pakistan during February 2019. Laboratory reared culture of *D. giffardii* (18 days old) and peach fruit fly pupae fresh (1h old), 1 d old, 2 d old, 3 d old and 4d old were used as a stock culture during the experiment following five replications under laboratory conditions (28°C±2 and 65%±5 RH). Parasitoids were released in pair form (3 pairs of *D. giffardii*)

on every 60 pupae of *B. zonata* for different exposure periods (6h, 12h, 18h and 24h).

Rearing of *Bactrocera zonata*

The fruit fly *B. zonata* was mass-reared on peach and artificial diet containing wheat bran (26%), sugar (12%), dried torula yeast (3.6%), sodium benzoate (0.1%), methyl p-hydroxybenzoate (0.1%) and water (58%). Two days old eggs of fruit flies were put directly on the diet trays having an artificial diet (3 ml). These eggs were collected in plastic glasses having 0.5 mm holes around them smeared internally with peach juice and put in adult fruit fly cages. The hatched larvae were fed on the diet until complete maturation. Mature larvae pop out from the diet trays on the substrate (sand/sawdust) for pupation, then pupae were sieved from the sawdust and used in maintaining the culture and experiments. The adult peach fruit flies then emerge from these collected pupae, which reared on protein hydrolysate, water and sugar in the cages.

Rearing of *Dirhinus giffardii*

The colonies of parasitoid *D. giffardii* being well maintained at NIA bio-control agents rearing lab from the last several years were reared in glass cages on the pupae of *B. zonata* and artificial diet. A fresh diet solution of 30% honey and 70% water was offered to the parasitoids through soaked cotton wigs.

Data analysis

After the mentioned exposure periods, mean parasiti-

-sm, per female parasitism, no. of emerged parasitoids, percent parasitism and developmental period of *D. giffardii* from parasitism to emergence were recorded. The results were analyzed by using the software Statistix 8.1. Tukey’s honestly significant difference (HSD) test was used for the comparison of means among different treatments.

Results

Mean parasitism

The investigations on pupae of peach fruit fly (*B. zonata*) were conducted to check the mean parasitism of *D. giffardii* by using different age pupae and exposure periods. The highest mean parasitism was recorded 40 on 3-day old pupae at the exposure time of 24 h (Table 1-4), while the minimum mean parasitism, which was observed in fresh pupae at an exposure time of 6 h (Table 1). It was also observed that after the exposure time of 12 and 18 h, maximum mean parasitism was 28 and 31, respectively in 3-day old pupae (Table 2; 3). The mean parasitism results showed that the performance of *D. giffardii* was good enough in the case of 3-day old pupae while 2, 3 and 4-day old pupae parasitism results were also better compared to fresh pupae (1 h old) of *B. zonata*.

Per female parasitism

Per female parasitism directly depends on the fecundity of female parasitoid. The maximum per female parasitism (13.33) was observed on 3-day old pupae at the exposure time of 24 h and minimum (1.33) on fresh pupae at 6 h exposure time (Table 1;

Table 1 Influence of host age and exposure time on parasitism, per female parasitism and emergence of *Dirhinus giffardii* against pupae of *Bactrocera zonata* that were offered for 6h.

Age of <i>B. zonata</i> pupae	Mean parasitism	Per female parasitism	No. of emerged parasitoids	Parasitism (%)
Fresh (1 h old)	4.00 ± 1.00b	1.33 ± 0.33b	1.66 ± 0.66b	6.66 ± 1.66b
1 d old	8.00 ± 2.64ab	2.66 ± 0.88ab	6.00 ± 2.08ab	13.33 ± 4.40ab
2 d old	14.00 ± 2.65ab	4.66 ± 1.15ab	11.00 ± 2.64ab	23.33 ± 4.41ab
3 d old	19.00 ± 4.35a	6.33 ± 1.45a	15.66 ± 4.05a	31.66 ± 7.26a
4 d old	15.00 ± 3.46ab	5.00 ± 1.16ab	12.00 ± 2.88ab	25.00 ± 5.77ab

Table 2 Influence of host age and exposure time on parasitism, per female parasitism and emergence of *Dirhinus giffardii* against pupae of *Bactrocera zonata* that were offered for 12h.

Age of <i>B. zonata</i> pupae	Mean parasitism	Per female parasitism	No. of emerged parasitoids	Parasitism (%)
Fresh (1 h old)	7.00 ± 1.00c	2.33 ± 0.33c	4.00 ± 0.57c	11.66 ± 1.66c
1 d old	10.00 ± 2.64bc	3.33 ± 0.88bc	8.33 ± 2.60bc	16.66 ± 4.40bc
2 d old	19.00 ± 3.60abc	6.33 ± 1.20abc	15.66 ± 3.52abc	31.66 ± 6.00abc
3 d old	28.00 ± 4.35a	9.33 ± 1.45a	24.66 ± 4.05a	46.66 ± 7.26a
4 d old	22.00 ± 2.00ab	7.33 ± 0.66ab	19.33 ± 2.18ab	36.66 ± 3.33ab

Table 3 Influence of host age and exposure time on parasitism, per female parasitism and emergence of *Dirhinus giffardii* against pupae of *Bactrocera zonata* that were offered for 18h.

Age of <i>B. zonata</i> pupae	Mean parasitism	Per female parasitism	No. of emerged parasitoids	Parasitism (%)
Fresh (1 h old)	9.00 ± 1.73c	3.00 ± 0.57c	7.00 ± 1.73c	15.00 ± 2.88c
1 d old	16.00 ± 3.60bc	5.33 ± 1.20bc	13.66 ± 3.38bc	26.66 ± 6.00bc
2 d old	21.00 ± 3.00abc	7.00 ± 1.00abc	18.66 ± 2.84abc	35.00 ± 5.00abc
3 d old	31.00 ± 4.35a	10.33 ± 1.45a	29.00 ± 4.35a	53.33 ± 7.26a
4 d old	25.00 ± 2.00ab	8.33 ± 0.66ab	23.00 ± 2.00ab	41.66 ± 3.33ab

Table 4 Influence of host age and exposure time on parasitism, per female parasitism and emergence of *Dirhinus giffardii* against pupae of *Bactrocera zonata* that were offered for 24h.

Age of <i>B. zonata</i> pupae	Mean parasitism	Per female parasitism	No. of emerged parasitoids	Parasitism (%)
Fresh (1 h old)	13.00 ± 2.00c	4.33 ± 0.66c	11.66 ± 1.85c	21.66 ± 3.33c
1 d old	19.00 ± 4.35bc	6.33 ± 1.45bc	16.66 ± 3.48c	31.66 ± 7.26bc
2 d old	26.00 ± 4.36abc	8.66 ± 1.46abc	24.00 ± 4.04bc	43.33 ± 7.27abc
3 d old	40.00 ± 2.00a	13.33 ± 0.66a	38.33 ± 1.86a	66.66 ± 3.33a
4 d old	32.00 ± 2.33ab	10.66 ± 0.67ab	29.66 ± 1.76ab	51.66 ± 3.34ab

4). The maximum per female parasitism after the exposure time of 12 h and 18 h was 9.33 and 10.33 while the lowest was recorded 2.33 and 3.00, respectively (Table 2; 3). However, it was found that host pupal age and exposure time have significant effects on the rate of per female parasitism.

No. of emerged parasitoids

The emergence of adult *D. giffardii* was recorded from the parasitized pupae of *B. zonata* on daily basis. The no. of emerged parasitoids ratio revealed that their emergence differed significantly among various age pupae of host and also depend on the exposure time. The maximum emergence of parasitoids (38.33) was observed from 3-day old pupae after the exposure time of 24 h while minimum (1.66) was observed from fresh pupae after the exposure time of 6 h (Table 1; 4).

Percent parasitism

To test the ideal pupal host age for parasitism preference of parasitoid *D. giffardii*, different age pupae of *B. zonata* were offered for parasitism and the data were recorded during various exposure periods. The percent parasitism in 3 and 4-day old pupae was significantly higher compared with the fresh (1h old), 1-day and 2-day old pupae in all exposure periods (6, 12, 18 and 24 h). The highest percent parasitism of 66.66 was observed on 3-day old pupae followed by 4-day old pupae after the exposure period of 24 h (Table 4). The lowest percent parasitism of 15.00 was observed on fresh pupae of

B. zonata after the exposure period of 6 h (Table 1). The results concluded that pupal age has a significant role in percent parasitism.

Developmental period of *D. giffardii*

The development time, from oviposition to adult emergence of parasitoid *D. giffardii* was recorded in relation to different exposure periods and host age. The mean longest duration of development (409 h) was observed in 6 h pupal exposure time on the fresh pupae of *B. zonata* and this differed significantly from all the other exposure periods. A shorter duration of development was 305.7 h in 4 d old pupae at an exposure time of 24 h (Fig. 1).

Discussion

The results of the current study revealed that at the maximum exposure time, pupal parasitoid, *D. giffardii* showed higher rate of parasitism on 3-day old pupae and lower rate of parasitism on the fresh pupae of *B. zonata*. These findings have similarities with the result of some prior studies. According to their results, host age and exposure time have a pronounced effect on the rate of parasitism [22]. The parasitism of *D. giffardii* ordinarily at its peak, when exposed on the older pupae as compare to fresh pupae of *B. zonata* [5]. It is also worth stating that too much old and too many fresh pupae are not considered good for rearing purpose, because it was observed that parasitoid *D. giffardii* have maximum parasitism potential against medium age pupae. According to the findings of Pfannenstiel et al. [23],

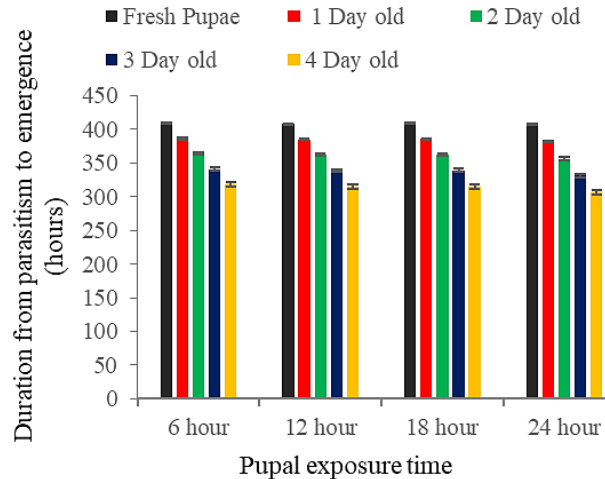


Fig. 1 Effect of host (pupal) age and exposure time on the developmental period (hours) of *Dirhinus giffardii* from parasitism to emergence.

some species prefer medium-aged host pupae for their progeny's development. Parasitism depends upon the oviposition, done by the female *D. giffardii* on the pupae of the host, this indicates that there is a linear association between per female parasitism and percent parasitism [24]. However, it is also imperative to point out that *D. giffardii* has good searching efficiency for *B. zonata* pupae and gave maximum parasitism, per female parasitism and the emergence of parasitoids [25, 26]. The current studies showed an increase in the rate of per female parasitism with an increase in the age of pupa and exposure time. Actually, per female parasitism is directly linked with fecundity. To accomplish the egg-laying process, female *D. giffardii* locates, visit and finally select the suitable host for oviposition. Earlier workers reported that during the selection of the host, its species, age, instar and inside nutritional qualities of yolk material also play a role [27, 28]. Needle-like ovipositor of female parasitoid pierces the pupa and egg are laid between puparial skin and layer of yolk material. Larvae of *D. giffardii* remains inside the puparium until the emergence and consume the flesh of the pupa [21]. During the present study, it was found that parasitoid emergence was maximum in case of older pupae compare to fresh because *D. giffardii* female prefers to oviposit in the older pupa. The present study further revealed that parasitoid offspring that is produced from younger pupae take more time for its complete development compared to older pupae. Results have a resemblance to some prior studies which reported that the growth time of parasitoids usually varied

with the host age. For example, it was observed in *Nasonia vitripennis* [29], *Dinarmus basalis* [30], *Mythimna separata* (Walker) [31] and *Diadro muscollaris* [32]. In conclusion, our results confirmed that *D. giffardii* can develop effectively in 2, 3 and 4-day old host pupae but it prefers to parasitize the 3-day old host pupae of fruit flies. The maximum parasitism, emergence and shorter development time were observed in 3-day old pupae of *B. zonata*. Further, our results are useful in the reduction of the peach fruit flies population in Pakistan and for the designing of mass rearing protocols and biological control studies of *B. zonata* under laboratory and field conditions.

Conflict of interest

The authors declare no conflict of interest.

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