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

Muhammad Awais*, Niaz Hussain Khuhro, Muhammad Hamayoon Khan, Raza Muhammad Memon, Muhammad Usman Asif

Plant Protection Division, Nuclear Institute of Agriculture (NIA), Tandojam, Sindh, Pakistan

**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.
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 worldwide 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 giffardi (Hymenoptera: Chalcididae) is a durable insect pest management platform of the tephritids and has quarantine prominence [18]. Parasitoid, D. giffardi is an efficient pupal parasitoid of fruit-infesting tephritids, that is inhabitant of West Africa and local host of this parasitoid is Ceratitis capitate. It has been used for the first time in West Africa to control Black Soldier Fly and dipterous house flies. D. giffardi 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. giffardi female lay eggs inside the pupae by rupturing the puparial wall of the fruit fly pupae. After hatching from the egg, larvae of D. giffardi 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. giffardi against different age pupae of B. zonata at different time intervals.

Materials and Methods

To evaluate the parasitism of D. giffardi 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. giffardi (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. giffardi)
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. The colon

**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 parasitism, 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 B. zonata 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 B. zonata 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 |
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.

**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 18 h.

| Age of *B. zonata* 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 24 h.

| Age of *B. zonata* 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],
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, it’s 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 Diadromus muscellaris [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.

References

[1] Fletcher BS. Temperature development rate relationships of the immature stages and adult of tephritid fruit flies. Robinson AS and G. Hooper (Eds.) Fruit Flies: their biology, natural enemies and control. Elsevier World Crop Pests 3A, Amsterdam 1987; Pp 273-289.

[2] Younes MWF, Shehata NF, Mahmoud YA. Histopathological effects of gamma irradiation on the peach fruit fly, Bactrocera zonata (Saund.) female gonads. J Appl Sci Res 2009; 5(3):305-310.

[3] Hardy DE. Taxonomy and distribution of the oriental fruit fly and related species (Tephritidae, Diptera). Proc Hawaiian Entomol Society 1997; 20:395-428.

[4] Rasool B, Rafique M, Asrar M, Rasool R, Adeel M, Rasul A, Jabeen F. Host preference of Bactrocera flies species (Diptera:Tephritidae) and parasitism potential of D. giffardii and Pachycopepoides vindemmiae under laboratory conditions. Pak Entomol 2017; 39(1): 17-21.

[5] Awaies M, Khuroho NH, Khan MH, Memon RM, Asif MU. Assessment of parasitizing potential of pupal parasitoid Dirhinus giffardii (Silvestri) against different age pupae of fruit fly and their post emergence sex ratio under lab conditions. J Entomol Zool Stud 2018; 6(5):159-162.

[6] White I, Elson-Harris MM. Fruit Flies of Economic Significance: Their Identification and Bionomics. CAB International Wallingford, UK. 1992; p. 601.

[7] Yasuda KHI, Mathuda K. Changes of fruit injury rate after the implementation of the Dacus dorsalis (Hendel) eradication control project. Proc Assoc Plant Prot Kyushu 1981; 70 (11):139-141.

[8] Latif A. Integrated management of Fruit flies (Diptera: Tephritideae) in Pakistan. Ann Reppt Agricultural Linkages Programme. Pak Agri Res Council, Islamabad 2004; pp. 1-51.

[9] Joomaye AN, Price NS, Stone house JM. Quarantine pest risk analysis of fruit flies in Indian Ocean: the of Bactrocera zonata. Proc. Indian Ocean Commission regional fruit fly Symposium. 2000; pp. 179-183.
The ectoparasitic pupal wasp (Hymenoptera: Chalcididae) against the pupae of Bactrocera cucurbitae (Coquillett) (Diptera: Tephritidae). Pure Appl Biol 2020; 9(1):609-617.

[23] Pfannenstiel RS, Browning HW, Smith JW, Jr. Suitability of Mexican rice borer (Lepidoptera: Pyralidae) as a host for Pediobius faveri (Hymenoptera: Eulophidae). Environ Entomol 1996; 25:672-676.

[24] Tillman PG. Functional response of Microplitis croceipes and Cardiochiles nigriceps (Hymenoptera: Braconidae) to variation in density of Tobacco Budworm (Lepidoptera: Noctuidae). Biol Control 1996; 25:254-258.

[25] Khan MH, Khuho NH, Awais M, Memon RM, Akbar W. Assessment of the parasitism potential of three parasitoids of fruit fly, Bactrocera spp. (Diptera: Tephritidae) under laboratory conditions. Pure Appl Biol 2019; 8(2):1579-1587.

[26] Khan MH, Khuho NH, Awais M, Memon RM, Asif MU. Functional response of the pupal parasitoid, Dirhinus giffardii towards two fruit fly species, Bactrocera zonata and B. cucurbitae. Entomol Gen 2020; 40(1):87-95.

[27] Harvey JA. Dynamic effects of parasitism by an endoparasitoid wasp on the development of two host species: implications for host quality and parasitoid fitness. Ecol Entomol 2001; 25:267-278.

[28] Harvey JA, Strand MR. The developmental strategies of endoparasitoid wasps vary with host feeding ecology. J Ecol 2002; 83:2439-2451.

[29] Wyfie H. Effect of host age on rate of development of Nasonia vitripennis (Walk.) (Hymenoptera: Pteromalidae). Can Entomol 1964; 96:1023-1027.

[30] Islam W. Effect of host age on rate of development of Dinarmus basalis (Rond.) (Hymenoptera: Pteromalidae). J Appl Entomol 1994; 118:392-398.

[31] Husni Y, Kainoh, Honda H. Effects of host pupal age on host preference and host suitability in Brachymeria lasus (Walker) (Hymenoptera: Chalcididae). Appl Entomol Zool 2001; 36:97-102.

[32] Wang X, Liu S. Effects of host age on the performance of Diadromus collaris, a pupal parasitoid of Plutella xylostella. Bio Control 2002; 47:293307.