

Full Length Research Paper

## Role of *Dirhinus giffardii* Silv. age on the parasitism preference to different days old pupae of *Bactrocera zonata* and *Bactrocera cucurbitae*

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Apprehensions are mounting about the effects of pesticides on human and environment. Therefore, interest is being revived to use biological control, which is recognized as an important component of sustainable pest management. *Dirhinus giffardii* has been effectively used as pupal parasitoid for the management of fruit flies. Experiments were conducted to determine the suitable host age for rearing of the pupal parasitoid on the two species of fruit flies, *Bactrocera zonata* and *Bactrocera cucurbitae*. Results indicated that *D. giffardii* preferred the pupae of *B. zonata* than *B. cucurbitae* at all the tested host (pupae) ages of the fruit flies. Maximum parasitism per female was observed at the age of 3 days old pupae. The parasitism increased significantly up to the pupal age of 3 days and then the parasitism started declining. Parasitism of the *D. giffardii* was recorded on fruit flies pupae up to the age of 5 to 6 days on both the fruit fly species and no parasitism was recorded from day 7 onwards. The results revealed that age of parasitoid, *D. giffardii* also had significant effect on pupal parasitism of *B. zonata* and *B. cucurbitae*. The mean parasitism per female was increasing with age of parasitoid and reached to its peak at the age of 5 days of parasitoids. Thereafter the parasitism started declining with the subsequent age of parasitoid and it reached lowest at the age of 30 days. The studies suggested that the parasitoids *D. giffardii* should be discarded after the age of 15 days for good mass rearing.

**Key words:** Pupal parasitoids, fruit flies, host and parasitoid ages, parasitism.

### INTRODUCTION

The insect pests adversely affecting agricultural productions are commonly controlled by the application of large quantities of pesticides. Production is affected in the field, prior to harvest, and after harvest. In general, globally 30 to 40% losses in field and post harvests by the insects are common (Mathew, 1999). Due to frequent and

injudicious application of pesticides insect pests develop resistance that jeopardized their efficiency and also deteriorate the environment. (Van Emden et al., 2004). Mainly the urban public is fervently more disparate to the present control strategy of insect pests in the field crops and the fruit orchards. Further, concerns regarding the

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impact of poisonous chemicals on biodiversity and the environment in particular have augmented the necessity of implementing non-insecticide control programs. Thus it is imperative to search for the pest control strategies that would reduce the quantity of cruel and wide-range usage of pesticides for suppression of the agricultural pests. With expression focused on alternative control program, there has been a renewed interest in biological control. Appropriate application of biological control tends to be effective, environmentally safe and sustainable approach for pest management. Releases of the natural enemies at appropriate stage and time in the field are another critical component for successful application of biocontrol technology (Van Lenteren et al., 2006).

Fruit flies (Diptera: Tephritidae) are very common pests of economic importance in nearly all tropical, subtropical and various temperate regions of the world (De Meyer et al., 2010). The cosmopolitan nature of fruit fly species highlights their international importance in sustainable fruit and vegetable production as well as trade issues. Most of the economic species of fruit flies (*Bactrocera zonata*, *Bactrocera dorsalis*, *Bactrocera cucurbitae*, *Dacus ciliatus*) are polyphagous in nature and damage a wide range of fruits and vegetables affecting their production (Imran et al., 2013). High value exports of fruit (citrus, guava, and mango) and vegetables significantly contribute in the national economy of Pakistan (Anonymous, 2009). To disinfest the fruits, expensive quarantine treatments are often a prerequisite for such exports that is, long duration cold storage, heat treatment, controlled atmospheres, irradiation; such post-harvest tactics increase the expenditure and ultimately reduce quantity of the products. Generally, organophosphate insecticides are recommended to control fruit flies in Pakistan (Mian et al., 1986). In recent decades, biological control offers one of the most promising, environmentally sound, and sustainable tools for control of arthropod pests (Van Driesche et al., 2008). It is therefore, imperative that biological control also be exploited for the management of fruit flies in Pakistan to the fullest extent.

The importance of parasitoids in the augmentative release of biological control of many pests has been reported by various workers (Wang and Messing, 2004). The fruit flies pupal parasite, *Dirhinus giffardii* (Hymenoptera: Chalcididae) has the potential to be exploited as bio-control agents against different fly species of Pakistan but its parasitism on different hosts may be variable and needs to be determined. Dresner (1954) reported that *D. giffardii* could attack *B. dorsalis* puparia previously parasitized by *Fopius vandenboschi* in Hawaii and has only a slight preference for un-parasitized over parasitized puparia. However, detailed information is lacking on potential interactions between *D. giffardii* and other principal fruit fly parasitoids. Some aspects of the biology of *D. giffardii* have been documented by Dresner (1954), Podoler and Mazor (1981). Sangvorn et al. (2004) performed laboratory investigations on the pupal parasitoid (*Spalangia endius* Walker) of fruit fly

*Bactrocera correcta* (Bezzi) and *B. dorsalis* (Hendel). They mainly find out the effect of parasitoid age, pupal age and host-parasitoid density on the rate of parasitism and reported the peak of parasitism by the females at the age of 3 days. The rate of parasitism of *B. dorsalis* was in the increasing order turned down to below 50% with the pupae age of 7 days, while that of *B. correcta* remained above 90%. Their studies on varying host density revealed that the numbers of parasitized pupae increased with host bulk, but the percentage parasitism went on the decline and was inversely density dependent. In the experiments on variable host (or parasitoid) density, the percentage parasitism was significantly higher in *B. correcta* compared to *B. dorsalis* at all densities they tested. As *D. giffardii* is a very important parasitoid, therefore, the present studies were planned to evaluate the comparison of parasitism and development of the parasitoid at different parasitoid and host ages on the pupae of *B. zonata* and *B. cucurbitae*, the two economical and predominant fruit fly species of Pakistan.

## MATERIALS AND METHODS

The experiments were conducted at nuclear institute of agriculture (NIA), Tando Jam. The parasitoids and pupae of the two fruit fly species, *B. zonata* and *B. cucurbitae* were obtained from their respective colonies being maintained at NIA for the last several years. The fruit flies, *B. zonata* and *B. cucurbitae* were mass cultured on sugar, water and protein hydrolysate. On the pop out day, the trays were kept in pupation substrate in the large trays to collect the pupae. By this method, the pupae of the same age were collected through sieving the pupation substrate daily and used for experimentations. The strains of pupal parasite, *D. giffardii* is also being maintained at NIA fruit fly rearing laboratory since the last four years. The parasitoids are being mass cultured on the pupae of *B. zonata* for releases in the guava and mango orchards against fruit flies. The parasitoids used in the studies were cultured on *B. zonata* pupae and sexed immediately after emergence and used according to the requirement of each experiment. After sexing, the newly emerged parasitoid wasps were held in screen cage separately at  $25 \pm 2^\circ\text{C}$ , 60% R.H, 10:14 D/L and provided with water and honey. All the experiments were conducted under the same environmental condition in which parasitoid and the host pupae were kept.

### Effect of age of the host (pupae) for parasitism

A sample of 300 pupae each of *B. zonata* and *B. cucurbitae* were exposed to five pairs of 2 days old parasitoid *D. giffardii* at different ages of the host pupae. For this purpose a stock of 5000 selected pupae of the each fruit fly species were separated carefully from the oviposition substrate on day one just after the larval pop out and kept in plastic containers separately. From the stock culture, 300 pupae of the fruit fly species were offered to the two days old five pairs of the parasitoids on each day starting from 1 to 8 day of their ages for parasitism in  $20 \times 20 \times 20$  cm perplex sheet cages having wire screen on one side. To obtain the similar age of the parasite, newly emerged parasitoids were sexed by examining the abdominal

**Table 1.** Effect of host age on parasitism of *Dirhinus giffardii* on the pupae of *Bactrocera zonata* and *Bactrocera Cucurbitae*.

Pupae offered on day (age)	<i>Bactrocera zonata</i>	<i>Bactrocera cucurbitae</i>
1	12.34 <sup>d</sup> ± 0.45	7.23 <sup>c</sup> ± 0.38
2	31.67 <sup>b</sup> ± 0.68	16.18 <sup>b</sup> ± 0.43
3	43.84 <sup>a</sup> ± 0.82	36.45 <sup>a</sup> ± 1.46
4	24.05 <sup>c</sup> ± 0.46	16.2 <sup>b</sup> ± 0.56
5	7.45 <sup>e</sup> ± 0.43	4.25 <sup>d</sup> ± 0.24
6	0.52 <sup>f</sup> ± 0.12	0.58 <sup>e</sup> ± 0.11
7	0	0
8	0	0
LSD values	1.430	1.753

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through analysis of variance followed by DMRT (P=0.05).

tips of the wasps and kept separately in two perplex cages. The adult wasps were fed with 30% water and honey solution. The pupae were exposed to the parasitoids in the cages have wire netting on one side and the remaining side of the cages is made by transparent perplex glass. Every day 300 pupae of the two fruit fly species with four replications were offered to two day old parasitoids for parasitism for twenty four hours. The experiment was continued up to 8 days of the pupal age. The rate of parasitism on each fruit fly pupae was recorded at the time of emergence of the parasitoid wasps from the pupae.

#### Effect of the parasite age to parasitize the host pupae

Freshly emerged parasitoids of *D. giffardii* were sexed and kept separately in oviposition cages and fed with 30% water and honey solution. These emerged male and female adult wasps of *D. giffardii* were kept in the laboratory maintained at 27 ± 2°C temperature and 65 ± 5% relative humidity. From the stock culture, 5 pairs of the parasitoids were released at their different ages ranging from 1 to 30 days with 5 time intervals in perplex glass cages measuring 20 × 20 × 20 cm and then they were offered 300 pupae of 2 days old of *B. zonata* and *B. cucurbitae* separately. For each set of the age, fresh un-mated parasitoids pairs were obtained from the stock and they were presented 300 pupae of the respective fruit fly species. The pupae, to each age of the parasitoids were exposed for 24 hrs in four replications and kept separately till emergence of the parasitoid wasps. The data recorded was calculated on parasitism per female basis (parasitoid emergence) at each tested age of the parasitoids starting from 5 to 30 days.

In both set of experiments, four replications were performed for evaluation of the effects. The experiments were conducted in completely randomized design (CRD) and analyzed using the analysis of variance (ANOVA). Duncan's Multiple Range was used to distinguish the means.

## RESULTS

#### Effect of the ages of the host (pupae) on parasitism

The results on the effect of different ages of pupae on the parasitism of *D. giffardii* revealed that the age of pupae played a significant role for parasitism

(Table 1). The parasitism rate increased as the age of the pupae of *B. zonata* and *B. cucurbitae* advanced. The peak of parasitism on pupae of both the fruit fly species were recorded at the age of 3 days and thereafter the parasitism started decreasing gradually. The results showed that on a very first day the mean parasitism by *D. giffardii* on pupae of *B. zonata* and *B. cucurbitae* was 12.34 and 7.23 per female, respectively. The trend was then increased gradually and on the 2 days old pupae of both the fruit fly species, it was 31.67 and 16.18 per female, respectively. The maximum numbers of pupae of the fruit fly species were parasitized at the age of 3 days (43.84 of *B. zonata* and 36.45 of *B. cucurbitae*) and then a decreasing trend in the parasitism rate was investigated with the successive ages of the pupae. The parasitism per female at the pupal age of 4 days of *B. zonata* and *B. cucurbitae* was 24.05 and 16.2, respectively. The same was much reduced when the parasitoids were offered pupae of the age of 5 days (7.45 on *B. zonata* and 4.25 on *B. cucurbitae*). The parasitism reached to negligible level at the age of 6 days of the pupae of both the fruit flies species (0.52 and 0.58, respectively). No parasitism was recorded on the pupae at the age of 7 and 8 days. The present research findings also confirmed that the parasitism rate was relatively much higher on the pupae of *B. zonata* compared to *B. cucurbitae* which suggest that the former is preferred host for the parasitoids and can efficiently be used for mass rearing of *D. giffardii* under laboratory conditions. The parasitoid preferred to attack 2 to 3 days old pupae in which the host pupae had fully established instead of 1 day old pupae. It has been observed that after complete development of the adult in the puparia that is, at the age of 6 day of the host pupae the parasitism by *B. giffardii* was almost negligible and no parasitism was recorded at the pupal age of 7 days onward. An identical trend of age effect for parasitism of *D. giffardii* was recorded on pupae of both the fruit

**Table 2.** Effect of parasitoid, *Dirhinus giffardii* age to parasitized the two day old pupae of *Bactrocera zonata* and *Bactrocera cucurbitae*.

Age of the parasite (Days)	Mean parasitism per female by	
	<i>Bactrocera zonata</i>	<i>Bactrocera cucurbitae</i>
1	20.90 <sup>c</sup> + 0.24	15.90 <sup>c</sup> + 0.34
5	46.60 <sup>a</sup> + 0.65	37.80 <sup>a</sup> + 0.50
10	28.30 <sup>b</sup> + 1.12	22.35 <sup>b</sup> + 0.48
15	22.15 <sup>c</sup> + 0.40	14.05 <sup>d</sup> + 0.30
20	11.00 <sup>d</sup> + 0.52	5.40 <sup>e</sup> + 0.32
25	3.00 <sup>e</sup> + 0.18	1.35 <sup>f</sup> + 0.17
30	1.50 <sup>e</sup> + 0.13	0.52 <sup>f</sup> + 0.10
LSD values	1.662	0.917

Means followed by different letters into the same column indicate a significant difference. Data was analyzed through analysis of variance followed by DMRT (P=0.05).

fly species tested. However, the parasitism preference by *D. giffardii* was higher on *B. zonata* pupae in comparison to the *B. cucurbitae*.

#### Effect of parasitoid *D. giffardii* age to parasitized the host pupae.

The results revealed that the age of parasitoid, *D. giffardii* had a significant effect on the pupal parasitism of the fruit fly species, *B. zonata* and *B. cucurbitae*. The mean parasitism per female was in the increasing order with the age of parasitoid and reached to its maximum level at the age of 5 days of parasitoids (Table 2). Thereafter the parasitism started declining gradually with the subsequent age of parasitoid. The results showed that number of pupae of *B. zonata* and *B. cucurbitae* parasitized by *D. giffardii* at the age of 1 day was 20.90 and 15.90 per female, respectively. The peak parasitism by the parasitoids was recorded at the age of 5 days where the mean parasitism of both the fruit fly species was 46.60 and 37.80 per female, respectively. The decreasing trend was observed with further progress in age of the parasitoids and at the age of 10 days of *D. giffardii*, the parasitism on *B. zonata* and *B. cucurbitae* pupae was reduced to 28.30 and 22.35 per female, respectively. The same was much reduced at the age of 15 days of the parasitoids where mean parasitism per female on *B. zonata* and *B. cucurbitae* was 22.15 and 14.05, respectively. The pupal parasitism decreased further at the latter stages of life and the lowest was recorded at the parasitoid age of 30 days (1.5 of *B. zonata* and 0.52 of *B. cucurbitae*). The present research findings confirmed that parasitoids up to the age of 15 days resulted in considerable rate of parasitism of the host pupae with maximum level of parasitism at the age of 5 days. It is also important to mention that rate of parasitism by *D. giffardii* at all ages tested was relatively

higher on the pupae of *B. zonata* compared to *B. cucurbitae* which represent inclination of parasitoids towards the pupae of former fruit fly species.

#### DISCUSSION

In the present studies *D. giffardii* preferred the *B. zonata* pupae than the *B. cucurbitae*, although *B. zonata* pupae are smaller than the *B. cucurbitae*. These findings are in contradiction with that of Wang and Messing (2004). They observed that body size of the host species showed positive correlation with host size and the parasitoids emerged in case of pupal parasite *D. giffardii*. They reported that the parasitoids consumed almost all the host resource when emerged from the host puparia of either species that is the parasite gained maximum fitness when reared on the larger host. However, they did not observe any effect on the development time of both male and female *D. giffardii* when reared on different sizes of host species. Their studies also showed that *D. giffardii* preferred to parasitize the larger pupae of *Bactrocera litifrons* than to parasitize the pupae of *Ceratitis capitata*. These findings showed smoothness in the body growth of this generalist parasitoid. It suggests that there was no obvious exchange in the body size and development time in *D. giffardii*, although it may vary in respect to assortment and ecological distinction. There is another possibility that *D. giffardii* can prefer *B. zonata* pupae for parasitism as compare to the *B. cucurbitae* is may be due to the presence of grooves on *B. cucurbitae* pupae, which are more prominent on *B. cucurbitae* pupae. Sangvorn et al. (2004) performed laboratory investigations on the pupal parasitoid (*Spalangia endius* Walker) of fruit fly *B. correcta* (Bezzi) and *B. dorsalis* (Hendel). They observed the effect of parasitoid age, pupal age and host-parasitoid density on the rate of parasitism and recorded the peak of parasitism by the females at the age of 3 days. They reported that the rate

of parasitism of *B. dorsalis* was in the increasing order and turned down to below 50% with the pupae age reached at 7 days old, while that of *B. correcta* remained above 90% at this age. Their studies on varying host density revealed that the numbers of parasitized pupae increased with host bulk, but the percentage parasitism went on the declining trend and was inversely density dependent. In the experiments on variable host (or parasitoid) density, they observed that the percentage parasitism was significantly higher in *B. correcta* compared to *B. dorsalis* at all densities tested. We also observed the peak parasitism per female at the parasitoids age of 5 days on 3 days old pupae. The studies suggested that the parasitoids *D. giffardii* should be discarded after the age of 15 days for the maintenance of good mass rearing colony.

### Conflict of Interests

The author(s) have not declared any conflict of interests.

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