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Assessment of parasitizing potential of pupal parasitoid *Dirhinus giffardi* (Silvestri) against different age pupae of fruit fly and their post emergence sex ratio under lab conditions

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Abstract

Fruit flies being serious pests of fruits and vegetables are responsible for huge economic losses in the world. Biological control is a sustainable pest management technique which offers naturally sound and effective management of these pests. *Dirhinus giffardii*, pupal parasitoid of fruit fly, is one of the most significant biological control agents which have been effectively used in controlling fruit flies. Laboratory studies were conducted to investigate the preference of *D. giffardii* on different aged pupae (1, 2, 3, 4 and 5 days old) of *B. zonata*, at different exposure times (24 h, 48 h & 72 h). Results revealed that mean rate of parasitism was highest on 3 days old pupae in all the exposure periods of 24, 48 and 72 h of *B. zonata* with maximum parasitism recorded after exposure period of 72 h. In all the exposure periods, the rate of parasitism was in the increasing order from 1-3 days old pupae after which the same was declined significantly with the lowest parasitism rate recorded in 5 days old pupae. In all the different aged parasitized pupae the post emergence sex ratio of male parasitoids was recorded higher compared to females. The studies manifested that the exposure time had a significant effect on parasitism and that the parasitoid *D. giffardi* can be more efficiently reared on 2-3 days old pupae of *B. zonata* with exposure time of 72 h for parasitization.

Keywords: Fruit flies, pupal parasitoid, parasitism, host age, sex ratio

1. Introduction

Fruit flies (Diptera: Tephritidae) cause huge losses to fruits and vegetables throughout the world, and are recognized as major insect pests of the horticultural industries. They are reported to have more than 4,000 species distributed all over the world [9, 19]. Being serious pests, they have pecuniary importance in tropical, subtropical and several temperate zones of the biosphere [14]. *Dacus ciliates*, *Bactrocera cucurbitae*, *Bactrocera zonata* and *Bactrocera dorsalis* are common and serious species of fruit flies in Pakistan [20], where they are causing losses worth more than 200 million US dollar annually to fruit and vegetable industry [23]. Due to infestation of maggots and post-harvest losses, market value of fruits like citrus, mango and guava is decreasing day by day [12]. The incidence of fruit flies reduces the yield and quality of fruits [11]. Generally, deterioration starts when female fruit fly pierce the skin of fruits through their needle like ovipositor and deposit their eggs. Then after hatching of eggs larvae make holes into the fruits to nourish his body on the pulp and make them unsuitable for human use [28]. Full fed larvae fall on the soil and pupate, adult fruit flies which emerge from pupae mostly active at the time of sunrise and their movement declines slowly at mid-day [2].

In general, huge amount of pesticides are being used for the suppression of this quarantine pest. It is a known fact that agrochemicals and pesticides became salient part of global agriculture systems during the previous century and at the same time realize that agrochemical remnants did disperse in the environment, causing astonishing disfigurement of land ecosystems and adulterating the human foods [4, 8]. In all agro-ecosystems agrochemicals residues are present, due to which the human health is on real risk and facing some complex problems [10]. Different human health related concerns are related with pesticides, such as nausea, headaches, infertility, birth defects, endocrine disruption and cancers [3]. Particularly children health is more endangered by little and constant exposure of pesticides [13]. There are few alternatives to pesticides that do not present the same environmental problems.

One of these alternative include biological control techniques which provide one of the most efficient, naturally safe, and conceivable mechanisms against pests [15].
Pupal parasitoid, *Dirhinus giffardii* (Silvestri) efficiently work in declining the population of fruit flies [24]. *D. giffardii* (Hymenoptera: Braconidae) primitive of West Africa, is considered an efficient biological control agent in reducing the (Dipteran) pest population [27, 17, 21]. Through appropriate application of this beneficial insect at suitable time and stage, fruit growers can get sustainable and successful control of fruit fly [25]. Larvae of *D. giffardii* emerged inside the puparium, consume the flesh of host and accomplish his life from egg to pupa inside host pupae. Biological control through use of *D. giffardii* is the durable management program of tephretid and has quarantine importance [26]. Keeping in view, the present study was designed to evaluate the effectiveness *D. giffardii* against different aged pupae of fruit flies with respect to exposure time.

2. Materials and methods

In order to evaluate the parasitizing potential of *Dirhinus giffardi* towards different aged pupae of *B. zonata* after different exposure periods (24 h, 48 h & 72 h) and their post emergence sex ratio, experiment was performed at Bio-Control Research Laboratory of Fruit Fly, Plant Protection Division, Nuclear Institute of Agriculture (NIA), Tandojam, Sindh during 2017. Laboratory reared culture of biological control agent *D. giffardi* (12 days old) and fruit fly pupae (1, 2, 3, 4 and 5 day old) were used as a stock culture during the experiment following five replications, under laboratory conditions (28 ± 2°C and 65 ± 5% RH).

2.1 Rearing of *Bactrocera zonata*

The fruit flies, *B. zonata* were mass reared on 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%). Two days old eggs of fruit flies (3 ml) were put 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 fed on the diet till complete maturation. Mature larvae pop out from the trays on the substrate (sand/saw dust) for pupation from where the pupae were collected through sieving [16] and used for maintaining the culture and experiments. The adult fruit flies were provided protein hydrolysate and sugar.

2.2 Rearing of *Dirhinus giffardii*

The colony of parasitoids *Dirhinus giffardii* well maintained at NIA from the last several years. *D. giffardii* were reared in glass cages on pupae of *Bactrocera zonata* 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 [6].

2.3 Effect of host age (pupae) and exposure time on the parasitization rate of *D. giffardii*

To study the effect of host age and exposure time on the parasitism rate of *D. giffardii*, pupae of
Bactrocera zonata having age 1 to 5 days old were placed in glass cages. Five adult pairs of parasitoid whose age was 12 days were collected from stock culture and released in cages for 24, 48 and 72 h (n = 5). After the said exposure periods, the pupae were collected from petri dishes and kept separately in empty cages till the emergence of parasitoids. The particular diet (honey and water) in each treatment was provided to adult parasitoids throughout the course of experiment.

2.4 Sex ratio of parasitoid *D. giffardii* after emergence from parasitized pupa

After parasitoid emergence in cages of respective host ages and exposure periods for parasitism, sex ratio and percentage of parasitization was recorded. In both set of experiments, five replications were performed, following CRD design and results were analyzed by using the software statistix 8.1 through anova, while means were separated through LSD test.

3. Results

The results on the parasitism rate of *D. giffardii* on different aged pupae of *B. zonata* after 24 h exposure period (Table 1) revealed that parasitism rate by 5 pairs of females was in the increasing order from 1 to 3 days old pupae with maximum parasitism recorded on 3 days old pupae (76.20 ± 0.37). The rate of parasitism was lowest on 5 days old pupae (27.40 ± 0.67) followed by parasitism on pupae of age of 4 days (35.40 ± 1.43). In addition the results regarding sex ratio of parasitoids after 24 hours exposure period revealed that maximum male parasitoid percentage was 51.97 ± 0.33 in 4 day old pupae and minimum (51.02 ± 0.18) in 2 days old pupae while maximum females parasitoid percentage 48.97 ± 0.18 and minimum (48.02 ± 0.33) was observed in 2 and 4 day old pupae, respectively. Pupal age and exposure time have significant effect on the parasitism rate of *D. giffardii* while they have no significant interactions with sex ratio of parasitoids.
The results showed that maximum parasitism of (81.60 ± 1.07) by 5 pairs of *D. giffardii* was observed on 3 days old pupae of *B. zonata* while minimum parasitism of 31.20 ± 1.42 was observed on 5 days old pupae after 48 h exposure time. The trend of parasitization was in increasing order from 1 to 3 days old pupae while parasitization on 4 day and 5 days old pupae was the lowest. After the 48 hours exposure period, maximum males parasitoids (51.92 ± 0.30) were emerged from 5 days old pupae and maximum females (49.11 ± 9.17) were recorded on 3 days old pupae (Table 2).
After 72 h exposure period, the results depicted that mean parasitization by *D. giffardii* on 1, 2, 3, 4 and 5 days old pupae were respectively, 67.00 ± 1.00, 82.20 ± 3.30, 88.00 ± 0.70, 49.00 ± 0.70 and 36.80 ± 0.66 whereas regard to sex ratio, maximum male parasitoids (52.03 ± 10.35) were observed from parasitization of 3 days old pupae and minimum (51.44 ± 1.80) from 4 days old pupae. Similarly highest female emergence of (48.56 ± 2.04) was recorded from 4 days old pupae and lowest (47.97 ± 10.31) from 3 days old pupae (Table 3).
Results regarding combined mean parasitism and sex ratio after 24, 48 and 72 h exposure time revealed that highest parasitization (81.93 ± 0.53) was recorded on 3 days old pupae while the lowest (31.80 ± 0.65) on 5 days old pupae. Sex ratio showed that maximum male parasitoids (52.03 ± 0.22) emergence from 5 days old pupae while highest females emergence (48.86 ± 0.71) were observed from 4 days old pupae (Table 4).

Table 1: Parasitism rate and post emergence sex ratio of *D. giffardi* on different aged pupae of *B. zonata* after 24 h exposure period.

Host age (Pupae)	Parasitization by 5 Females	Per Female Parasitization	Post Emergence Sex Ratio of Parasitoids	
			Male %	Female %
1 Day Old	64.20 ± 0.37C	12.84 ± 0.07C	51.40 ± 0.30BC	48.59 ± 0.30B
2 Day Old	68.20 ± 0.73B	13.64 ± 0.14B	51.02 ± 0.18E	48.97 ± 0.18A
3 Day Old	76.20 ± 0.37A	15.24 ± 0.07A	51.18 ± 0.25D	48.55 ± 0.24C
4 Day Old	35.40 ± 1.43D	7.08 ± 0.28D	51.97 ± 0.33A	48.02 ± 0.33E
5 Day Old	27.40 ± 0.67E	5.48 ± 0.13E	52.54 ± 0.42B	47.45 ± 0.42D

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

Table 2: Parasitism rate and post emergence sex ratio of *D. giffardi* on different aged pupae of *B. zonata* after 48 h exposure period.

Host age (Pupae)	Parasitization by 5 Females	Per Female Parasitization	Post Emergence Sex Ratio of Parasitoids	
			Male %	Female %
1 Day Old	65.00 ± 0.54C	13.00 ± 0.10C	51.08 ± 0.19E	48.91 ± 0.19C
2 Day Old	74.20 ± 1.06B	14.84 ± 0.21B	50.94 ± 0.16C	49.05 ± 0.16B
3 Day Old	81.60 ± 1.07A	16.32 ± 0.21A	50.89 ± 9.00D	49.11 ± 9.17A
4 Day Old	41.00 ± 1.92D	8.20 ± 0.38D	51.72 ± 0.30B	48.27 ± 0.30D
5 Day Old	31.20 ± 1.42E	6.24 ± 0.28E	51.92 ± 0.30A	48.07 ± 0.30E

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

Table 3: Parasitism rate and post emergence sex ratio of *D. giffardi* on different aged pupae of *B. zonata* after 72 h exposure period.

Host age (Pupae)	Parasitization by 5 Females	Per Female Parasitization	Post Emergence Sex Ratio of Parasitoids	
			Male %	Female %
1 Day Old	67.00 ± 1.00C	13.40 ± 0.20C	51.63 ± 0.34C	48.37 ± 0.34C
2 Day Old	82.20 ± 3.30B	16.44 ± 0.66B	51.70 ± 8.35B	48.30 ± 8.39D
3 Day Old	88.00 ± 0.70A	17.60 ± 0.14A	52.03 ± 10.35A	47.97 ± 10.31E
4 Day Old	49.00 ± 0.70D	9.80 ± 0.14D	51.44 ± 1.80E	48.56 ± 2.04A
5 Day Old	36.80 ± 0.66E	7.36 ± 0.13E	51.60 ± 0.28D	48.40 ± 0.28B

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

Table 4: Combined mean parasitism rate and post emergence sex ratio of *D. giffardi* on different aged pupae of *B. zonata* after 24, 48 and 72 h exposure period.

Host age (Pupae)	Parasitization by 5 Females	Per Female Parasitization	Post Emergence Sex Ratio of Parasitoids	
			Male %	Female %
1 Day Old	65.40 ± 0.45C	13.08 ± 0.09C	51.30 ± 0.18B	48.62 ± 0.18D
2 Day Old	74.86 ± 1.13B	14.97 ± 0.22B	51.22 ± 2.76C	48.77 ± 2.81B
3 Day Old	81.93 ± 0.53A	16.38 ± 0.10A	51.37 ± 6.48A	48.63 ± 6.52C
4 Day Old	41.80 ± 0.83D	8.36 ± 0.16D	51.14 ±0.60D	48.86 ± 0.71A
5 Day Old	31.80 ± 0.65E	6.36 ± 0.13E	52.03 ± 0.22E	47.96 ± 0.22E

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

4. Discussion

The preferences of *D. giffardi* on five different aged pupae of fruit fly as hosts and the effect of exposure time for parasitization were tested using laboratory experiments. A pronounced effect of host age and exposure time on parasitism rate of *D. giffardii* was noticed. Results showed that the order of preference of adult female parasitoid for parasitization were in order 3 day old > 2 day old > 1 day old > 4 day old > and 5 day old pupae at all the exposure periods (24 h, 48 h & 72 h). Parasitoid preference was highly significant for 3 day old pupae at all exposure periods with highest recorded after 72 h exposure period [6]. The females of *D. giffardi* oviposit on selected hosts on which their progeny fare best [1]. The relationship of pupal age and exposure time with parasitization rate revealed that it affect the parasitism significantly. The parasitism of parasitoids was at peak level on 2 and 3 day old pupae of fruit fly and then its start decreasing gradually. The maximum number of parasitized pupae and per female parasitization was observed in the cage of 2 and 3 days old pupae after the exposure time of 72 hours. The parasitism rate in 2 and 3 day old pupae of *B. zonata* suggests that *D. giffardi* as its suitable true pupal parasitoid. Mass rearing and field releases of this biological control agent before the pest out breaks can efficiently suppress pest population and can contribute effectively in integrated management of fruit fly. At the time of oviposition *D. giffardii* do not kill its host, and in such a way parasitized host can continue to metamorphose until the parasitoid egg hatchout, at which time the larva becomes forever paralyzed while the parasitoid larva feeds on it [7]. Our results showed that decreasing trend of parasitization started in 4 and 5 days old pupae. The possible reason for this could be the inception of post embryonic development of fruit fly [22] while at early embryonic stage parasitoids oviposit their eggs inside pupa, egg hatch in 2 days and its larvae consume all the puparial material as a food source. Apparently small pupae could not provide enough nutrition to parasitoid so as a result unhealthy parasitoids emerge. Use of natural hosts against parasitoides has major impact on larval ontogenesis of particular species [18]. It was observed that post emergence sex ratio of male parasitoides was dominant in number [5] as compare to females of *D. giffardi*, in all aged parasitized pupae after 24, 48 and 72 hours.

5. Conclusions

Based on findings from this study, the maximum parsitization was observed in case of 2 and 3 days old pupae of *Bactrocera zonata,* while rate of parasitization of *D.giffardi* could be arranged in following order: 3 day old > 2 day old > 1 day old > 4 day old > and 5 day old pupae. The study conceptualizes that mass production of pupal parasitoid *Dirhinus giffardii* will be helpful in reduction of the fruit flies population.

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