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Parasitism potential of *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae) on pupae of the fruit fly species, *Zeugodacus cucurbitae* (Coquillett) and *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), during variable exposure durations

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Abstract

Background: Fruit flies are important insect pests of horticultural crops. Pesticides used to control them which cause environmental and health hazards; therefore, other alternative sustainable management measures are required.

Main body: Successful implementation of an integrated pest management program, using biological control agents, needs synchronization of parasitoids with hosts selection and exposure time. The present study was conducted to assess the biological activity of the parasitoid species, *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae), against the melon fruit fly, *Zeugodacus cucurbitae* (Coquillett), and the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), at different exposure durations (24, 48, and 72 h). The experiments were conducted under standard laboratory conditions. Significant differences in the parasitism rate of *D. giffardii* were observed for both species at different exposure durations. The maximum parasitism rate of *D. giffardii* ($52.60 \pm 2.84\%$) and ($42.73 \pm 2.74\%$) was observed at 48-h exposure period for *Z. cucurbitae* and *B. dorsalis*, respectively. Also, a comparison between both species showed a difference in parasitism rate at 24 and 48 h and a positive correlation between percent parasitism and exposure duration. Adult emergence of *D. giffardii* showed a maximum emergence rate from pupae of *Z. cucurbitae* ($63.55 \pm 4.02\%$) and *B. dorsalis* ($51.61 \pm 2.33\%$) at 10 days interval, respectively. However, the daily emergence of the parasitoid was not correlated to exposure duration in both species. The results of the present study may serve in the mass rearing of *D. giffardii*.

Conclusion: The biological activity of *D. giffardii* was maximum at 48 h of exposure for both fruit fly species, and this important information may improve the mass rearing technology of *D. giffardii*.

Keywords: *Dirhinus giffardii*, *Bactrocera dorsalis*, *Zeugodacus cucurbitae*, Fruit flies, Exposure time

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Background

Fruit flies are positioned as quarantine pests of horticultural crops worldwide (Mariana et al. 2020). In South Asia, fruit fly species are reported for more than 100 host plants (Hui and Liu 2005). In Pakistan, losses of US\$ 200 million annually are reported due to fruit fly species mainly the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), and the melon fruit fly, *Bactrocera* (*Zeugodacus*) *cucurbitae* (Coquillett) (Diptera: Tephritidae) (Sarwar 2014). Most of the available literatures use *Bactrocera* (*Zeugodacus*) *cucurbitae* as a scientific name. However, considering the revised taxonomic position for the *B. cucurbitae* as proposed by Virgilio et al. (2015), this manuscript will consider *Zeugodacus cucurbitae* as a scientific name for the melon fly.

Currently, chemical sprays and pheromone traps are considered the first line of defense against fruit fly infestation. However, lack of awareness and injudicious use of insecticides caused many problems including reduced chemical efficacy, resistance in fruit fly, and residual effects (Aziz and Hussain 2018). These mentioned problems triggered the efforts to create awareness among the farming community and use other alternatives eco-friendly and sustainable management techniques (Vargas et al. 2012). In this regard, Centre for Agriculture and Bioscience International (CABI) through its regional bioscience center in Pakistan implemented a USAID/USDA funded project entitled “Phytosanitary Risk Management Programme in Pakistan (PRMP)” in 2014–2019. The main objective of this program was to explore and utilize the local species of biocontrol agents for the management of apple codling moth, papaya mealy bug, and fruit flies.

For the fruit fly management, the extensive surveys were conducted in different fruit and vegetable growing areas of Pakistan, which resulted in the exploration of *Dirhinus giffardii* (Silvestri) (Hymenoptera: Chalcididae) associated with fruit fly species as a pupal parasitoid. In the past, *D. giffardii* initially described from Nigeria has been considered as a generalist parasitoid for integrated pest management programs. It attacks several families, i.e., Tephritidae, Glossinidae, and Muscidae of order Diptera and Noctuidae of order Lepidoptera (Noyes 2014). In the management of fruit flies, *D. giffardii* showed good parasitism rates on pupae of different fruit fly species, i.e., *B. dorsalis*, *B. cucurbitae*, *B. zonata*, *B. oleae*, and *B. correcta* (Naveed et al. 2014; Rasool et al. 2017). *Dirhinus giffardii* completes a major part of its life cycle (egg to pupa) inside the host pupae. The parasitoid consumes the host resource completely and gained more fitness when reared on a large host (Imran et al. 2013). However, utilizing biological control agents in pest management programs requires successful mass rearing

techniques. Mass rearing optimization, synchronization of *D. giffardii* with host selection, and exposure time are key factors (Montoya et al. 2007).

In this regard, the present study was designed to determine suitable host and time required for maximum parasitism and for developing mass rearing protocol for *D. giffardii*.

Main text

Materials and methods

Fruit fly cultures

Parent colonies of the two fruit fly species, i.e., *Z. cucurbitae* and *B. dorsalis*, were maintained at CABI's Biological Control Laboratory, Rawalpindi, Pakistan (33° 38' 39" N, 73° 04' 57"), since 2016. The flies were kept in rearing cages (22 × 18 × 18) (inches) equipped with iron mesh (16 μm) to facilitate the ventilation and fine muslin cloth sleeve opening to provide the food for culture handling and maintenance. A standardized diet was provided for adult feeding (Table 1) pasted on small circular (10 cm diameter) rexine sheets and replenished on a daily basis in rearing cages. Water was provided as ad libitum. The rearing conditions were maintained at 28 ± 1 °C, 65 ± 5% RH, and 14:10 (L to D) h photoperiod.

Parasitoid's culture

The parent colonies of *D. giffardii* were maintained since 2016. Initially, soil sieving was done for pupae collection from Sharaqpur district (31° 27' 48" N 74° 6' 0" E) Punjab, Pakistan. Later, sieved pupae were brought to the laboratory for further process. *D. giffardii* emerged from these pupae were kept in transparent cages for further propagation. An artificial diet containing solutions of 70% water and 30% honey was provided to adult parasitoid through soaked cotton on regular basis. The parent colony was reared at for 3 consecutive generations, and the rearing conditions were maintained at 28 ± 1 °C, 65 ± 5% RH, and 14:10 (L to D) h photoperiod, before the experimentation to avoid pre-imaginal conditioning.

Experimental units

The host pupae of *Z. cucurbitae* and *B. dorsalis* (3 days old) were exposed to *D. giffardii* at different exposure durations, i.e., 24, 48, and 72 h (separated for each

Table 1 Composition of standardized diet for feeding the adults of fruit flies

Ingredients	Quantity (gm or ml)—for 800 ml diet
Yeast	03
Banana	1392
Egg yolk	06
Vitamin B complex	02
Sugar	33

species). The experiment was conducted with 3 replicates for each exposure duration. Each replicate was 500 pupae (3 days old) of *Z. cucurbitae* and *B. dorsalis* (from each species), placed in a small Petri dish (6 cm diameter) as residing media for *D. giffardii* adults to parasitize them. Ten pairs of *D. giffardii* (5 days old) were introduced to individual replicates of *Z. cucurbitae* and *B. dorsalis* pupae separately and exposed for said exposure durations. Later, the exposed pupae were removed from the containers and kept in separate boxes to record the parasitism and emergence data for 15 consecutive days. The experiments were repeated twice in time and pooled together for precise results.

Statistical analysis

The parasitism rate was calculated using the following formula as per Farooq et al. (2019):

$$\text{Parasitism rate} = \frac{\text{number of parasitoids (emerged + unemerged)}}{\text{total number of pupae}} \times 100$$

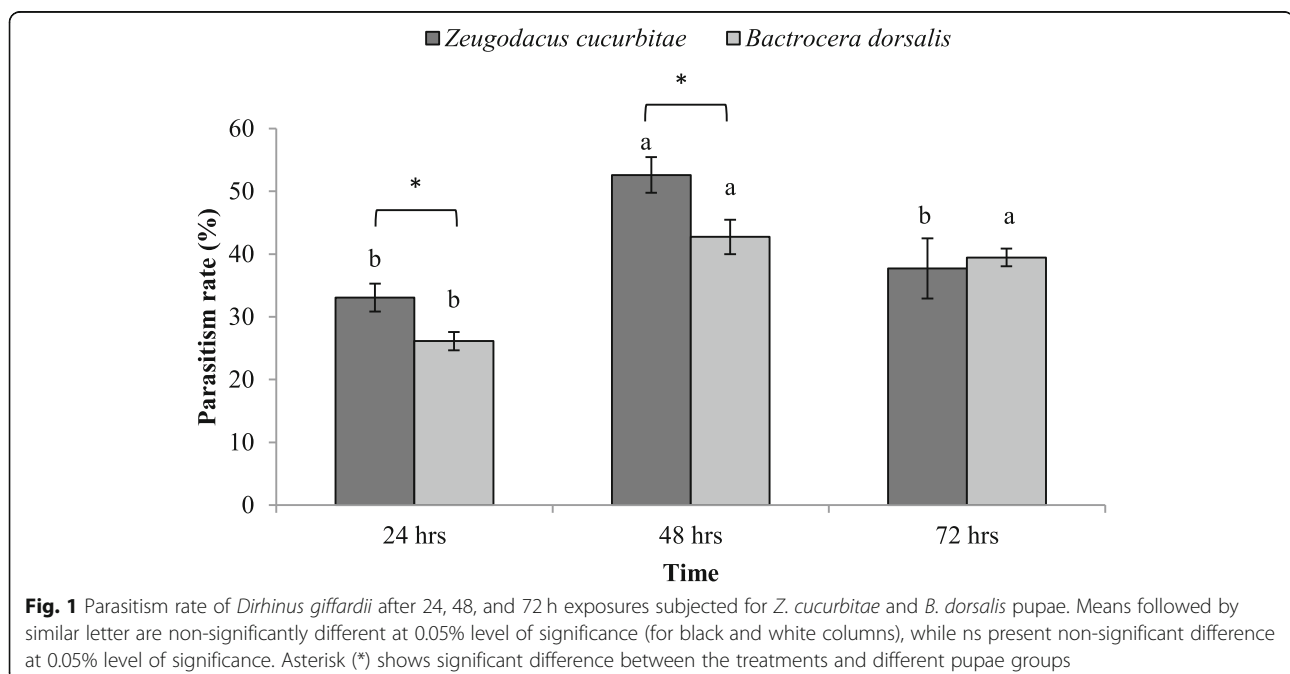
Experimental data was subjected to analysis of variance (ANOVA) in Statistix 8.1 (McGraw-Hill 2008), and multiple comparisons between means and groups were made with aid of LSD test at 0.05% level of significance. The linear regression was used to check the relationship between percent parasitism and percent emergence to varying exposure durations.

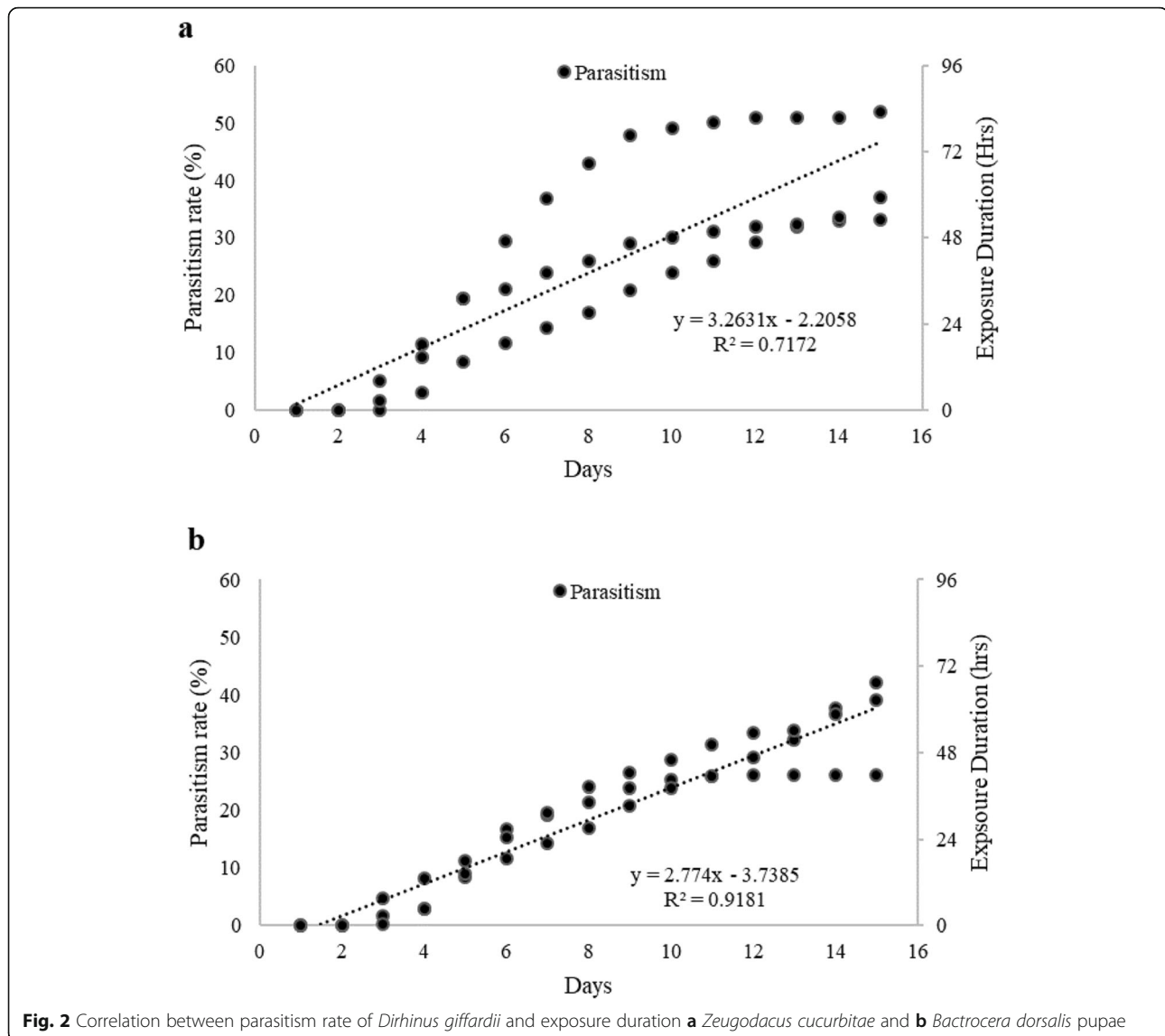
Results and discussion

Parasitism rate

The parasitism rate of *D. giffardii* after exposure to *Z. cucurbitae* pupae was determined. The results showed that the highest parasitism rate ($52.60 \pm 2.84\%$) by *D. giffardii* was observed after 48 h exposure to *Z. cucurbitae* pupae, followed by 72 h exposure ($37.73 \pm 4.80\%$) ($F = 8.67$; $df = 2, 8$; $P = 0.017$) (Fig. 1). For *B. dorsalis*, the highest parasitism rate ($42.73 \pm 2.74\%$) by *D. giffardii* was observed after 48 h exposure to *B. dorsalis* pupae, followed by 72 h exposure ($39.47 \pm 1.39\%$) ($F = 20.1$; $df = 2, 8$; $P = 0.002$) (Fig. 1). In addition, the comparison between parasitism rate for different pupae groups and different exposure durations showed significant difference at 24 and 48 h ($F = 4.75$; $df = 2, 8$; $P = 0.03$) (Fig. 1). In the context of correlation, the results showed a positive correlation for parasitism rate of *D. giffardii* vs. exposure duration for *Z. cucurbitae* pupae ($R^2 = 0.7172$) (Fig. 2a) and *B. dorsalis* pupae ($R^2 = 0.9181$) (Fig. 2b).

Parasitoids can discriminate between host pupae. They provide a survival advantage to parasitoids population and the preference of *D. giffardii* that may be influenced by different hosts as they possess different nutritional qualities (Harvey 2000). The present study highlighted on the parasitism activity of *D. giffardii* on *B. dorsalis* and *Z. cucurbitae*. The parasitism potential of *D. giffardii* against *B. dorsalis* and *Z. cucurbitae* might be a virtuous source for controlling fruit flies (Van Lenteren et al. 2006). The results of the present study showed significant differences in parasitism rates at different exposure





durations for both species of fruit flies. The maximum emergence rate of *D. giffardii* was observed on *Z. cucurbitae* than that on *B. dorsalis* pupae. Similar results were observed by Rasool et al. (2017), where *Z. cucurbitae* pupae showed a maximum parasitism rate of *D. giffardii*. The present study focused mainly on exposure duration and its effect on the parasitism activity of *D. giffardii*. The parasitoid showed the maximum parasitism rate of *Z. cucurbitae* and *B. dorsalis* pupae after 48 h of exposure. Kacar et al. (2017) described the parasitoids functional response in which the maximum number of parasitoids was observed after the hosts pupae were exposed for 48 h to parasitoids which support findings of the current study.

Adult emergence

Adult emergence data of *D. giffardii* from *Z. cucurbitae* pupae was determined at 5-, 10-, and 15-day intervals.

The maximum adult emergence of *D. giffardii* (63.55 ± 4.02) was observed on 10-day intervals after exposure of 24 h. The minimum adult emergence (13.85 ± 4.20) was observed at 5-day intervals after exposure of 72 h ($F = 2.78$; $df = 4, 26$; $P = 0.0473$) (Fig. 3).

Additionally, adult emergence data of *D. giffardii* for *B. dorsalis* pupae was also determined at 5-, 10-, and 15-day intervals. The maximum emergence rate of *D. giffardii* ($51.67 \pm 2.33\%$) was recorded at the 10-day intervals after exposure of 24 h. Minimum emergence ($11.66 \pm 0.50\%$) was observed at 15-day intervals after exposure of 72 h ($F = 4.61$; $df = 4, 26$; $P = 0.006$) (Fig. 3).

In the context of correlation, the results showed no correlation for the daily emergence of *D. giffardii* vs. exposure duration for *Z. cucurbitae* pupae ($R^2 = 0.0085$) (Fig. 4a) and *B. dorsalis* pupae ($R^2 = 0.004$) (Fig. 4b).

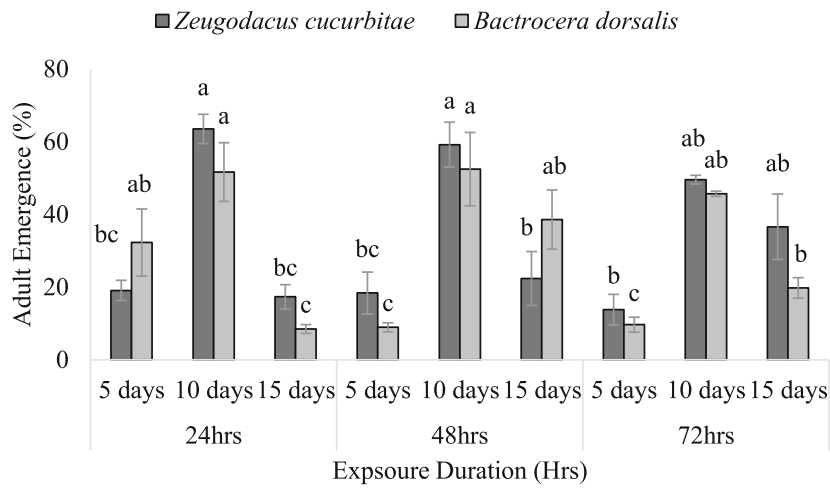


Fig. 3 Adult emergence of *Dirhinus giffardii* after 24, 48, and 72 h exposures subjected for *Zeugodacus cucurbitae* pupa and *Bactrocera dorsalis* pupae. Means followed by similar letter are not significantly different at 0.05% level of significance (for white and black columns)

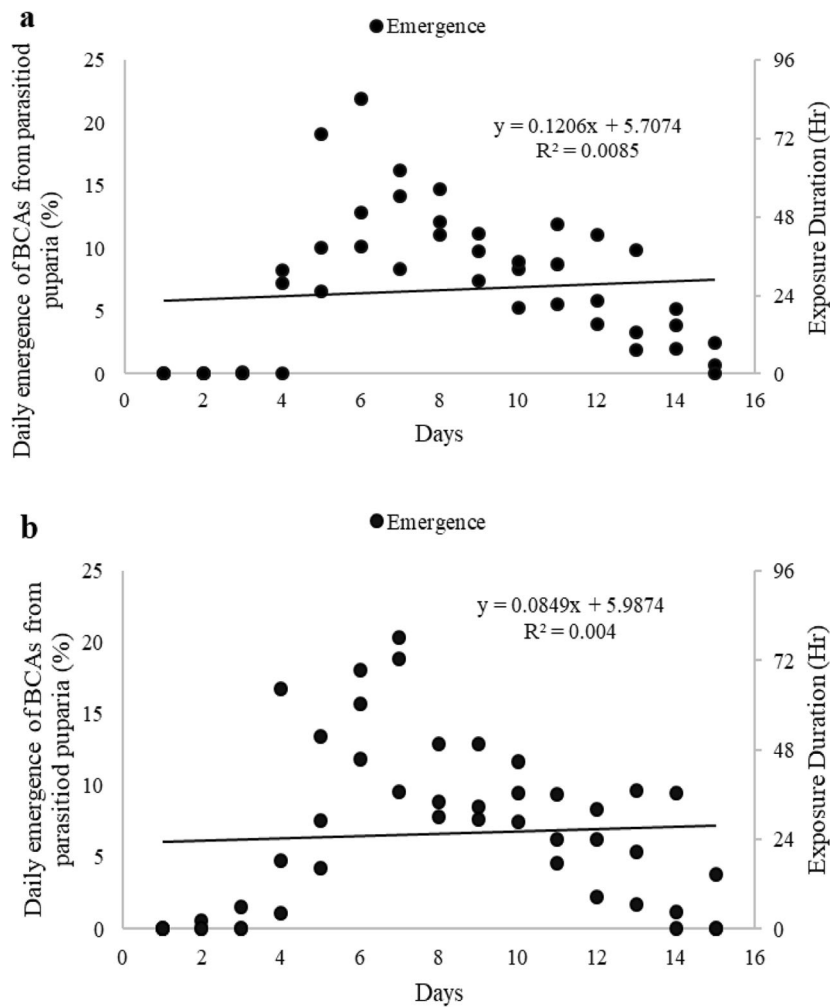


Fig. 4 Correlation between adult emergence of *Dirhinus giffardii* and exposure duration **a** *Zeugodacus cucurbitae* and **b** *Bactrocera dorsalis* pupae

The present study demonstrated the adult emergence of the parasitoids from infested hosts in relation to days. Interestingly, in the case of *D. giffardii* and *B. dorsalis*, a significant difference was observed for different exposure times; however, maximum emergence occurred at 10-day intervals for all exposure durations. Similarly, the results were observed for the *D. giffardii* and *Z. cucurbitae*, where a maximum emergence of flies and parasitoids occurred at 10-day intervals, although the present study showed that exposure duration had a significant effect on the adult emergence of parasitoids. However, Guillen et al. (2002) reported non-significant differences in parasitism rates of *Coptera haywardi* (Hymenoptera: Diapriidae) and *Pachycrepoideus vindemiae* Rondani (Hymenoptera: Pteromalidae) after exposure periods of 24, 36, 48, and 72 h.

Besides, the age of host, food source, and age of parasitoids are important factors that play a vital role in the development of parasitoids (Sitthichaiyakul and Amornsak 2017; Naveed et al. 2014). Keeping in view the importance of biological control and potential of *D. giffardii*, future studies will be conducted aiming on the assessment of parasitism and host preference of *D. giffardii* under choice and non-choice scenarios. Also, the present studies were conducted in the laboratory conditions and small arenas, which may not reflect field conditions. However, choose the scenario that could help us to identify some key factors, i.e., host selection and exposure time may aid in improving mass rearing protocols and comply with demands for rearing and maintenance within the context of research or commercialization.

Conclusion

In conclusion, the current study was performed to assess the biological activity of *D. giffardii* for fruit flies. *D. giffardii* emergence was maximum at 48 h of exposure duration for *Z. cucurbitae* ($52.60 \pm 2.84\%$) and *B. dorsalis* ($42.73 \pm 2.74\%$). Further, the results showed a correlation between parasitism to increasing exposure duration. The current study provides important information for mass rearing of *D. giffardii*, as comparing relative effectiveness and exposure duration of *D. giffardii* for fruit fly species could aid in improve rearing technology of this biocontrol agent.

Abbreviations

ANOVA: Analysis of variance; LSD: Least significance difference

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Authors' contributions

The concept and design of experiments were prepared by all authors. FA and NZ conducted the experiments in the laboratory. MF, SH, and FA wrote the manuscript and analyzed the data. All authors read and approved the manuscript.

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Availability of data and materials

All data of the study have been presented in the manuscript, and high-quality and grade materials were used in this study.

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Competing interests

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

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