



Research Article

Effect of Host Pupae Age and Depth in Plant Debris on the Searching Efficiency of Pupal Parasitoid *Dirhinus giffardii* (Silvestri) on Oriental Fruit Fly *Bactrocera dorsalis* (Hendel)

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Abstract | This research study reports the effect of pupal parasitoid *Dirhinus giffardii* on oriental fruit fly *Bactrocera dorsalis* (Hendel) at various host pupae depths (0, 1, 2, 3, 4 and 5 cm) in plant debris and at different host pupae ages (24, 48, 72 and 96 h) in laboratory at $25 \pm 2^\circ\text{C}$, $60 \pm 5\%$ relative humidity and a photoperiod of DL 14:10 h. The observations indicated a significant effect of different depths of host pupae and its age on the amount of parasitism by parasitoid. Maximum significant ($F = 10.78$, $P < 0.0001$) parasitized pupae were recorded at 72 h of age at surface of the debris, while minimum number of parasitized pupae were recorded in a day old pupae at 3 cm depth. Correlation of percent pupal parasitism with depths of host pupae in plant debris, showed a negative linear trend. With increasing depth, the percentage of parasitism decreased, showing the lowest significant ($F = 10.55$, $P < 0.0001$) 38.74% pupae parasitized at 3 cm depth. Whereas, correlation of percent of pupae parasitized with the age of host pupae exhibited a positive linear trend. Older host pupae were more parasitized showing significant ($F = 292.76$, $P < 0.0001$) peak of 54.60% at 72 h of age. Since, pupal parasitoid *D. giffardii* mass rearing is helpful for decreasing oriental fruit fly population. Hence, it can be suggested that parasitoids exposed to older pupae have a greater parasitism potential. However, parasitization rate is adversely affected by the presence of plant debris.

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Introduction

Increasing population has led to surge in the demand for food especially fruits and vegetables which provide all the essential nutrition for a healthy

lifestyle. However, insect pests, diseases and various other natural and unnatural factors affect both quality and quantity of the fruits and vegetables. Amongst the insect pests, fruit flies (Tephritidae: Diptera) are most destructive insect pests, causing

high economic losses to fruits and vegetables due to their polyphagous nature. Recently, approximately 11 tephritid pest species are reported as of economic importance posing threat alone to Australian horticulture industry (Sultana *et al.*, 2020). Fruits or vegetables damaged by fruit flies are neither suitable for feeding nor for marketing.

Though fruit fly species have wide range of host plants that they are able to attack, natural field infestation of one species, the oriental fruit fly *B. dorsalis* (Diptera: Tephritidae), alone has been recorded in 76 plant families (McQuate and Liquido, 2017). About 250 host plants, including citrus, peach, mango, mandarin, guava, chili pepper carambola and coffee, have been documented to be infested by *B. dorsalis* (Ye and Liu, 2005; Jin *et al.*, 2011).

A wide range of plants can be host for different fruit fly species. Natural means of managing insect pests of agriculture crops including biological control of insect pests. For instance, a classical biological method for the control of fruit flies outside Hawaii was explored that *Fopius arisanus* decreased the infestation and population rate of *B. dorsalis*, *B. tryoni* and *B. kirki* in guava fields (Vargas *et al.*, 2007). The native Australian braconid parasitoids *Diachasmimorpha tryoni* was applied against *B. tryoni* in the Australia, Hawaii and other regions (Zamek *et al.*, 2012). *Pachycrepoideus vindemmiae* can also be used as a biocontrol agent against *B. dorsalis* and it also has the capability to parasitize melon fly pupae at every stage (Zhao *et al.*, 2013).

Dirhinus giffardii (Hymenoptera: Chalcididae) is a pupal parasitoid that attacks a wide range of fruit-infesting tephritids, including *B. cucurbitae*, *B. dorsalis*, *B. tryoni* and *B. oleae* (Wharton and Yoder, 2021). This parasitoid is native to West Africa, and its original host is Mediterranean fruit fly *Ceratitis capitata*. Besides tephritids, *D. giffardii* has the ability to parasitize dipterous houseflies (Mohamed *et al.*, 2006; Wharton and Yoder, 2021). It has also been used for the first time in West Africa to control black soldier fly (Devic and Maquart, 2015). *D. giffardii* showed good management by parasitizing 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). This is a solitary parasitoids, well distributed in more than twenty countries particularly in Pacific and Central America, parasitizing puparia

of many cyclorrhaphous flies. They feed within puparia on host pupae that has developed from the host larvae exoskeleton. It does not kill its host till the larva develops from parasitoid egg (Wang and Messing, 2004a). An ectoparasitoid of Tephritidae fruit fly pupae, female *D. giffardii* oviposits onto the host pupa after piercing the puparia wall (Silvestri, 1914). Until the parasitoid larva hatches, the host continues to develop, while the host is paralyzed and consumed by the larva.

The minute eggs are elongated in form and somewhat narrower at one end. While dissection of pupae parasitized freshly, the developed embryo of parasitoid could be observed through the thin translucent eggshell with its head at the broader egg end (El-Husseini *et al.*, 2008). The larvae of this species which feed on host tissues, are transparent and white in color. The pupae complete life cycle in two to three days and male is born earlier than female. Fruit fly can survive from 18 to 30 days at 27°C temperature and 70-75% humidity (Wang and Messing, 2004a). Nutritional qualities differ in various hosts and host age (Morales-Ramos *et al.*, 2016). When parasitoid wasps were given a choice for oviposition, they preferred older puparia (Tang *et al.*, 2015). Whereas, maximum parasitism was recorded in older hosts pupae in various plant debris depths in case of *B. zonata* (Ali *et al.*, 2016). While given choice the parasitism rate of underneath pupae was adversely affected due the presence of pupae on surface (Okuyama, 2019).

B. dorsalis is among the insects known for underground pupation; and increasing density of larvae increases pupation depth. Therefore, depth can be considered among important factors influencing rate of parasitism (Okuyama, 2019). Considering the facts, this research was carried out to understand the searching ability of *D. giffardii*, pupal parasitoid wasp, on *B. dorsalis* at various depths of host pupae in plant debris and host pupae age.

Materials and Methods

Parent population of oriental fruit fly was maintained in the Insect Biocontrol Laboratory, Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University, Tandojam, Hyderabad in transparent plastic cages (18×12×12 inches) having proper ventilation at 25±2°C, 65±5% on food

supplement of suitable seasonal fruits for several years. For the experiment, fresh ber fruits were used for rearing larvae. Infested fruits were transferred in saw dust (purchased from local market) for pupation in the same sized cages. In following few days, larvae hatched out and for pupation were placed in saw dust. After few days, fresh pupae were sieved out of saw dust.

The stock culture of *D. giffardii* was also already maintained in the laboratory at $25 \pm 2^\circ\text{C}$, $65 \pm 5\%$ RH on artificial food supplements (solution of 30% honey and 70% water via soaked cotton) in (12×12×12 inches) transparent plastic cages for many generations.

The debris which was collected from horticulture orchard of the university, consisted of fallen leaves, fruits and twigs/shoots of chikoo tree and dead parts of other plants growing under these trees. This debris was kept in shade and airy place at room temperature for a week for drying out the surface moisture. This debris was then unevenly grinded and mixed manually by hand for constant distribution of all the constituents.

The experiment was carried out in the Insect Biocontrol Laboratory at $25 \pm 1^\circ\text{C}$, $60 \pm 5\%$ relative humidity. *B. dorsalis* pupae of different aged (24, 48, 72 and 96 h old) were buried at various depths (0, 1, 2, 3, 4 and 5 cm) of plant debris (collected from Horticulture orchard of the university) in (5 × 2.5 inches height × width) round plastic jars (each treatment factor was replicated five times). Since parasitoid *D. giffardii* prefers to attack older host puparia (Wang and Messing, 2004b), above mentioned age group of host pupae was selected for this study. *B. dorsalis* larvae prefer to pupate in soil depth of less than 4 cm (Hou et al., 2006), while pupation was not recorded 5 cm below surface (Okuyama, 2019), hence 0-5 cm depths were evaluated in this study.

In each jar, 30 un-parasitized pupae were placed gently on surface of a layer of soil at the bottom of the individual jar and then different depths of plant debris were managed on the pupae and 3 pairs of parasitoids *D. giffardii* (10 days old after emergence) in each jar were released for parasitism. The tops of the jars were covered with muslin cloths and were banded by round elastic. Each jar was supplied with a piece of cotton wool wet with water and honey as diet for the adult parasitoids. After 48 h period of parasitism, covers were taken off and plant debris was removed from the jars manually and pupae were sieved through sieving net and were kept into glass vials through camel hairbrush. The fruit fly pupae

were immediately dissected to check the presence of parasitoid egg or newly hatched pupa for determining the level of parasitism.

This study was designed as completely randomized design (CRD) having two factors; host pupae in 6 different plant debris depths (0, 1, 2, 3, 4 and 5 cm) and 4 ages of host pupae (24, 48, 72 and 96 h), each treatment factor was replicated five times. Data was subjected to statistical analysis by applying two-factor factorial ANOVA and post-hoc LSD test at $P \leq 0.05$ using software Statistix version 8.1 (Analytical Software, Tallahassee, Florida, USA).

Results and Discussion

Influence of various depths and ages of B. dorsalis pupae on its parasitization by D. giffardii

A significant difference was observed in number of parasitized *B. dorsalis* pupae by *D. giffardii* in combine effect of different plant debris depths and ages of host pupae ($F = 10.78$, $P < 0.0001$). Maximum parasitized pupae 18.46 were seen in 72 hours of host age at surface of the debris, followed by 17.53 in 2 cm and 17.45 in 4 cm depths in host pupae which were 48 and 72 hours old, respectively. Lowest parasitized pupae were 6.23 followed by 7.00 and 7.11 in 3, 1 and 2 cm depths, respectively in 24 hours old pupae (Table 1A).

Comparing the influence of debris depths on the number of parasitized pupae of fruit fly by wasp showed a significant difference in various depths ($F = 10.55$, $P < 0.0001$). The highest number of parasitized pupae 14.26 were at the surface of debris which gradually decreased to the 11.62 at 3 cm depth. Likewise, the effect of various host ages, on the number of pupae parasitized by parasitizing wasp, had significant difference ($F = 292.76$, $P < 0.0001$). The greatest number of parasitized pupae 16.38 were noted in 72 hours, while lowest were noted in a day old pupae.

Pupae of B. dorsalis left unparasitized by D. giffardii in various depths and ages of host pupae

Overall, there was a significant difference in interaction effect of different ages of pupae *B. dorsalis* and various depths of plant debris at which these pupae were placed, on the number of unparasitized pupae by parasitoid ($F = 15.96$, $P < 0.0001$). Maximum unparasitized pupae 17.27 were seen at 3 cm depth of the debris, followed by 15.83 in 1 cm and 14.93 in 2 cm depths in 24 hours old host pupae. Whereas, significantly lowest number of unparasitized pupae

were 5.12 which were recorded in 48 to 72 hours of host pupae ages at surface of debris and 2 cm depth, respectively, followed by 5.30 in 72 hours in 4 cm depth and 7.24 in 48 on debris surface (Table 1B).

Comparing the influence of debris depths on the number of unparasitized pupae of fruit fly by parasitoid wasp showed a significant difference in various depths ($F = 29.87$, $P < 0.0001$). The maximum number of unparasitized pupae 12.31 were at 3 cm depth of debris which gradually decreased to the 8.80 at surface. Similarly, the effect of various host ages also had significant difference on the number of unparasitized pupae by parasitoid wasp ($F = 312.53$, $P < 0.0001$). The greatest number of unparasitized pupae 14.68 were noted in a day old host pupae and minimum was noted in 72 h old pupae.

Correlation between percent of B. dorsalis pupae parasitized by D. giffardii with depths of host pupae in plant debris and age of host pupae

Correlation of various depths of oriental fruit fly in debris with its parasitization percentage by pupal parasitoid *D. giffardii* showed a negative linear trend ($R^2 = 0.2491$). With increasing depth, the percentage of parasitism decreased, showing the lowest 38.74% pupae parasitized at 3 cm depth (Figure 1A). Whereas, a positive linear trend ($R^2 = 0.3782$) was observed in correlation between different host ages of oriental fruit fly and its parasitization percentage by pupal

parasitoid *D. giffardii* (Figure 1B). Older pupae were more parasitized showing significant peak 54.60% at 72 hours of age.

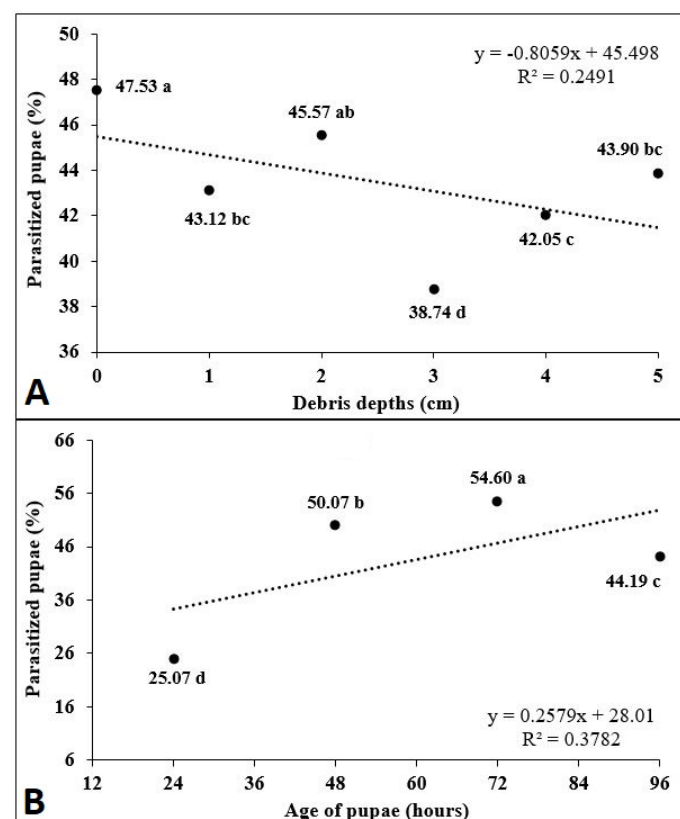


Figure 1: Correlation between percent of *B. dorsalis* pupae parasitized by *D. giffardii* with (A) depth of host pupae in plant debris and (B) the age of host pupae. Two-factor factorial ANOVA was conducted. Means sharing similar letters are not significantly different by post-hoc LSD at $P \leq 0.05$.

Table 1: Number of pupae (a) parasitized and (b) un-parasitized of *B. dorsalis* exposed to parasitoid *D. giffardii* in various depths and ages of host pupae.

Depths (cm)	Age (hours)				Total means
	24	48	72	96	
(A) Number of parasitized pupae					
0	8.00 ± 0.58 mn	16.25 ± 0.42 bcd	18.46 ± 0.46 a	14.33 ± 0.67 fgh	14.26 A
1	7.00 ±1.00 no	15.83 ± 0.27 cdef	16.85 ± 0.39 bc	12.07 ± 0.07 ij	12.94 BC
2	7.11 ± 0.49 no	17.53 ± 0.74 ab	14.55 ± 0.45 efgh	15.49 ± 0.29 cdefg	13.67 AB
3	6.23 ± 0.39 o	13.23 ± 0.52 hi	16.78 ± 0.69 bc	10.24 ± 1.27 kl	11.62 D
4	9.32 ± 0.09 lm	12.40 ± 0.29 ij	17.45 ± 0.56 ab	11.29 ± 0.29 jk	12.62 C
5	7.47 ± 0.79 no	14.89 ± 0.59 defg	14.20 ± 0.12 gh	16.12 ± 0.12 bcde	13.17 BC
Total means	7.52 D	15.02 B	16.38 A	13.26 C	
(B) Number of unparasitized pupae					
0	14.33 ± 0.67 cd	7.24 ± 0.05 j	5.12 ± 0.12 k	8.50 ± 0.29 ij	8.80 E
1	15.83 ± 0.27 b	8.53 ± 0.18 ij	7.49 ± 0.76 j	10.23 ± 0.91 gh	10.52 B
2	14.93 ± 0.30 bc	5.12 ± 0.12 k	8.20 ± 0.20 ij	8.32 ± 0.32 ij	9.14 DE
3	17.27 ± 0.15 a	10.23 ± 1.26 gh	7.52 ± 0.02 j	14.24 ± 0.63 cd	12.31 A
4	12.23 ± 0.12 ef	11.28 ± 0.02 fg	5.30 ± 0.15 k	12.23 ± 0.23 ef	10.26 BC
5	13.50 ± 0.26 de	9.18 ± 0.18 hi	8.22 ± 0.22 ij	7.83 ± 0.73 j	9.68 CD
Total means	14.68 A	8.60 C	6.97 D	10.22 B	

Means ± SE sharing similar small letters are not significantly different and total means sharing similar capital letters in respective rows and columns are not significantly different by post-hoc LSD at $P \leq 0.05$. $n = 30$.

Host age is one of the factors influencing the development of parasitoids (Rasool *et al.*, 2017). It is important for a parasitoid to select a host of suitable age for its development. Parasitoids can discriminate between host pupae of different ages, and then select a suitably aged host for parasitization. Nutritional qualities differ in various hosts, host instars or host pupal ages. Maximum fitness it achieved by parasitoids when they are attack relatively larger hosts (Wang and Messing, 2004c). This offers an apparent advantage for the survival of the parasitoid population. Our findings were concurrent with those of *B. zonata* and *B. cucurbitae* which showed increased amount of parasitism as the host pupae age advanced, showing peak parasitism on pupae of at the age of 3 days and a subsequent decline (Naveed *et al.*, 2014). It was also suggested that a greater potential regarding fecundity, parasitism performance and survival is achieved when parasitoids reared on older pupae of *B. cucurbitae*, as these parasitoids have been found to efficiently suppress *B. cucurbitae* populations in greenhouse (Mehmood *et al.*, 2018). Likewise, older puparia for oviposition are also preferred by some other parasitoid wasps while having other choices (Tang *et al.*, 2015). Although, we observed higher parasitism in older pupae in no choice experiment in consistence with two studies (Naveed *et al.*, 2014; Rasool *et al.*, 2017), yet host age preference was questioned even when directly exposed (Okuyama, 2021).

In accordance with our findings on oriental fruit fly, where percentage of parasitism decreased with increasing debris, different ages of host pupae of peach fruit fly to parasitoid *D. giffardii* displayed maximum parasitism in older pupae at different plant debris depths (Ali *et al.*, 2016). Host pupae density and variability in soil properties can affect fruit fly pupation depth (Hou *et al.*, 2006; Okuyama, 2019). This explains the importance of sanitation in the fields and orchards. Pests can take shelter in debris and making it hard for the natural parasites to search these pests. Based on these findings removal of plant debris can be included as one of the strategies of Integrated Pest Management to reduce the chance of pest to be out of reach of natural parasites .

In our results maximum parasitized pupae were observed at surface of the debris in 72 hours old host pupae and lowest parasitized pupae were observed at 3 cm in a day old host pupae. On the other hand, highest unparasitized pupae were seen at 3 cm in

a day old host pupae and minimum unparasitized pupae were observed at debris surface in 48 to 72 hrs. These observations are in accordance with a study which showed that by increasing exposure time and pupal age of peach fruit fly gradually increased the parasitism. Hence, supporting our findings, maximum parasitism was in three days old pupae, also indicating a positive correlation of increasing age with oviposition, development, emergence and parasitism of parasitoid pupa (Awais *et al.*, 2020a).

Another study showed that parasitoid density and exposure time had a significant impact on parasitism of melon fruit fly and emergence rate of parasitoids. Parasitism rate was highest when five pairs of *D. giffardii* parasitoid were incorporated and rate of emergence of parasitoid was also highest after six days of exposure (Awais *et al.*, 2020b).

Researcher have explored the parasitism rate and food preference of various *Bactrocera* species by parasitoids *D. giffardii*. It was observed that all species preferred soft fruits like guava and bitter gourd for feeding (Rasool *et al.*, 2017). In accordance with our observations, in this study, parasitism of *Bactrocera* fly species by *D. giffardii* was from 18 to 54% and it was maximum on 2-4 days old pupae. A study supported our results by showing that increasing weight of host which increases with age, showed a significant positive correlation between host pupal weight and the number of parasitoids emerging from a pupa (Syed *et al.*, 2018). Similarly, maximum parasitized melon fly pupae were 4 to 5 day old when exposed to parasitoid wasp *Spalangia endius* Walker. Both male and female had a longer development time on 6 to 7 days old host. A simultaneous linear decrease was observed in female (sex ratio) with an increase in host age (Tang *et al.*, 2015).

Our results are in consistent, when rearing of parasitoids of the peach fruit fly was examined for bulk production and biological control. The study showed that overall parasitism increased with increasing host larvae age by larval parasitoid *Trybliographa daci* Weld. Comparatively, higher parasitism was observed in 4 days old larvae than 5 days old (Sarwar *et al.*, 2015).

Further study is needed to understand the role of different soil properties on the host pupae population density, pupae depth and searching behavior of

parasitoid. Controlled research environment may provide an understanding of single factor but this may not be conclusive for natural field environment.

Conclusions and Recommendations

D. giffardii has been documented as an effective natural control agent of fruit flies. However, among other factors, host pupae age and host pupae depth in soil or debris can influence the extent of parasitism. Hence, presence of plant debris in orchards also affects parasitization rate. It can be further suggested that parasitoids exposed to older host pupae have a greater parasitism potential to suppress fruit fly populations.

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Novelty Statement

The selected fruit fly specie was exposed to parasitoid in various plant debris depths and ages of host pupae for the first-time in order to understand the searching behavior of parasitoid. Findings of the current study may provide a way forward for considering the influence of plant debris on natural pests in orchards.

Author's Contribution

Zarnosh Habib: Performed experiment and prepared first draft of manuscript.

Muhammad Ibrahim: Analyzed data and wrote the final manuscript.

Nasir Shah: Provided practical expertise.

Israr Ullah: Planned the experiment and gave final approval of manuscript.

Norman Javed Gill: Critically revised manuscript and gave final approval.

Conflict of interest

The authors have declared no conflict of interest.

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