Searching Ability of Pupal Parasitoid, *Dirhinus giffardii* (Silvestri) on *Bactrocera zonata* (Saunders) at Various Depths of Plant Debris

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Abstract: The present study was carried out to determine the searching ability of pupal parasitoid, *Dirhinus giffardii* of *Bactrocera zonata* in the Bio Control Research Laboratory, Department of Entomology, SAU, Tandojam, at temperature 27 ± 1ºC and 60 ± 5% relative humidity. Adults of fruit fly were fed with water, sugar and needo milk powder, whereas, *Dirhinus giffardii* were fed with honey and water solution. Ten days old *D. giffardies* were used against 20 pupae of *Bactrocera zonata* in the experiment and data recorded on parasitized pupae and un-parasitized pupae after 24, 48, 72 and 96 hours. The results in all treatments indicates that highest parasitized pupae were at peak level of (16.66) after 72 hours age of pupae on the depth of 0 cm in plant debris followed by 4 cm depth (16.33), whereas lowest parasitized pupae were recorded at 5cm depth (13.66) after 72 hours of age inside plant debris. Likewise, the highest un-parasitized pupae were at peak level of (14.00) after 24 hours of age of pupae on the depth of 3cm in plant debris followed by 1cm depth (13.00), whereas lowest un parasitized pupae were recorded at 4cm depth (11.66) after 24 hours of age inside plant debris. The analysis of variance indicated that there wasno significant difference among the parasitized and unparasitized pupae of flies in the different depths of plant debris and age intervals ($P<0.05$). It is concluded that the highest parasitized pupae were determined at plant debris of 0 cm, followed by 2cm, 5cm, 4cm, 1cm, and 3cm, respectively. In case of age intervals the highest parasitized pupae were recorded after 72 hours old pupae followed by 48 hours, 96 hours and 24 hours, respectively.

Keywords: Searching ability, *Dirhinus giffardii*, Parasitism potential, Guava fruit fly, artificial rearing.

INTRODUCTION

Pakistan has rich topographic and climatic endowments and variations in plant debris where, large ranges of horticultural crops are grown. Horticulture sector can provide opportunities to increase income and alleviate hunger, poverty and reduce socio-economic problems [1]. Total annual production of fruits and vegetables is 12 metric tons in Pakistan wherein, fruits production is about 5.71 metrictons. Important fruits produced in Pakistan are citrus, mango, dates, guava, banana, peach, plum, pear, apple, apricot, grapes, and persimmon [2]. Guava, *Psidium guajava* L. (Family: Myrlaceae) is one of the most common fruits commercially grown in different areas of Pakistan. It is a sub- tropical tree that grows up to the height of 35 feet [3].

Tephritidae is a large family of fruit flies nearly 4,500 described species arranged in about 500 genera [4]. Very common pests of economic importance in nearly all tropical, subtropical and various temperate regions of the world are (Diptera) Fruit flies [5]. *Bactrocera zonata* (Saunders) is considered as one of the most critical pests of fruits, which was spread in many regions of the world. It is also recorded in some countries where it causes huge problems to various fruits in Pakistan, and about 25 to 50% losses in guava fruits [6]. Most of the destructive species of fruit flies are *Bactrocera dorsalis*, *Bactrocera zonata, Bactrocera cucurbitae* and *Dacus ciliates*. All the species are polyphagous in nature and damage a wide range of vegetables and fruits by affecting their production [7]. Most of the fruit fly species are highly polyphagous attacking several important vegetables and fruits including citrus, guava, mango, avocado, tomatoes, cucurbits and pepper etc. Female adults of the fruit flies lay eggs underneath the skin of the vegetables and fruits and hence cause direct losses. The eggs develop into larvae that feed in the decaying flesh of the crop. Infested fruits and vegetables quickly rot and turn into inedible or drop to the ground. In additional to cause direct losses in the marketability and yield, which posses significant threats to quarantine security and thus to international trade in fresh vegetables and fruits world-wide [8]. Thus it is vital to search the control strategies for the pest to reduce the usage of pesticides against agricultural pests. With expression paying attention on alternative control program, there has been a renewed interest in bio control. Biological control appropriate application tenders effective, environmentally sustainable and safe approach for the management pests. Releases of the expected enemies at suitable stage and time in the field are another critical factor for successful use of bio control expertise

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[9].Recently, biological control efforts have been focused on augmentative release of *D. giffardii*, (Silvestri) and *Fopiusaris anus* (Sonan) (Hymenoptera: Braconidae) [10]. *Dirhinus giffardii* has been recorded in more than 20 countries in the world and is native to West Africa [11], to control the (Diptera) pests. sFor example, in Hawaii *Pachycrepoideus indemmiae* was introduced to control the house fly and horn fly from Asia, while *D. giffardii* was recorded from West Africa on attacking the pupae of fruit flies during the 1900s [12].

Keeping the above facts there is great need to work on "Searching ability of pupal parasitoid, *D. giffardii* (silvestri) of *Bactrocera zonata* (Saunders) at different depths inside plant debris". This research work will be helpful for field release to prevent the fruits from the attack of fruit fly.

MATERIALS AND METHODS

Experiment was conducted at Bio-Control Research Laboratory, Department of Entomology, Sindh Agriculture University Tandojam. Three different kinds of artificial diets namely water, sugar and needo milk powder were offered to adults of fruit fly while honey and water solution was provided to *D. giffardii*. The particular diet in each treatment was provided to adults throughout the course of experiment. Each treatment was repeated three times. In each replication three pairs of male and female were used. Ten days old *D. giffardies* were exploited in the experiment at 27 ± 1ºC, 60 ± 5% relative humidity.

Dirhinus giffardii, was reared on artificial diet (solution of 30% honey and 70% water), the food (cotton impregnated with honey and water) given to the *Dirhinus giffardii* presents in the cages, and these wigs were washed daily and fresh diet were offered to the parasitoids.

Larval Diet: Larvae were reared on different fresh fruits. Such as: Guava and banana. After 24 hours oviposition of the female flies, the infested fruits were transfer in the saw dust for pupation in the cage.

Saw dust: It was purchased from Tandojam Aara machine, after few days the full grown larvae were pop out and drop himself into saw dust for pupation. After few days the saw dust was sieved to separate the fresh pupae.

Plant Debris: Debris was collected from SAU, Tandojam horticulture garden. In the debris, dried leaves of guava and mango were collected from plant debris surface and opened on the clean ground on sun light for the conformation of moisture in leaves.

Experimental Design: CRD

Treatments: Different days old pupae were buried inside plant debris at the depth of: T1=0cm depth, T2=1cm depth, T3=2cm depth, T4=3cm depth, T5=4cm depth, T6=5cm depth.

Pupal age = 24, 48, 72 and 96 hours.

Replication = 3

Procedure

In this experiment 48 hours old un-emerged pupae of *B. zonata* were kept inside the plant debris at different depths as: 0, 1,2,3,4 and 5cm in plastic jars (replication). In each jar (replication) twenty un-parasitized pupae were seeded on soil surface inside the jars and then different depths of plant debris were covered and three pairs of pupal parasitoid *D. giffardii* was released for parasitism and diet were given on the sides of jars. The age of pupal parasitoids were ten days old.

In the same experiment next parameter (sub treatment) was the time period duration, in which different time interval pupae of *B. zonata,* 24 hours, 48 hours, 72 hours and 96 hours old were buried at 0cm, 1cm, 2cm, 3cm, 4cm and 5cm depths inside the debris for 48 hours for parasitization. The top of the jars were covered with muslin cloth and banded by round elastics. After 48 hours period of parasitism, covers were opened and plant debris was removed from the jars with the help of hands and pupae were sieved from the plant debris surface through the help of sieving net and kept into glass vials through camel hair brush to monitor the parasitization.

The collected data was subjected for statistical analysis and statistical differences existed between data sets (P<0.05), Fisher's Least Significant Differences (LSD) was used to separate the differing means according to [18].

RESULTS

Influence of the Parasitization after 24, 48, 72 and 96 Hours on Pupae of *Bactrocera zonata* at Various Plant Debris Depths

Parasitized pupae of *B. zonata* after 24, 48, 72 and 96 hours as influenced by various plant debris depths

Table 1: Influence of the Parasitization after 24, 48, 72 and 96 Hours on Pupae of *B. zonata* as by Various Plant Debris Depths

S. No.	Treatment	Age			
		24 hrs	48 hrs	72 hrs	96 hrs
1	T1= 0 cm depth	08.00	15.00	16.66	12.66
2	T2=1cm depth	07.00	14.33	15.00	11.33
3	T3=2cm depth	07.33	16.33	13.66	13.00
4	T4=3cm depth	06.00	12.00	15.00	08.00
5	T5= 4cm depth	08.33	11.00	16.33	08.33
6	T6=5cm depth	07.33	13.00	13.66	13.33

		Treatment	Age	Interactions
SE±	=	1.1189	0.9136	2.2379
LSD @ 0.05	=	2.2523	1.8390	4.5046

were determined and the data are depicated in Table **1**. The analysis of variance demonstrated a non-significant variation for parasitized pupae of *B. zonata* among the treatments and significant difference for 24, 48, 72 and 96 hours of age pupae. While interaction for parasitized pupae of *Bactrocera zonata* between various treatment and different age was non-significant (Appendix-I).

The results showed that the highest parasitized pupae were at peak level of (16.66) after 72 hours of age of pupae on the depth of 0 cm in plant debris followed by 4 cm depth (16.33), whereas lowest parasitized pupae were recorded at 5cm depth (13.66) after 72 hours of age inside plant debris. In addition, the results regarding 48 hours pupal age showed that highest parasitized pupae were (16.33) on the depth of 2 cm in plant debris followed by 0 cm depth (15.00), while lowest parasitized pupae were recorded at 4 cm (11.00) after 48 hours of age inside plant debris depth. In continuation to the 24 hours age of pupae maximum parasitization were recorded (08.33) on the depth of 4cm followed by (08.00) on the plant debris depths of 0 cm, while minimum parasitized pupae (06.00) were noted on the plant debris depths of 3cm after 48 hours age of the pupae. Whereas the highest parasitized pupae were (13.33) after 96 hours of pupal age on the plant debris depth of 5cm followed by (13.00) on the depth of 2cm, while lowest parasitization was recorded on 3cm were (08.00) inside plant debris depths. According to the findings 96 hours pupal age is concerned, the results showed strange termed as we increase the pupal age after 72 hours, the parasitization dramatically decreased (see 96 hours) in (Table **1**).

Un- parasitized pupae of *B. zonata* after 24, 48, 72 and 96 hours as influenced by various plant debris depths was determined and the data is reported in Table **2**. The analysis of variance demonstrated a non-significant variation for un-parasitized pupae of *B. zonata* among the treatments and significant difference for 24, 48, 72 and 96 hours of age, while interaction for un-parasitized pupae of *B. zonata* between various treatment and different age was non-significant (Appendix-II). The results indicates that the highest un parasitized pupae were at peak level of (14.00) after 24 hours of age of pupae on the depth of 3cm in plant debris followed by 1cm depth (13.00). Whereas lowest un parasitized pupae were recorded at 4cm depth (11.66) after 24 hours of age inside plant debris. The results regarding 96 hours pupal age showed that the highest un parasitized pupae were (12.00) on the depth of 3cm in plant debris followed by 4 cm depth (11.66), while lowest un parasitized pupae were recorded at 5 cm (06.66) after 96 hours of age inside plant debris depth. In continuation to the 48 hours age of pupae, maximum un parasitized pupae were recorded (09.00) on the depth of 4cm followed by (08.00) on the plant debris depths of 3 cm, while minimum un parasitized pupae (03.66) were noted on the plant debris depths of 2cm after 48 hours age of the pupae. Whereas the highest un parasitized pupae were (06.33) after 72 hours of pupal age on the plant debris depths of 2cm and 5cm followed by (05.00) on the depth of 1cm. While lowest un-parasitization was recorded on 0 cm were (03.33) inside plant debris depths.

Table 2: Influence of the Un- Parasitized Pupae of *Bactrocerazonata* after 24, 48, 72 and 96 Hours as by Various Plant Debris Depths

S. No.	Treatment	Age			
		24 hrs	48 hrs	72 hrs	96 hrs
1	T1= 0 cm depth	12.00	05.00	03.33	07.33
2	T2=1cm depth	13.00	05.66	05.00	08.66
3	T3=2cm depth	12.66	03.66	06.33	07.00
4	T4=3cm depth	14.00	08.00	05.00	12.00
5	T5= 4cm depth	11.66	09.00	03.66	11.66
6	T6= 5cm depth	12.66	07.00	06.33	06.66

		Treatment	Age	Interactions
SE±	=	1.1189	0.9136	2.2379
LSD @ 0.05	=	2.2523	1.8390	4.5046

DISCUSSION

In the current finding indicated in the all treatments that maximum parasitization of pupae were at peak level of (16.66) after the age of 72 hours on the depth of 0 cm in plant debris followed by 4 cm depth (16.33), whereas lowest parasitized pupae were recorded at 5cm depth (13.66) after 72 hours of age inside plant debris. Whenever, highest un parasitized pupae were at peak level of (14.00) after 24 hours of age of pupae on the depth of 3cm in plant debris followed by 1cm depth (13.00), whereas lowest un parasitized pupae were recorded at 4cm depth (11.66) after 24 hours of age inside plant debris respectively. This findings are in accordance with those of Purcell *et al*.[19] who observed the effects of guava ripening on large quantity and parasitism ratio of oriental fruit fly, *Bactrocera dorsalis* (Hendel) parasitoids. The egg parasitoid, *Biosteres arisanus* (Sonan) was the leading parasitoid raising from tree harvested guavas fruits at all sites and composed 90-98% of all parasitoids recovered but minimum in profusion as guava fruit aged on the field surface, the impact of this parasitoid is usually underestimated by sampling. The eulophid parasitoid, *Tetrastichus giffardianus* (Silvestri), was more plentiful in 4- to 9-day-old ground fruit. Dani *et al*. [20] reported that larvae characteristically burrowed no >2 cm before puparium and seldom burrowed >5 cm. At 4 field sites, pupae of the most commonly encountered were placed on the surface of plant debris at 2.5 and 5 cm depths and were sampled 10 days. At the end of the sampling period the quantity of pupae residual ranged from 15 to 70%. Differences in the species of parasitoids and predators were located plant debris surface and attacked many of the insect pest species. Guillen *et al*. [13] reported that time (24, 36, 48, and 72 h) had no effect on parasitism percent but *C. Haywardi* was also capible to parasitize the pupae that were buried up to 5 cm depth. Wang *et al*. [17] reported the two solitary parasitoids, *Pachycrepoideus vindemmiae* (Rondani) and *Dirhinus giffardii* (Silvestri) are effective for many fruit flies pupae, they get food from the host pupae inside and host remain alive until its egg develops into larva and permanently paralysed the host embryo. As a result, ovipositing into an immature host puparium (1 day old) in which the host pupa has not yet fully produced. Results in complete death of offspring in *P. vindemmiae*, but *D. giffardii*, even if anguish higher death than in older host puparia. According to their age interval observed its host preference to 2- to 3-day-old pupae due to fully development of host and liked un-parasitised pupae rather than other parasitized previously. Baig *et al*. [22] recorded highest parasitism (28.3%) at 15 pupae of *D. giffardii*, while emergence was highest (13.3) under laboratory condition. Justin *et al*. [23] suggested that mulching impacts on *R. mendax* significantly on pupation depth with potential of the searching habbit of parasitoids implications for its management. Zhao *et al*. [24] proposed that 3 to 6 days-old pupae of *B. dorsalis* are suitable host ages for *P. vindemmiae* and suitable for mass rearing of *P. vindemmiae* in lab circumstances

for bio control techniques. Naveed *et al*. [25] recorded highest parasitism at 3 days old pupae and respectively observed on the age of 5 to 6 days pupae of the fruit fly species and from day 7 to onwards no parasitism was recorded. The studies optionally suggested that the *D. giffardii* parasitoids should be redundant after the age of 15 days for superior rearing. Liang *et al*. [26] showed that *S. endius* females deposited eggs successfully in different age's pupae of *B. zonata* and *B. cucurbitae* and disturbed the progeny of host inside the pupae. There was an oviposition liked in 3 to 5 days old pupae. The maximum ratio of parasitization occurred on 4 and 5 day old hosts, followed by 2 and 3 days old pupae of hosts. The regular emergence time for both males and females was significantly longer in 6 to 7 days old hosts than in the immature host stages. These outcomes suggest that *S. endius* and most of the tephritids are best partners for the biological control against fruit flies by releasing parasitoids.

CONCLUSION

It is recommended that the mass production of pupal parasitoid *Dirhinus giffardii* will be helpful for the reduction in the population of the fruit flies. The availability of plant debris in the orchard does not effect on the parasitization rate, however, sanitation in orchard will be helpful to increase rate of parasitization of pupae of fruit flies in the orchards.

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