

## Research Article

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# Assessment of the parasitism potential of three parasitoids of fruit fly, *Bactrocera* spp. (Diptera: Tephritidae) under laboratory conditions

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### Abstract

The parasitism potential of a pupal parasitoid, *Dirhinus giffardii* Silvestri and two larval-pupal parasitoids, *Diachasmimorpha longicaudata* (Ashmead) and *Aganapis daci* (Weld) was assessed against *Bactrocera* spp. under laboratory conditions. Three different types of hosts, viz. *Bactrocera zonata* (Saunders), *Bactrocera dorsalis* (Hendel) and *Bactrocera cucurbitae* (Coquillett) were reared on artificial larval diet and known number of pupae and larvae of each fruit fly species were offered to the respective parasitoids in glass cages in a no choice test. Results on the parasitism potential of *D. giffardii* towards pupae of different fruit fly species revealed that the highest per female parasitism was recorded on *B. zonata* ( $16.72 \pm 1.67$ ). Adult emergence percentage of *D. giffardii* did not differ significantly among all the three *Bactrocera* spp. Sex ratio of the emerged parasitoids revealed maximum percent females ( $61.64 \pm 2.67$ ) from pupae of *B. cucurbitae*. Similarly per female parasitism by *D. longicaudata* was also significantly the highest on larvae of *B. zonata* ( $26.40 \pm 1.79$ ) with maximum adult emergence percentage of  $93.41 \pm 2.54$ . Sex ratio of the emerged *D. longicaudata* did not differ significantly. Parasitism rate of *A. daci* was significantly the highest on larvae of *B. zonata* ( $24.88 \pm 2.01$ ) with insignificant differences in the adult emergence percentage. Sex ratio of *A. daci* showed that percent females from the emerged parasitoids were the highest ( $54.2 \pm 3.44$ ) on *B. dorsalis*. Relative collective parasitism per female by all the three fruit fly parasitoids revealed that highest parasitism rate ( $23.7 \pm 2.26$ ) was exhibited by *D. longicaudata* followed by *A. daci* ( $22.72 \pm 2.14$ ). The study manifested that *B. zonata* could be the ideal host for laboratory rearing of these parasitoids.

**Keywords:** *Bactrocera*; Emergence; Fruit fly; Host; Parasitoids; Parasitism; Sex ratio

### Introduction

Fruit flies, *Bactrocera* spp. (Diptera: Tephritidae) are pests of great economic

importance in the tropical and subtropical regions of the world [1]. This cosmopolitan distribution of fruit flies highlights their

international importance in sustainable fruit and vegetable production as well as trade issues [2, 3]. High value exports of fruit (citrus, guava, mango etc.) and vegetables (especially cucurbits) significantly contribute in the economy of Pakistan [4]. However, being major quarantine pests of fruits and vegetables, fruit flies not only create hindrances in fruit and vegetable exports but also reduce their average ha<sup>-1</sup> yield. In favorable conditions, production from the entire crop can be wiped out by these pests and the whole agricultural economy of the infested area could be ruined [5]. The genus *Bactrocera*, with about 651 described species, have been reported the most economically significant fruit fly genus. About 50 species in this genus are considered to be highly destructive and major polyphagous pests of horticultural and vegetable crops [6-8]. Among these the *Bactrocera cucurbitae* (Coquillett), *Bactrocera dorsalis* (Hendel) and *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) inflict heavy losses to a wide range of fruits and vegetables in Pakistan.

Injudicious and frequent use of pesticides each year for the control of these pests are continuously affecting the biotic and abiotic factors of the environment [9]. Biological control is a fundamental component of Integrated Pest Management system [10]. Unlike chemical control method, it has no ill effects on the environment. In certain cases, biological control alone produced magnificent results in suppressing pest population eliminating the need for other control methods [11]. It has been demonstrated in previous studies that *Aganapis daci* (Weld) (Hymenoptera: Eucilidae), *Dirhinus giffardii* Silvestri (Hymenoptera: Chalcididae) and *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) are important natural enemies of *Bactrocera* spp. Of these biological control agents, *A. daci* (originally

described as *Trybliographa daci* Weld), was first collected in Malaysia and Borneo, and introduced into Hawaii as a potential biocontrol agent for *B. dorsalis* [12]. It has also been successfully reared on and released against Medfly in many European countries including Greece and France [13, 14]. *D. giffardii* is also an efficient pupal parasitoid of many fruit fly species. It is primitive of West Africa and has been effectively used in the biological control program in many countries for reducing Dipteran pest population [15-17]. *D. longicaudata* is a larval-pupal parasitoid of several fruit fly species and is native to Southeast Asia [17, 18]. It has been used in augmentative biological control programs against *B. dorsalis* [19]. Similarly, it is also widely spread in Pakistan with parasitism rate exceeding 36% on *B. dorsalis* in the foot hills. In Pakistan, *A. daci* together with *D. longicaudata* resulted in more than 44 % parasitism of *B. zonata* in unsprayed orchards in the plains [20].

Production of natural enemies in an efficient and economical way is a prerequisite for biological control program. Considerable technological advances have been made in mass rearing of parasitoids and predators for augmentative biological management of the pests [21-23]. Similarly production of laboratory reared natural enemies may be enhanced by the provision of suitable host. From the last several years *D. giffardii*; *D. longicaudata* and *A. daci* have been documented from the mango and guava orchards of Sindh. Keeping this in view, the present study was designed to find out the parasitism potential of these parasitoids and hence, a suitable host for their proficient rearing.

#### **Materials and methods**

The experiment on parasitism potential of three parasitoids, viz. *D. giffardii*, *D. longicaudata*, and *A. daci* was conducted in the fruit fly rearing laboratory of Nuclear

Institute of Agriculture (NIA) Tandojam under controlled conditions ( $26 \pm 1$  and  $65 \pm 5\%$  RH and 16 h L: 8 h D photoperiod). Three different kinds of hosts, *B. zonata*, *B. dorsalis* and *B. cucurbitae* were reared on artificial larval diet containing wheat bran (26%), sugar (12%), dried torula yeast (3.6%), Sodium benzoate (0.1%), Methyl-p-hydroxybenzoate (0.1%) and water (58%). The adult fruit flies were provided with sugar and protein hydrolysate. For oviposition, plastic glasses having 0.5 mm holes around them were smeared internally with guava juice and placed in the adult cages. Eggs laid by females in these jars were collected and removed by fine hair brush. These eggs were further used for maintaining the culture by directly seeding (about 2.5 g) on artificial diet (2.5 kg) in metallic trays. The trays were kept above the pupation substrate (saw dust) under laboratory conditions. The larval pop out for pupation started on 8-10 days after the eggs being seeded. The pupae were collected by sieving the saw dust regularly on daily basis till complete pupation of all the larvae. The strains of pupal parasite, *D. giffardii*, *D. longicaudata* and *A. daci* were maintained at the fruit fly rearing laboratory. *D. giffardii* were reared in glass cages (30×30×30 cm) on pupae of *B. zonata* whereas culture of *D. longicaudata* and *A. daci* were maintained on larvae of *B. zonata* in glass cages (30×30×30 cm). For adult parasitoids artificial diet containing fresh solution (30% honey and 70% water) were provided through soaked cotton wigs impregnated with honey and water.

To find out the parasitism potential of *D. giffardii* five pairs of the parasitoid (5-6 days old) were released separately in glass jars (18×18×18 cm) containing 3 days old pupae (200) of one of the three fruit fly species, viz. *B. zonata*, *B. dorsalis* and *B. cucurbitae*. After 24 h the pupae were removed and placed in another jar for parasitoid emergence. The experiment was replicated 5

times. The data were regularly recorded on parasitism rate and emergence of wasps. The sex ratio of the emerged parasitoids was also accomplished carefully. Similarly for other two parasitoids, *D. longicaudata* and *A. daci*, 3-4 days old 300 larvae of each of the above mentioned fruit fly species were provided separately in Petri dishes on artificial diet placed in glass jars containing 5 pairs of one of the two species of parasitoids for 24 h period. After 24 h, the larvae were removed in petri dishes and placed in another glass jar having saw dust for pupation. The pupae were collected after pupation and placed back in the glass jar for emergence. Data were regularly recorded on parasitism rate, emergence and sex ratios of the parasitoids.

#### Statistical analysis

Data recorded on parasitism rate, emergence and sex ratio of different parasitoids were subjected to analysis of variance (one-way ANOVA) using Statistical Software program IBM SPSS Statistics 20. Multiple comparisons among the means were made using Tukey's HSD test ( $P < 0.05$ ).

#### Results

Results on the parasitism potential of *D. giffardii* on pupae of different *Bactrocera* species revealed that the highest per female parasitism was recorded on *B. zonata* ( $16.72 \pm 1.67$ ) followed by parasitism of  $15.04 \pm 0.87$  on *B. zonata* (Table 1). Significantly the lowest per female parasitism ( $10.76 \pm 0.89$ ) was recorded on *B. cucurbitae* ( $P < 0.05$ ). Percent parasitism also showed the same trend. Adult emergence percentage of *D. giffardii* did not differ significantly among all the three *Bactrocera* spp. However, the highest ( $94.17 \pm 2.67\%$ ) was recorded on pupae of *B. zonata* followed by  $91.20 \pm 3.23\%$  on pupae of *B. dorsalis* ( $P > 0.05$ ).

Sex ratio of the emerged parasitoids (Figure 1) revealed that maximum percent females ( $61.64 \pm 2.67$ ) were recorded from pupae of *B. cucurbitae* which were statistically at par with percent females emerged from pupae of

*B. zonata* ( $59.27 \pm 3.17$ ). Whereas significantly the lowest female percentage was recorded from pupae of *B. dorsalis* ( $52.47 \pm 2.58$ ) ( $P < 0.05$ ).

Per female parasitism by *D. longicaudata* was significantly the highest (Table 2) on larvae of *B. zonata* ( $26.40 \pm 1.79$ ) followed by parasitism on the larvae of *B. dorsalis* and *B. cucurbitae* ( $22.16 \pm 1.07$  &  $20.52 \pm 0.81$ , respectively) ( $P < 0.05$ ). It is important to note that parasitism rate of *D. longicaudata* on *B. dorsalis* and *B. cucurbitae* were found insignificantly different. The same sequence was also true for percent parasitism. Likewise, maximum adult emergence percentage was recorded on *B. zonata* ( $93.41 \pm 2.54$ ) followed by *B. dorsalis* ( $92.04 \pm 1.59$ ). The lowest adult emergence percentage ( $91.26 \pm 2.35$ ) was recorded on *B. cucurbitae*. However, the same was found non-significantly different among all the treatments ( $P > 0.05$ ).

Sex ratio of the emerged parasitoids revealed that the percent females of *D. longicaudata* from all the three species of *Bactrocera* did not differ significantly (Figure 2). However highest percentage of females ( $57.75 \pm 3.6$ ) was recorded on *B. cucurbitae* followed by *B. dorsalis* ( $55.18 \pm 4.13$ ).

Parasitism rate (per female) of *A. daci* was significantly the highest on larvae of *B. zonata* ( $24.88 \pm 2.01$ ) followed by parasitism of  $21.72 \pm 1.12$  and  $21.56 \pm 1.23$  on larvae of *B. dorsalis* and *B. cucurbitae*, respectively ( $P < 0.05$ ). Parasitism rate of *A. daci* for the latter two species of *Bactrocera* was found statistically insignificant. Percent adult emergence of *A. daci* also did not differ significantly among all the treatments, however, the highest ( $93.84 \pm 3.14$ ) was recorded on *B. zonata* and the lowest ( $91.59 \pm 3.23$ ) on *B. cucurbitae* (Table 3).

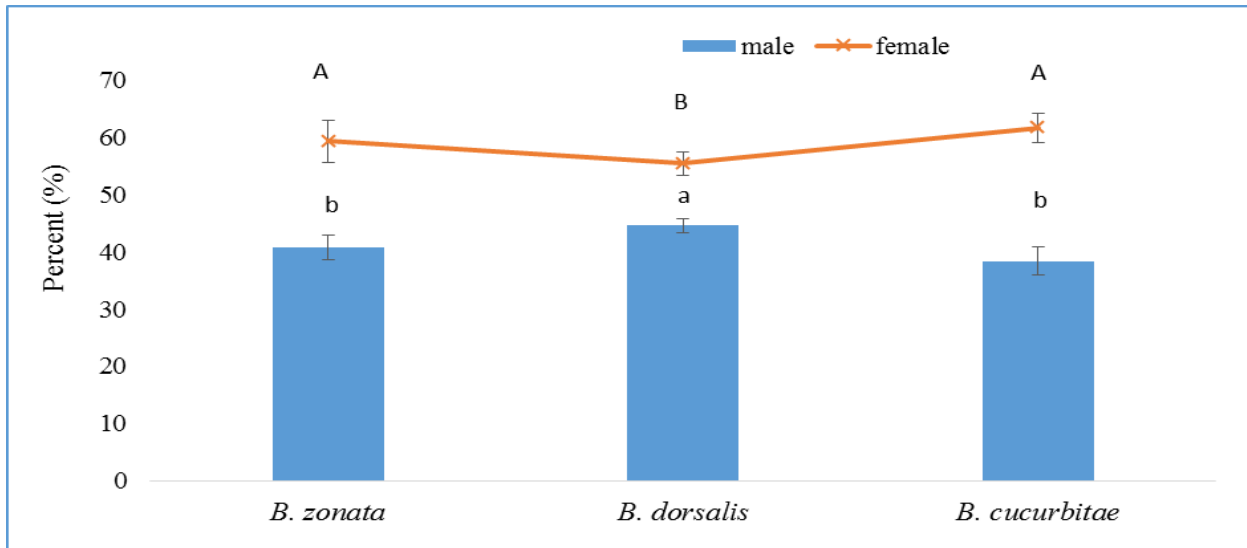
Sex ratio of *A. daci* showed that percent females from the emerged parasitoids were the highest ( $54.2 \pm 3.44$ ) on *B. dorsalis* followed by *B. zonata* ( $53.73 \pm 4.16$ ). Lowest female percentage ( $52.64 \pm 3.56$ ) was recorded on *B. cucurbitae*. However, sex ratio in term of percent females did not show significant differences (Figure 3).

Relative collective parasitism per female by all the three fruit fly parasitoids revealed that highest parasitism rate ( $23.7 \pm 2.26$ ) was exhibited by *D. longicaudata* followed by *A. daci* ( $22.72 \pm 2.14$ ). Significantly the lowest parasitism rate was showed by *D. giffardii* ( $14.43 \pm 1.89$ ) (Figure 4).

**Table 1. Parasitism potential of *D. giffardii* on pupae of three *Bactrocera* species**

Treatment	Parasitism/female	% Parasitism	% Emergence
<i>B. zonata</i>	$16.72 \pm 1.67$ a	$8.36 \pm 1.12$ a	$94.17 \pm 2.67$ a
<i>B. dorsalis</i>	$15.04 \pm 0.87$ a	$7.90 \pm 1.05$ a	$91.20 \pm 3.23$ a
<i>B. cucurbitae</i>	$10.76 \pm 0.89$ b	$5.38 \pm 0.84$ b	$88.32 \pm 4.12$ a
F-ratio	13.95	16.23	0.96
P value	0.001	0.001	0.410

Means followed by different letters within a column show significant differences ( $P < 0.05$ )

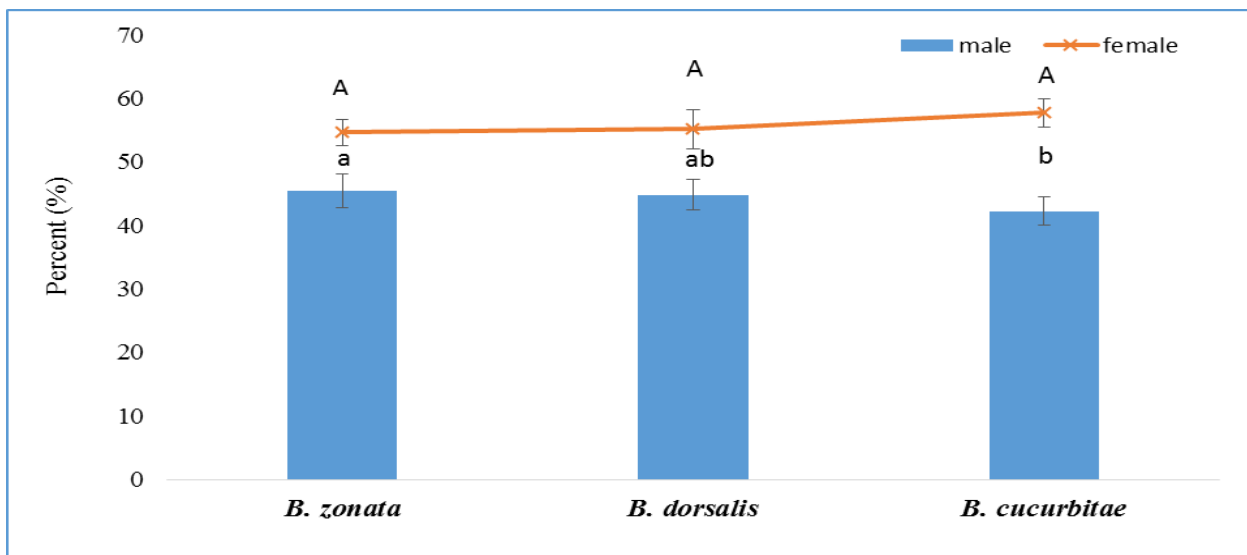


**Figure 1. Sex ratio (percent males & females) of *D. giffardii* on pupae of different *Bactrocera* spp. (capital and small letters show comparison among female and male parasitoids, respectively)**

**Table 2. Parasitism potential of *D. longicaudata* on larvae of three *Bactrocera* species**

Treatment	Parasitism/female	% Parasitism	% Emergence
<i>B. zonata</i>	26.40 ± 1.79 a	13.70 ± 1.23 a	93.41 ± 2.54 a
<i>B. dorsalis</i>	22.16 ± 1.07 b	11.08 ± 0.88 b	92.04 ± 1.59 a
<i>B. cucurbitae</i>	20.52 ± 0.81 b	10.26 ± 1.04 b	91.26 ± 2.35 a
F-ratio	15.59	12.43	0.47
P value	0.0001	0.001	0.63

Means followed by different letters within a column show significant differences ( $P < 0.05$ )

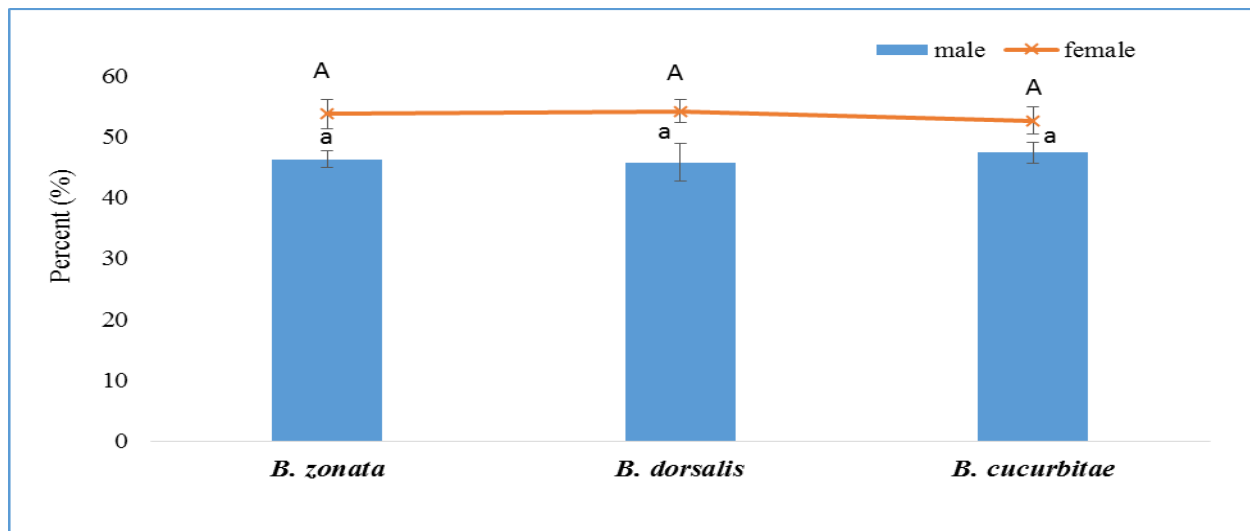


**Figure 2. Sex ratio (percent males & females) of *D. longicaudata* on larvae of different *Bactrocera* spp. (capital and small letters each show comparison among female and male parasitoids, respectively)**

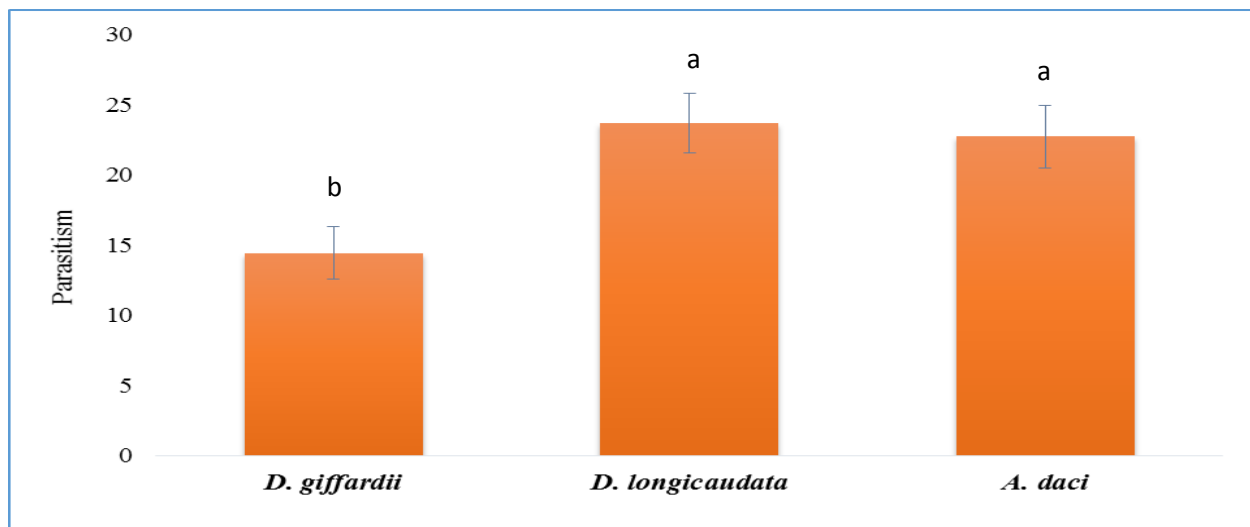
**Table 3. Parasitism potential of *A. daci* on larvae of three *Bactrocera* species**

Treatment	Parasitism/female	% Parasitism	% Emergence
<i>B. zonata</i>	24.88 ± 2.01 a	12.44 ± 1.67 a	93.84 ± 3.14 a
<i>B. dorsalis</i>	21.72 ± 1.12 b	10.86 ± 1.03 b	92.47 ± 2.65 a
<i>B. cucurbitae</i>	21.56 ± 1.23 b	10.78 ± 1.11 b	91.59 ± 3.23 a
F-ratio	13.17	11.64	0.41
P value	0.001	0.001	0.68

Means followed by different letters in each column show significant differences ( $P < 0.05$ )



**Figure 3. Sex ratio (percent males & females) of *A. daci* on larvae of different *Bactrocera* spp. (capital and small letters each show comparison among female and male parasitoids, respectively)**



**Figure 4. Relative collective per female parasitism rate of *D. giffardii*, *D. longicaudata* and *A. daci* towards different fruit fly species**

## Discussion

Results of the present studies manifested that the pupal parasitoid, *D. giffardii* exhibited higher rate of parasitism on pupae of *B. zonata* and *B. dorsalis*, which are almost of identical size, compared to *B. cucurbitae*. Pupae of the latter is bigger compared to other two fruit fly species. This higher parasitism rate of *D. giffardii*, irrespective of the size, could be attributed to nutritional fitness of the host for the parasitoid [24-26] due to which a particular host receive high degree of parasitism. While locating host for oviposition, most of the parasitoids usually determine the quality of the host in term of nutrition [27, 28] which affect their preferences and hence parasitism potential. It is also worth mentioning that different host pupae have different cues perceived by the parasitoids which in turn may affect parasitism rate of a particular parasitoid [26]. However, contrary to our findings, many earlier workers reported higher parasitism rate on bigger size host while referring to positive correlation of host size with parasitism rate [29-31].

Larval-pupal parasitoids, *D. longicaudata* and *A. daci* showed significantly higher parasitism on *B. zonata* compared to *B. dorsalis* and *B. cucurbitae*. Whereas parasitism rate on larvae of *B. dorsalis* and *cucurbitae* did not differ significantly. Apart from factors discussed above for *D. giffardii*, higher parasitism potential of *A. daci* for *B. zonata* could also be attributed to innate preference of the parasitoid for this particular fruit fly species. Difference in larval cues for attraction of parasitoid may also be one of the reasons that led to higher parasitism on *B. zonata* compared to other two species. These chemical cues emanated from host larvae play important role in the process of host acceptance [32]. Contrary to this, [33] reported parasitism of *D. longicaudata* at similar rates on larvae of *C. capitata* and *A. fraterculus* in no choice test. It is also worth

mentioning that the time spent by a particular parasitoid for oviposition may be different for different host species which may affect the rate of parasitism. The sex ratio of the emerged parasitoids (*D. giffardii*, *D. longicaudata* and *A. daci*) from all the fruit fly species was slightly skewed in favour of females. Statistical comparison of female parasitoids of *D. longicaudata* and *D. giffardii* emerged from all three species of fruit flies showed non-significant differences. The reason for higher female percentage could be the higher sensitivity of males than females as some males might have died in the embryonic stage. [17] Reported that the biased sex ratio towards females of *D. longicaudata* may be linked to good quality and suitable age of the host that encourage parasitoids for female eggs oviposition. Conversely, females with poor food quality usually produce more male offspring.

In conclusion of the present studies, *B. zonata* appeared to be the most favorable host for laboratory rearing and multiplication of all the three parasitoids, *D. giffardii*, *D. longicaudata* and *A. daci* on account of higher parasitism rate and adult emergence percentage. On the other hand, *B. cucurbitae* could be considered as least favorable host compared to *B. dorsalis* where lowest parasitism rate was recorded. However, further studies are also needed to provide a better understanding about the parasitism behavior of these parasitoids.

### Authors' contributions

Conceived and designed the experiments: MH Khan & NH Khuhro, Performed the experiments: MH Khan, M Awais & MU Asif, Analyzed the data: MH Khan & RM Memon, Contributed reagents/ materials/ analysis tools: MH Khan, M Awais, RM Memon & MU Asif, Wrote the paper: MH Khan & NH Khuhro.



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