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Md. Forhad Hossain
1. Insect Biotechnology Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Dhaka-1349, Bangladesh
2. Department of Applied Bio Sciences, Macquarie University, NSW-2109, Sydney, Australia

M Aftab Hossain
Insect Biotechnology Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Dhaka-1349, Bangladesh

Kajla Seheli
Insect Biotechnology Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Dhaka-1349, Bangladesh

Luc Leblanc
Department of Entomology, Plant Pathology and Nematology University of Idaho, (EPPN), 875 Perimeter Drive MS 2329, Moscow, Idaho, USA

Corresponding Author:
Md. Forhad Hossain
1. Insect Biotechnology Division, Institute of Food and Radiation Biology, Bangladesh Atomic Energy Research Establishment, Bangladesh Atomic Energy Commission, Dhaka-1349, Bangladesh
2. Department of Applied Bio Sciences, Macquarie University, NSW-2109, Sydney, Australia

Response of the braconid parasitoid *Diachasmimorpha longicaudata* (Ashmead) to fruits infested by oriental fruit fly, *Bactrocera dorsalis* (Hendel)

Md. Forhad Hossain, M Aftab Hossain, Kajla Seheli and Luc Leblanc

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Abstract
Biological control is an increasingly employed method in the management of tephritid pests because it is ecologically benign and target specific. The parasitoid *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) is considered an effective biological control agent of tephritid pests, but there are few studies on its behavioral responses to the local host types. This information is necessary for the success of the post-release establishment of the parasitoid. The parasitoid's behavior on oriental fruit fly *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) infested (1 and 4d old) and uninfested fruits were assessed to understand the efficacy and preference of *D. longicaudata* for different host fruits. The guava was considered reference fruit to compare with banana, carambola and apple. In olfactory bioassays, the parasitoid preferred banana and guava over apple and carambola, and guava that had been infested for a longer period of time. In no-choice and dual-choice bioassays, the parasitism rate, sex ratio, and fertility were compared and in dual-choice bioassays, the parasitoid exhibited significant differences of the above parameters in guava-banana and guava-carambola but not in the guava-apple trial. In the no-choice bioassays, nothing differs substantially between guava and other fruits except the parasitism rate of guava and banana which was significantly greater in guava. This study will help to understand the post-release behavior of the parasitoid to implement the integrated pest management programs of the oriental fruit fly.

Keywords: Oriental fruit fly, parasitoid, *Diachasmimorpha longicaudata*, host fruit

1. Introduction

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is one of the most severe horticultural pests of tropical and subtropical regions [1-4]. *Bactrocera dorsalis* is the most problematic species among the 34 fruit fly species documented in Bangladesh [5-8]. In addition to causing substantial direct losses to a wide variety of horticultural crops, it inhibits or threatens the development of agricultural economy in many countries because of rigorous quarantine regulations [9-10]. This quarantine is the legal application of measures intended to stop the spread of pests due to the importation from one country to another. The invasive pests lead to economic losses by direct losses and the costs for the prevention, surveillance, research and the control. In addition, it has a negative impact on the exporting country's reputation [9-10]. Numerous management approaches have been implemented worldwide, including chemical control, male annihilation, protein bait sprays, sterile insect technique, and biological control [11]. Intensive chemical management strategies may lead to insecticide residues in fruits and vegetables, ecosystem population, increasing pesticide resistance, adverse consequences on non-target organisms, and the ultimate emergence of secondary pest species strengthen this concept [12]. Consumers are also urging the deployment of ecologically friendly pest management strategies to reduce the use and impacts of pesticides [13-14]. Biological control is considered a potentially effective alternative to pesticides. Prior to release, however, the candidate biological control agents should be evaluated whether it has prominent host searching capacity and well establishment ability after the release.

Diachasmimorpha longicaudata (Ashmead) (Hymenoptera: Braconidae) is an effective, koinobiont, solitary endoparasitoid of tephritid fruit flies [15-18]. This species is increasingly used as a biological control agent in area-wide integrated pest management (IPM) programmes targeting economically significant tephritid fruit flies [15-17]. Plant cues are known to influence host foraging behavior [19-20], with plant volatiles being the initial cues in locating host patch [21]. Recently, the preference-performance or host fruit hierarchy of *D. longicaudata* has been studied in the laboratory and semi-field cages [21]. Generally, adult parasitoids are released at the age of 6 to 8 d [22]. Other factors that should be taken into account in investigations include environmental factors, availability, distribution or density of the pest's host plants, host plant-derivative odors, competition for the host, and age of the parasitoid [23]. Numerous accounts have been published on the biology of *D. longicaudata* [3, 4, 15, 17, 21], but to control oriental fruit flies in different host fruits it is necessary to document the behavioral responses of the parasitoid on the pest infesting different hosts as well as its infestation age for a successful post-release establishment of the parasitoid. We hereby report behavioral investigations performed on four locally available host fruits of *B. dorsalis*.

2 Materials and Methods

2.1 Parasitoid rearing

The *D. longicaudata* was collected and identified first in September 2019 from larvae of *B. dorsalis* infesting guava at the Atomic Agency Research Establishment compound, in Savar, Dhaka, Bangladesh [7]. The laboratory colony of *D. longicaudata* bred on *B. dorsalis* larvae, was established in 2021. Plastic cages (30 × 20 × 16 cm) covered by fine mesh organdy screen on both lateral sides were used to contain the parasitoid colony and kept at 25 ± 1°C, 75 ± 5% RH, and 12:12 (L:D) h photoperiod. Water, 10% sugar solutions and artificial diet (water, honey, agar, ascorbic acid and sodium benzoate) [24] were provided regularly. A cotton ball, soaked with the artificial diet, were placed in a Petri dish.

2.2 Host rearing

A colony of *B. dorsalis* was maintained on an artificial diet following the rearing methods described by Khan *et al*. (2011) [25]. Adults were kept in a cage (60 × 60 × 60 cm) maintained at 25 ± 1 °C, 75 ± 5% RH, and 12:12 h (L:D) photoperiod. Water and diet were supplied and the dead bodies (if any) were removed regularly.

2.3 Fruits

The four fruit species i.e., apple (*Malus domestica* Borkh), banana (*Musa acuminata* Colla), carambola (*Averrhoa carambola* L.), and guava (*Psidium guajava* L.) were chosen for this study because of their economic importance on the Indian Subcontinent and their wide availability in the local markets. Apples were bought from a local fruit market and all fruits were cleaned or washed using tap water before been used in the experiments. The other fruits were purchased from local organic suppliers, which were harvested just prior to the ripening stage and brought to the laboratory as early as possible and then washed. Washed fruits were allowed to dry for about an hour and stored at 9 ± 1 °C and 50 ± 5% RH until use. On the day of the experiment, fruits were artificially infested with 25 oriental fruit fly larvae [26]. Five equidistant holes (5 mm in diameter and 10-12 mm in depth) were drilled using an auger in every fruit. Five late second instars or third instars were placed inside each of the holes on fruits assigned for infestation, after which the holes were sealed with the removed flesh of the fruits. All of the infested fruits were kept in a controlled condition within the fruit fly-proof cage to prevent secondary infestations.

2.4 Olfactory response assay

A plastic Y-tube olfactometer (the length of the base and arms were 130 and 55 mm, respectively; the diameter of the tube was 24 mm) was used for this olfactory bioassay. Each arm was connected to a 5-L plastic container housing a test fruit. A mild unidirectional airflow was provided from the arms toward the base. After each assay, the olfactory tube was thoroughly cleaned with dishwashing liquid and acetone, dried, and reused. In the next assay, the treatments were alternated in opposite containers avoid spatial biases.
Eight days old female parasitoids were isolated individually in ventilated 5-ml plastic sample collection jars and kept in the olfactory testing room for 2 h prior to the experiment to allow acclimatization to laboratory conditions. Parasitoid were individually released at the base of the olfactory tube and the base was sealed with a fine mesh lid to prevent parasitoid escape and allow air flow. The movement of the parasitoid was observed until it crossed 30 mm of the arms (a positive response) or for 15 min after release. Non-responder parasitoids, which did not pass the 30-mm mark 15 min after release, were not considered in data analysis.
Two trials were conducted. In the first trial, 1-d-old infested guavas, 4-d-old infested guavas, and uninfested guavas were compared. In the second trial, infested guavas, carambolas, bananas, and apples (all 1-d-old) were compared. Each trial was repeated until 20 positive responses were obtained for each of the treatment combinations.

2.5 Laboratory trials

Host-fruit preference was determined in no-choice and dual-choice bioassays. For no-choice bioassays, the fruit fly-infested fruits were kept in ventilated plastic cages separately on a 250-ml plastic cup covered by cloth. A thin layer of sawdust was placed on the floor of the cages as a pupating substrate. In the dual-choice bioassays, guava paired with carambola, apple, or banana. Three pairs of 6- to 8-d-old parasitoids were released in each cage and allowed to parasitize the oriental fruit fly over seven consecutive days. The pupae were collected by sieving the sawdust, and then counted and incubated until the emergence of adult flies and/or parasitoids. Adult parasitoids were collected, counted, and sexed daily. Each fruit species combination was replicated three times. The parasitism percentage, fertility, and sex ratio were calculated using the following formulae.

Parasitism (%) = [(total no. of parasitoids emerged)/ (total no. of recovered pupae)
Fertility= total no. of parasitoids that emerged in each batch
Sex ratio= [(total no. of female parasitoids emerged/total no. of parasitoids) x 100]

2.6 Statistical analysis

For the olfactory bioassays, Pearson's chi-square test of independence was used to compare the olfactory responses using the Microsoft Excel. For the laboratory bioassays, the data was subjected to an independent two-sample t-test using software IBM SPSS Statistics (Version 25).

3 Results

3.1 Olfactory response assay

In olfactory trials, the female *D. longicaudata* significantly preferred banana to guava ($\chi^2 = 9$; $p<0.001$), banana to apple ($\chi^2 = 9$; $p<0.001$), carambola to apple ($\chi^2 = 16$; $p<0.001$), but the attraction combinations of guava-apple, guava-carambola, and banana-carambola were found insignificant (p>0.05) despite numerically more females positively responded to guava over apple and carambola, and banana over carambola (Figure 1). The effects of the infestation stage and age of guava (4-d-infested vs 1-d-infested, 4-d-infested vs uninfested, and 1-d-infested vs uninfested) were found highly significant (p<0.001). In this olfactory trial, the host fruit preference hierarchy was banana > guava > carambola > apple.

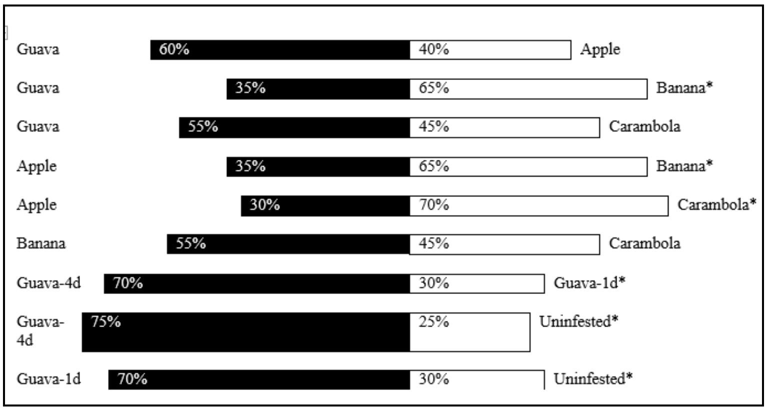

Fig 1: Olfactory assay/response of *D. longicuadata* female to different fruits. (* states significant differences at p<0.05)

3.2 Laboratory trials

The host fruit choice preference of the parasitoid was assessed among guava, banana, apple, and carambola under controlled laboratory conditions, comparing the parasitism rate, fertility, and sex ratio of the parasitoid applying the no-choice and dual-choice trials. In this case, the guava was used as a reference fruit.

In the no-choice test, the fertility and sex ratio did not differ significantly between guava and banana but the parasitism rate was significantly higher in guava than banana (Table 1) even though in the olfactory test the banana was found to be more attractive to parasitoids. All of these parameters were not significantly (p>0.05) differed in the guava-apple or guava-carambola comparisons.

Table 1: The parasitism rate (%), fertility, and sex ratio of *D. longicaudata* for different fruits in no-choice or dual-choice assay. (* states significant differences at p<0.05)

Fruits		No Choice			Dual Choice		
		Parasitism (%)	Fertility	Sex ratio (%)	Parasitism (%)	Fertility	Sex ratio (%)
Guava/Banana	Guava	55.94±13.3	4.67±1.45	44.29±2.97	18.98±5.77	5.33±1.76	50±0
	Banana	29.42±5.86	5.67±1.2	46.67±3.33	62.01±5.1	10.33±0.88	67.41±7.07
	t	3.41	-0.87	-0.38	-7.68	-3.27	-2.46
	p	0.038*	0.239	0.37	0.008*	0.041*	0.066
Guava/Apple	Guava	55.94±13.3	4.67±1.45	44.29±2.97	25.06±5.6	6±2	50±0
	Apple	65.83±13.72	10.67±2.85	64.98±13.37	39.74±12.09	5.67±1.45	55.56±5.56
	t	-0.7	-1.73	-1.94	-0.95	0.38	-1
	p	0.277	0.113	0.096	0.221	0.371	0.211
Guava/Carambola	Guava	55.94±13.3	4.67±1.45	44.29±2.97	36.97±8.88	6.67±1.45	45.77±2.17
	Carambola	34.98±9.46	4.67±1.2	42.06±4.83	31.20±8.51	4±1.15	50±0
	t	2.51	0	0.29	0.42	8	-1.95
	p	0.064	0.5	0.401	0.359	0.008*	0.095

In the case of dual-choice, the parasitism rate for the guava-banana combination was opposite to the no-choice test. Interestingly the parasitism rate was significantly higher in bananas (see Table 1). Although the fertility of dual-choice and no-choice tests were higher in bananas over guava. In contrast, in dual-choice, it was found significant. In spite of the sex ratio being numerically higher in bananas than in guava, this was not statistically significant.

The parasitism rate, fertility, and sex ratio of the parasitoid in the guava-apple combination were found insignificant both in no-choice and dual-choice trials at P = 0.05 level. Similarly, in the guava-carambola combination, those parameters were found insignificant except the fertility of dual-choice trial, which was significantly better in guava than carambola (P=0.008).

4. Discussion

Despite that *D. longicaudata* is considered an effective parasitoid against tephritid fruit flies, we needed to evaluate its efficacy on Oriental fruit flies infesting different common host fruits. This preliminary behavioral study included olfactory and laboratory trials. In the olfactory assay comparing infested and uninfested fruits, or 4d vs 1d infested fruits, a significant difference in response was found, which indicates that the parasitoid can differentiate the olfactory signal of Oriental fruit fly-infested vs uninfested fruits. These results are in line with the findings of several previous studies [21, 24, 27] that focused on Mediterranean fruit fly (*Ceratitis capitata* Wiedemann). However, unlike these previous studies, the olfactory response to different host fruits in our experiment was significant and female parasitoids were more attracted to 4d-infested fruits. This difference may be related to different pest species, or that the 4d infested fruits were producing more volatile compounds as the older infested guava rotted earlier. Because these types of volatiles, such as acetic acid, acetaldehyde, or ethyl alcohol, are known to attract *D. longicaudata* [10, 28, 29]. Moreover, larvae of some tephritid fruit flies, including *B. dorsalis*, release para-ethyl acetophenone which stimulates the host-searching activity of this parasitoid [20]. These types of volatile compounds, released from tephritid larvae and their host fruit, can affect parasitoid host searching capacity, fertility, mating, and dispersion [30]. Our results suggest that extract from host fruit should be included in the pre-release diet of the parasitoid to enhance their capability [17, 31-33]. In the case of parasitism rate in the no-choice trial, apple and carambola did not differ significantly from guava, unlike banana vs guava (P=0.038). The previous reports also showed no significant difference in parasitism rate in relation with host fruit, and rather it is a density-depended factor [3, 19, 34-37], though Harbi *et al*. (2019) [21] found a significant difference in apple vs clementine and apple vs peach infested by C. capitata larvae. However, the parasitoid could produce offspring in all types of host fruits that implies that this parasitoid can be released as it is capable to infest the oriental fruit fly even if the pest larvae present in different host fruits.

5. Conclusion

In a nutshell, by this study it was tried to find the behavioral responses of the parasitoid to host-fruit types and their infestation ages to know the post-release establishment of the parasitoid for the suppression of oriental fruit flies. The result showed that the parasitoid can parasitize and produce offspring although the oriental fruit fly larvae infested different types of fruits. This ability along with the nice foraging and host searching skill of this parasitoid recommend its application to control oriental fruit flies. However, further study is needed to know the behavioral responses in semi-field trials and also their dispersion ability.

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7. Conflicts of Interest

The authors declare no conflict of interest.

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