



ORIGINAL ARTICLES

Comparative Host Susceptibility, Oviposition, and Colour Preference of Two Polyphagous Tephritids: *Bactrocera cucurbitae* (Coq.) and *Bactrocera tau* (Walker)

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ABSTRACT

The present study aimed to understand the comparative host susceptibility of female *B. cucurbitae* and *B. tau* in terms of pupal number, and subsequent adult emergence, while given access to same host resources simultaneously under controlled laboratory condition. Oviposition and colour preference test for both the polyphagous fly species was also observed. Nine different fruits and vegetables viz., cucumber, bitter gourd, sweet gourd, sponge gourd, ash gourd, bottle gourd, wall gourd, egg plant, and tomato were chosen as natural hosts for comparable studies using choice and no-choice test. Five different colours, viz., green, yellow, dark yellow, orange, and transparent eggging devices were used for colour preference test. Distinct host preference was recorded for *B. cucurbitae* and *B. tau* with more or less susceptibility among nine hosts tested. Based on highest pupal yield, bitter gourd, and cucumber observed as the most susceptible host for both *B. cucurbitae*. For *B. tau*, sponge gourd appeared as most preferred host. Odour and colour seems to have significant effect on egg laying. Both the fly species showed strong preference to cucumber paste when used for artificial egg collection. Green coloured eggging devices appeared as most attractant for oviposition while smeared with cucumber paste. Significantly higher eggs were produced by *B. cucurbitae* than *B. tau*. It was revealed that host itself, and host paste exert differential influence on tephritid fruit flies, and vary in terms of susceptibility and eggging stimulant due to their nutritional and chemical composition. The experimental results were discussed in reference to the possible application in Integrated Pest Management (IPM) of tephritid fruit flies.

Key words: *Bactrocera cucurbitae*, *Bactrocera tau*, host susceptibility, colour and oviposition preference.

Introduction

Knowledge of the mechanisms responsible for host use patterns is critical in understanding the insect-plant relationship and developing successful population management strategies. Preference-performance hypothesis known to have significant role in host use patterns of phytophagous insects. Inter-intraspecific resource competition is also considered as important factor affecting host-plant use by phytophagous insects and necessary for understand the invasive phenomenon of fruit flies. Several studies have revealed that behavioural and environmental factors (biotic and abiotic) play important role in host use. Key factors affecting host acceptance behaviour and subsequent host use of dactine fruit flies include pre-alighting factors (e.g., fruit colour, host plant structure, shape and size) and post-alighting factors (e.g., pericarp toughness). Moreover, oviposition behaviour of dactine fruit flies is decisive in the choice of proper host plant to the immature, once they have relatively little mobility and depend on the nutritional resources selected by the adult females for their survival (Kostal, 1993; McInnis, 1989). A conjugation of plant physical and chemical factors influences on that choice and the balance between positive and negative stimuli determines the final selection (Renwick, 1989; Singer, 1986). Solomon (2007) reported that Queensland fruit fly, *Bactrocera tryoni*, (Froggatt) and cucumber fruit fly, *Bactrocera cucumis* (French) preferentially used certain host plants even when hosts of purportedly equivalent status were presented to them simultaneously and exhibited significant host discrimination.

The host use patterns of tephritids fruit flies range from highly specific (monophagous, oligophagous) to user of a wide range of hosts from different plant families (polyphagous). The melon fly, *Bactrocera cucurbitae* (Coq.) and the pumpkin fruit fly, *Bactrocera tau* (Walker) (Dipera:Tephritidae) were polyphagous species and considered serious horticultural pests in Bangladesh. More than 125 species of fruits and vegetables mostly belong to the family cucurbitaceae and Solanaceae have been recorded as hosts of *B. cucurbitae* (Dhillon, 2005). Similarly the pumpkin fruit fly, *Bactrocera tau* (Walker) is also considered as a serious pest of cucurbitaceous plants including ash gourd, bitter gourd, kankrol, bottle gourd, cucumber, ribbed gourd, sponge gourd, snake

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gourd and sweet gourd. Host plants of *B. tau* are Anacardiaceae, cucurbitaceae, Elaeocarpaceae, Monaceae, Myrtaceae, Oxalidaceae, Rutaceae, Sapotaceae, Solanaceae Carrol *et al.*, (2004).

Usually *B. cucurbitae* and *B. tau* co-exist in the field condition and share almost similar host range. In review of relationship between interspecific competition and invasions in fruit flies Duyck *et al.*, (2004) recommended on the emphasis of further research on different species competing in the same area and should be compared with respect to i. demographic parameters, ii. the out come of experimental co-infestations on the same fruit, and iii. behavioral and chemical interference. Several studies have already been done on the host susceptibility and oviposition preference of different *Bactrocera* species (Huque, 2006; Kabir, 1991; Khan, 2000; Saha, 1996; Saha, 2007). But, most of the authors considered one species of fly while investigating host suitability or oviposition preference. In the present experiment we try to understand whether *B. cucurbitae* and *B. tau* show differential performance in terms of host susceptibility when given access to same host resources at a time rather than separate study. The aim of present study were i. To compare the host susceptibility of female *B. cucurbitae* and *B. tau* among nine different natural hosts in choice and no-choice test, ii. To determine most suitable natural host paste for artificial egg collection, and iii. To understand the influences of colour to develop artificial eggging devices for both the fly species.

Materials and Methods

Stock Rearing of Insects:

Adults of *B. cucurbitae* and *B. tau* were maintained in the laboratory of Insect Biotechnology Division (IBD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh. Both *B. cucurbitae* and *B. tau* used in the present study originated from a population collected from infested cucumber, and sponge gourd respectively, and continued for several generation on respective host for laboratory adaptation. About 1500-2000 adult flies were maintained in an aluminum framed cage (76.2x 66x 76.2 H x L x W) covered with nylon net. The flies were supplied with protein based diets viz., (i) baking yeast: sugar: water at 1:3:4 ratio, and (ii) casein: yeast extract: sugar at 1:1:2 ratio. Water was supplied in a conical flask socked with cotton ball. The temperature and the relative humidity of the rearing room maintained at $28\pm 2^{\circ}\text{C}$ and $70\pm 10\%$.

Test Natural Hosts:

Nine different natural hosts used for the experiment purpose were: Cucumber *Cucumis sativus* (L.), Bitter gourd *Momordica charantia* (L.), Sweet gourd *Cucurbita maxima* (D.), Sponge gourd *Luffa cylindrica* (L.), Tomato *Lycopersicon esculentum* (Mill), Ash gourd [*Benincasa hispida* (Thunb.) Cogn.], bottle gourd *Lagenaria siceraria* (Molina), Egg plant *Solanum melongena* (L.), and pointed gourd *Trichosanthes dioica* (Roxb.).

Host Preference Study:

The experiments were conducted from January to December, 2010 in the control laboratory of Insect Biotechnology Division (IBD), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh.

Choice Test:

For this experiment, 50 pairs of *B. cucurbitae* and *B. tau* of 15-20 days old were placed inside small cage (31x21x31cm). Fifty grams of each of the nine different vegetables were weighed and offered simultaneously for egg laying for 20 minutes. After 20 minutes vegetables were collected and each of them was kept separately in a Petri dish and then placed on a layer of saw dust (pupation media) inside a plastic bowl. Plastic bowls were covered with thin clothes to permit pupation. After 2 to 3 days plastics bowls were checked regularly for drop out the rotting host-juice from the Petri-dish to avoid the unnatural death of larvae. After 6-8 days pupae were collected by sieving the sawdust. Total number of pupae produced by both *B. cucurbitae* and *B. tau* from each host were counted and subsequent adult emergence was recorded.

Non-choice Test:

The same weighted (50gm) nine different vegetables were kept separately in nine different small cages mentioned above containing 5 pairs of adult *B. cucurbitae* and *B. tau* for 20 minutes for oviposition. Then same

procedure was followed for pupal collection and subsequent adult emergence as described for choice test. In both cases, the percentage of the adult emergence was calculated by using the following formula:

$$\text{Percentage of the adult emergence} = \frac{\text{Number of adult emergence}}{\text{Number of pupae found}} \times 100$$

Oviposition Preference Test to Hosts Smearred Egging Devices:

The same nine different natural hosts were used in the present oviposition preference test viz, cucumber, bitter gourd, sweet gourd, sponge gourd, tomato, ash gourd, bottle gourd, egg plant and pointed gourd.

Choice Test:

In choice test, transparent plastic bottles (1.5 cm in diam.) having 30-40 holes (0.5 mm) each smearred with each of the nine host pastes were used as egging receptacles. These were finally put inside the cage (31x 21x 31 cm) containing 250-300 flies by placing them on a large Petri dish (14.5 x 2.5 cm) in an alternate fashion for 30 minutes containing 50 pairs of gravid *B. cucurbitae*. After that eggs from each egging receptacles were collected in water and counted under stereo-microscope. This experiment was performed separately for *B. tau*.

Non-choice Test:

Cages containing the same number of *B. cucurbitae* were used for non-choice test for oviposition preference using nine different host pastes for 30 minutes separately. In this case, only one egging receptacle was placed smearred with each type of host paste. The number of eggs from each egging receptacles were collected and counted as mentioned for choice twst. The experiment was also performed for *B. tau* separately.

Colour Preference Test for Oviposition:

In the present test similar egging devices use for oviposition test but of five different colour viz., green, dark yellow, yellow, orange, and transparent were used as egging receptacles. These were finally saturated with cucumber juice and put inside the cage containing 250-300 20 days old *B. cucurbitae* in an alternate fashion for 30 minutes. After that egg laying period eggs from different coloured egging receptacles were collected in water and total number was counted under stereo-microscope and recoded. The experiment was also performed for colour preference test of *B. tau*.

All the experiments mentioned above were repeated for three times with three replicates for each treatment.

Data Analysis:

Data collected from all experiments were analyzed using Analysis of Variance (ANOVA). Tukey's pair-wise comparison test was done by statistical software Minitab -Version 15.

Results:

Data in Table 1 showed comparative host preference of the melon fly, *B. cucurbitae* and the pumpkin fruit fly, *B. tau* in terms of mean (\pm SE) number of pupal production, and subsequent adult emergence from nine different natural hosts. In the present study host susceptibility of *B. cucurbitae* and *B. tau* differed due to pupal yield and subsequent adult emergence from the same host. One host favour higher egg laying and better larval development for one species over another. In the present study we determined the most susceptible host for *B. cucurbitae* and *B. tau* on the basis of higher pupal yield and subsequent adult emergence from corresponding host.

In choice test, pupal yield of both *B. cucurbitae* and *B. tau* was higher from bitter gourd followed by cucumber, sponge gourd, egg plant, sweet gourd, bottle gourd, pointed gourd, and ash gourd. It was lowest in case of tomato. Whereas, while counted percentage (%) adult emergence, bottle gourd and sponge gourd found to be most preferred /susceptible host for *B. cucurbitae*, and *B. tau* respectively.

In non-choice test cucumber ranked highest position in terms of total pupae produced by both *B. cucurbitae*, and *B. tau* pupae. Sponge gourd showed second higher susceptible host for both the species followed by bottle gourd, tomato, pointed gourd, sweet gourd, bitter gourd, ash gourd, and egg plant in terms of pupal yield. Whereas, percentage adult emergence of *B. cucurbitae* was highest from wall gourd, and ash gourd

(85.36%), and lowest from sponge gourd (9.16%). In contrast higher adult emergence of *B. tau* was recorded from sponge gourd, ash gourd and egg plant. Overall higher pupal production and subsequent adult emergence were recorded for *B. cucurbitae* than *B. tau*.

Table 1: Mean (\pm SE) number of pupae and % adult emergence of *B. cucurbitae* and *B. tau* from nine different natural hosts in both choice and non-choice test.

Natural hosts	Mean (\pm SE) number of pupae and % adult emergence in					
	Choice test			Non-choice test		
	Mean (\pm SE) Pupae	% Adult emergence		Mean (\pm SE) Pupae	% Adult Emergence	
		<i>B. cucurbita</i>	<i>B. tau</i>		<i>B. cucurbitae</i>	<i>B. tau</i>
Bitter gourd	202 \pm 16.74	75.24	12.37	48 \pm 1.15	31.25	14.54
Cucumber	193 \pm 13.86	63.21	7.77	170 \pm 5.77	47.05	24.7
Sponge gourd	179 \pm 7.21	54.74	25.13	131 \pm 3.48	9.16	34.35
Bottle gourd	115 \pm 0.66	86.95	1.73	112 \pm 2.33	57.14	27.67
Pointed gourd	92 \pm 4.63b	75	4.34	74 \pm 0.02	85.13	5.4
Sweet gourd	118 \pm 6.64	72.03	0.84	52 \pm 0.33	40.38	28.84
Ash gourd	64 \pm 0.57b	75	4.34	41 \pm 1.15	85.36	0
Egg plant	124 \pm 6.06	82.81	4.68	2 \pm 2.3	50	0
Tomato	49 \pm 3.46	26.53	16.32	97 \pm 2.88	72.16	15

Figure 1 and 2 illustrated the oviposition response of both *B. cucurbitae* and *B. tau* to artificial eggging devices smeared with same nine different hosts paste in choice and non-choice tests. In choice test significantly ($P < 0.05$) higher egg production of *B. cucurbitae* was recorded on tomato and cucumber paste smeared eggging devices and lowest in sponge gourd. In case of *B. tau*, cucumber paste and ash gourd paste showed highest egg collection. Rest of the host pastes showed more or less responses for egg collection. Again in non-choice test cucumber paste showed significantly higher egg collection in eggging device for *B. cucurbitae*. For *B. tau*, sweet gourd paste had significantly ($P < 0.00$) highest egg collection. However, *B. tau* showed no response to ash gourd and egg plant smeared eggging devices in present non-choice test.

Figure. 3 clearly indicated that both the fly species were attracted to green colour eggging receptacles smeared with cucumber paste. Significantly ($P < 0.05$) higher egg production was recorded in green colour eggging bottles for both *B. cucurbitae* and *B. tau* (41.94 % and 35.29 %, respectively). In case of *B. cucurbitae* dark yellow, and yellow had nearly same percentage of oviposition attraction. The order of attraction was dark-yellow (27.53%), yellow (27.12%), and orange (9.01%), respectively. While in *B. tau* the order of color preference for oviposition recorded as orange (25.22%), yellow (16.80%), and dark yellow (12.08%), respectively. Transparent bottle found as least preferred colour eggging device where only 1.13 % and 3.88 % eggs were deposited by *B. cucurbitae*, and *B. tau*, respectively (Figure 3).

Discussion:

Host fruit species and quality both affect immature instarts of fruit fly development and adult behaviour. Based on pupal yield, the present experiment suggest that all the nine natural hosts can be use by both *B. cucurbitae* and *B. tau*, depends on host availability, but the host quality seems to have major impact on larval development of particular species (Table 1). Similar to the present findings, in a study of five species of Dacini fruit flies, Fitt (1986) also noted that the abundance of species on different host fruits was due more to the choices made by females than to larval specialization. However, while many host plants can sustain the full development of different tephritid species, host quality governs major differences in survival rate, larval specialization.

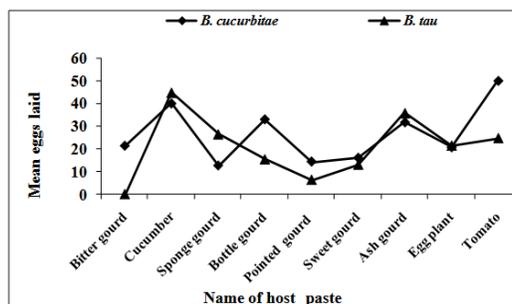


Fig. 1: Oviposition response of *B. cucurbitae* and *B. tau* in eggging devices smeared with paste of nine different natural hosts in choice test.

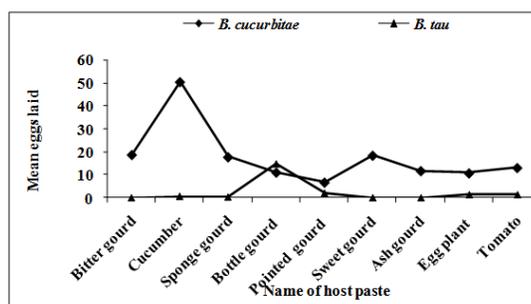


Fig. 2: Oviposition response of *B. cucurbitae* and *B. tau* in eggging devices smeared with paste of nine different natural hosts in non-choice test.

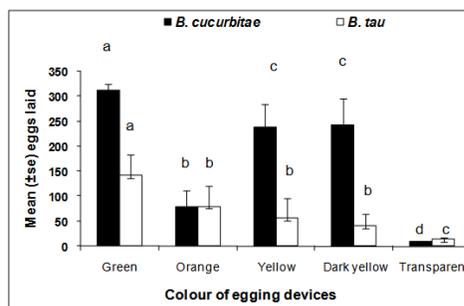


Fig. 3: Mean (\pm SE) number of eggs laid by *B. cucurbitae* and *B. tau* into different colour eggging devices smeared with cucumber paste.

In the present choice test bitter gourd, and cucumber observed to be most susceptible host for *B. cucurbitae* in terms of higher pupal yield. Doharey, (1983) reported bitter gourd, muskmelon, snap melon, and snake gourds as most preferred host for *B. cucurbitae*. Conversely, Kabir *et al.* (1991) noted sweet gourd as most preferred host for *B. cucurbitae* in the presence of sponge gourd, and cucumber. Sweet gourd also reported as most suitable natural host for *B. cucurbitae* in respect of higher ovariole number, pupal quality, and adult emergence (Saha, 2007). Based on pupal yield, sponge gourd appeared as strong and most susceptible host for *B. tau*, in the present choice and non-choice test which contradicts with the findings of Hoque (2006) reported Kankol as most susceptible host. However, none of the above mentioned authors use *B. cucurbitae* and *B. tau* simultaneously for host susceptibility test. Moreover, different natural hosts were used by different authors at different time. One of the possible reasons for the variation of host preference may be due to previous experience of stock fly rearing. In the present experiment stock rearing of *B. cucurbitae* and *B. tau* developed from cucumber and sponge gourd, respectively.

Recognition of the appropriate hosts by fruit fly for oviposition is a matter of controversy. Some investigations propose that secondary substances and or flavor play a more relevant role while others believe that the most important determinant is the nutritional value. A set of factors acting together may be involved in host recognition by the adult female (Dethier, 1982). Although, the choice of a host by ovipositing females is

known to have strong genetic explanation. Learning is possible and there are cases in which females were able to learn the characteristics of different biotypes of the same host (Prokopy, 1998). Female age and experience also noted to influence the oviposition preference hierarchy in *C. capitata* (Wiedemann) Jacome-Bravo *et al.*, (2001). In the present experiment flavor of cucumber paste/juice appeared to be stimulant for egg laying of *B. cucurbitae*, and *B. tau*. Significantly higher egg collection of *B. cucurbitae* on eggging devices containing tomato juice support the findings of Saha *et al.*, (2007) who reported tomato juice has an oviposition stimulant effect on the egg laying of *B. cucurbitae* adult flies.

Experiments conducted by several authors (Drew, 2003; Katsoyannos, 1985; Katsoyannos, 2001) indicated that *Bactrocera* species are able to discriminate colours and some visual characteristics of host colours play an important role on oviposition site selection. In the present experiment both *B. cucurbitae* and *B. tau* were observed to significantly attracted to green coloured eggging devices for oviposition and hardly eggs were laid into transparent bottles in choice test. Saha *et al* (1996) also noted a very low response of adult *B. cucurbitae* for oviposition to yellow, orange or white coloured eggging substrates except green one where significantly highest number of eggs was laid. In case of *C. capitata* strong response to yellow and green coloured eggging devices for oviposition was reported by Marchine and Wood (1983) indicated different *Bactrocera* species of different geographical origin responses to different colours for oviposition.

However, from the overall findings, we concluded both *B. cucurbitae* and *B. tau* exhibited distinct host preference when given access to same host resources, and partially contradict with existing reports on host susceptibility of both the fly species. Both fly species showed strong preference to cucumber paste used as artificial egg collection, although *B. cucurbitae* showed better response to tomato paste. Females of both the species preferred green coloured eggging devices for oviposition and fecundity of *B. cucurbitae* is much higher than *B. tau*. Experimental results might be useful in developing phytochemical bait to use in attract and kill technique to combat against these pestiferous fruit flies. Moreover, the present findings shed the light on the necessity of further research to collect extensive data on demographic patterns and niche differentiation of these polyphagous, invasive fruit flies.

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