



ATTRACTANCY OF MELON FRUIT FLY (DIPTERA: TEPHRITIDAE) TO BACTERIA ISOLATED FROM GUT AND OVIPOSITOR

JILU V. SAJAN, KIRTI SHARMA* AND R.K. SHARMA

Division of Entomology, ICAR-Indian Agricultural Research Institute, New Delhi 110012

*Email: kirtisharma2@yahoo.com

ABSTRACT

Bactrocera cucurbitae is an economically important pest of cucurbitaceous vegetables. They harbour a diverse group of microorganisms as endosymbionts. Bacteria associated with the pest have the potential to be used in different pest management strategies. Laboratory cage bioassays were conducted to study the attractancy of melon fruit flies to their associated bacteria in laboratory conditions. Among the bacteria isolated from laboratory reared flies, *Providencia rettgeri* showed maximum attractancy followed by *Enterococcus faecalis* in all age and sex groups of flies tested. Among the bacteria isolated from wild population of fruit flies, *Klebsiella pneumoniae* and *Enterobacter cloacae* were more attractive. All bacterial cultures attracted more female compared to male flies in the attractancy bioassay, and thus showing the potential to be utilized as a female targeted management strategy.

Key words: melon fruit fly, gut bacteria, olfactory studies, attractancy, *Bactrocera cucurbitae*

Tephritid fruit flies are widely distributed all over the world including tropical, subtropical and temperate regions (Prabhakar et al., 2012). The melon fruit fly *Bactrocera cucurbitae* was discovered in 1984 in Solomon Islands (Eta, 1985) and India is considered as the original home of the species. More than 50% of the cucurbits either partially or totally damaged by fruit flies and become unsuitable for human consumption (Sapkota et al., 2010). In nature, diverse kinds of relationships are found between microorganisms and insects (Piper et al., 2017). The study of bacterial symbionts in the fruit fly along with the knowledge of their physiological role in the host would help in devising an efficient trapping technique for this pest (Marchini et al., 2002). Melon fruit flies have a mutualistic association with bacteria and they harbour bacteria in their gut and ovipositor. Certain components of bacterial odour have an important role in fruit fly behaviour either as feeding or as ovipositional stimulants (Naaz et al., 2016) and these components can be used in pest management as baits or traps.

During the past 2 decades, numerous bacteria associated with fruit flies of the family Tephritidae have been identified (Kuzina et al., 2001; Marchini et al., 2002; Sharma and Reddy, 2012; Hadapad et al., 2016; Gujjar et al., 2017; Yong et al., 2017). Tephritid gut bacteria mostly belong to family Enterobacteriaceae and mainly two species, viz. *Klebsiella* and *Enterobacter* are the predominant ones (Reddy et al., 2014).

In many cases, bacteria have been demonstrated to be attractive to the species from which they were isolated (Robacker et al., 1991; MacCollom et al., 1992; Martinez et al., 1994; Reddy et al., 2013; Hadapad et al., 2016). Factors affecting the attraction of two fruit fly species, viz., *B. cucurbitae* and *B. papayae* to the odour of the bacterium *E. cloacae* were studied by Thaochan and Chinajariyawong (2011). The experimental factors studied were sex, mating experience, feeding status and host fruit provision. For *B. cucurbitae*, interaction between mating experience and feeding status affected the attractancy. They have further reported that the mated male flies and not given host fruit showed high response to bacterial odour. For the interactive factors, mated male and protein-fed flies had higher net attractancy to bacterial odour compared with the virgin female and protein-fed flies.

As per the previous studies, both sexes of fruit flies are reported to be attracted to the odour produced by associated bacteria. The odour is mostly due to the metabolites produced by bacteria. As all the available mass trapping techniques are male targeted there is a need to develop traps which attract female flies also. The current study was conducted to search the possibility of finding the bacteria which can be utilized for extracting the beneficial volatiles helpful in female-targeted trapping method.

MATERIALS AND METHODS

Fruits infested with *B. cucurbitae* were first collected from vegetable garden maintained by Division of Horticulture, IARI, New Delhi (28.64° N, 77.15° E) during June, 2013 and maintained in the laboratory condition for more than 20 generations before the isolation of bacteria from their gut. For wild population, infested fruits were collected from farmer's field, Yamuna Bank (28.62° N, 77.27° E), New Delhi during April, 2015.

Field collected maggots were reared in glass jars (15 cm dia and 20 cm height) and provided adequate pumpkin for its development. Rearing was carried out at 27±1°C and 70±5% relative humidity with a natural photoperiod in the culture room. After emergence, adults were transferred to rearing cages (30×30×30 cm). The rearing cage was designed with glass in one side and wood on opposite side and bottom of the cage. Other three sides were fitted with fine wire mesh. One sliding door was provided in the middle of wooden surface opposite to glass surface for servicing and for transfer of adult flies to cage. Artificial diet developed in our laboratory (under publication) was kept in a small petridish and covered with parafilm and kept inside cage overnight for oviposition by the adults. Maggot rearing was carried out on artificial diet in glass jars, 5 cm of which was filled with sterile sand to facilitate pupation. Glass jars were covered with markin cloth. Adults were provided with sugar, yeast autolysate and water and were replaced at four days interval.

Infested fruits collected from Yamuna Bank were kept on trays inside a modified rearing cage and provided extra fruits for the development of maggots. After pupation, each pupa was separated from sand and they were kept separately in small glass vials (25 ml capacity) having 1 cm of sand and covered with markin cloth for adult emergence. Flies were handled separately in all stages to avoid transfer of bacteria.

The *B. cucurbitae* test flies were obtained from the laboratory colonies maintained on artificial diet for more than twenty generations. During experimentation, newly emerged adults were released in separate cages meant for experimentation. Fifty adults (sex ratio=1:1) of different age groups such as 0-2 days old, 6-8 days old and 20 to 30 days old were used for the bioassay. Both protein starved and protein fed flies of all age groups were used for the experiment, separately. The protein-fed flies (newly emerged and mated) were continuously supplied with

adult diet and water, whereas the protein-starved flies had access only to sugar and water. Test flies were maintained at 27±1 °C and 70±5 % relative humidity with a natural photoperiod in the culture room. The test chamber was sterilized before the commencement of the experiment. Airflow appliances were switched off and even light were provided for maintaining uniform environment during the attractancy bioassay.

The pure cultures of bacteria isolated from the gut and ovipositor of lab reared and wild population of *B. cucurbitae* as described in Table 1 were used as attractant source. From stock culture these bacterial isolates were inoculated into 10 ml of sterile NB media and were incubated in a shaker at 150 rpm for 144 hr at 37°C. Bacterial cultures were centrifuged at 10,000 rpm for 15 min and separated into pellet and supernatant. Supernatant was used to test the attractancy.

The bioassay experiment was conducted in laboratory rearing cages. The mini-traps were made of small plastic bottle of 50 ml capacity. A small semi-circular hole of 5 mm dia was made on cap of bottle sufficient for fly to enter but not big enough to allow easy escape. For bioassay, 10 ml of supernatant of each bacterial species was poured into a small cotton ball and placed in the mini-trap separately. The positions of the traps were interchanged after every 1 hr to ensure that bacterial culture was solely responsible for attraction of the flies. The experiment was performed using completely randomized design with three replications. After each bioassay, flies were discarded. All tests were carried out between 10.00 and 16.00 hr under a combination of fluorescent and natural light. After every 1 hr, trapped flies were removed with sterile forceps and counted by sex.

The analyses was performed using the statistical software SPSS. The mean number of fruit flies attracted towards each attractant sources in the bioassay was square root transformed to meet the homogeneity of variances and compared by one-way analysis of variance. Significant results were followed by LSD for multiple comparison of mean.

RESULTS AND DISCUSSION

In this study, eight different bacterial species isolated from laboratory reared flies and seven bacterial species isolated from wild population of melon fruit flies, were used to test the attractancy of adults towards the bacterial odours (Table 1). Among these *K. oxytoca*,

Table 1. Bacteria species used to test attractancy of *B. cucurbitae*

Sl. No.	Species	Isolated from	Family
Lab reared flies			
1.	<i>Providencia rettgeri</i>	Ovipositor	Enterobacteriaceae
2.	<i>Providencia vermicola</i>	Ovipositor	Enterobacteriaceae
3.	<i>Bacillus niabensis</i>	Ovipositor	Bacillaceae
4.	<i>Klebsiella variicola</i>	Gut	Enterobacteriaceae
5.	<i>Bacillus methylotrophicus</i>	Gut	Bacillaceae
6.	<i>Enterococcus faecalis</i>	Gut	Enterococcaceae
7.	<i>Serratia marcescens</i>	Gut	Enterobacteriaceae
8.	<i>Bacillus pumilus</i>	Gut	Bacillaceae
Wild flies			
1.	<i>Enterococcus faecalis</i>	Ovipositor	Enterococcaceae
2.	<i>Klebsiella pneumoniae</i>	Ovipositor	Enterobacteriaceae
3.	<i>Elizabethkingia meningoseptica</i>	Ovipositor	Flavobacteriaceae
4.	<i>Klebsiella oxytoca</i>	Ovipositor	Enterobacteriaceae
5.	<i>Bacillus amyloliquefaciens</i>	Ovipositor	Bacillaceae
6.	<i>Raoultella ornithinolytica</i>	Gut	Enterobacteriaceae
7.	<i>Enterobacter cloacae</i>	Gut	Enterobacteriaceae

K. pneumoniae, *E. cloacae*, *Serratia* sp. and *Raoultella* sp. were also reported by Thaochan et al. (2010) from Thailand in *B. cucurbitae*. Other species i.e., *P. rettgeri*, *K. pneumoniae* (from Maharashtra), *K. oxytoca*, *K. variicola* (from Karnataka) and *Bacillus* sp. (from Himachal Pradesh and Kerala) were isolated by Hadapad et al. (2016) from *B. cucurbitae*. Gujjar et al. (2017) isolated bacteria associated with *B. dorsalis* from Karnataka viz., *E. cloacae*, *P. rettgeri*, *K. oxytoca* and *E. faecalis*. No significance difference was observed in the attractancy of 0-2 days old protein fed adults towards bacteria isolated from laboratory reared flies. Whereas, 6-8 days old adult flies, showed significantly higher attractancy towards *P. rettgeri* (5.33 flies) and *K. variicola* (5.00 flies) followed by other bacterial cultures and protein. Significantly less attractancy was observed towards blank media (1.00 flies) and sugar (0.33 flies) ($F=6.47$; $df=10, 32$; $P\leq 0.05$). The 20-30 days old protein fed adult flies followed the same trend and were attracted to *K. variicola* (7.67 flies) and *P. rettgeri* (7.00 flies) followed by *E. faecalis* (4.33 flies), *S. marcescens* (2.33 flies) and protein (2.33 flies).

The attractancy towards blank media and sugar was comparatively less ($F=6.00$; $df=10, 32$; $P\leq 0.05$). Protein starved 0-2 days and 6-8 days old flies were attracted more towards *P. rettgeri* (7.67 and 7.00 flies, respectively) and protein source (7.67 and 6.67 flies, respectively) compared to other treatments

($F=3.90$; $df=10, 32$; $P\leq 0.05$). The 20- 30 days old protein starved flies were attracted more towards *P. rettgeri* (5.67 flies) followed by protein (5.00 flies) ($F=3.95$; $df=10, 32$; $P\leq 0.05$). In all these experiments, the attractancy towards blank media and sugar remained minimum compared to other treatments (Table 2).

The bacteria isolated from wild population of *B. cucurbitae* were tested for their attractancy. The 0-2 days old protein fed flies showed maximum attractancy towards *K. pneumoniae* (7.33 flies) followed by *E. cloacae* (6.67 flies) ($F=7.10$; $df=9, 29$; $P\leq 0.05$). In a similar study conducted by MacCollom et al. (1994), washed bacterial cells of *E. agglomerans* showed significantly higher attractancy compared to control in field when tested against apple maggots. The 6-8 days old protein fed adult flies showed mean attractancy of 8.67 and 8.33 flies towards *K. pneumoniae* and *E. cloacae*, respectively ($F=14.80$; $df = 9, 29$; $P\leq 0.05$). The attractancy of 20-30 days old adults was higher towards *K. pneumoniae* (9.67 flies) followed by *E. cloacae* (7.67 flies) and *E. faecalis* (4.00 flies) ($F=10.19$; $df = 9, 29$; $P\leq 0.05$). All the flies showed significantly lower attractancy towards protein, sugar and blank media. Robacker and Bartelt (1997) conducted an experiment to test the attractancy of Mexican fruit flies and found that filtrates of *K.*

Table 2. Attraction of *B. cucurbitae* to various bacterial supernatants isolated from laboratory reared flies along with other control treatments

Bacteria species	Protein fed flies			Protein starved flies		
	0-2 days	6-8 days	20-30 days	0-2 days	6-8 days	20-30 days
<i>P. reitteri</i>	4.00*(1.93±0.64)	5.33 ^a (2.30±0.26)	7.00 ^{ab} (2.63±0.38)	7.67 ^a (2.77±0.10)	7.00 ^{ab} (2.62±0.48)	5.67 ^a (2.37±0.33)
<i>P. vermicola</i>	2.33(1.52±0.19)	1.67 ^{abc} (1.27±0.24)	2.00 ^c (1.38±0.37)	3.00 ^{ab} (1.71±0.30)	2.00 ^{ab} (1.38±0.37)	2.00 ^{abc} (1.38±0.37)
<i>B. niabensis</i>	2.67(1.58±0.52)	1.67 ^{abc} (1.27±0.24)	2.33 ^c (1.24±1.09)	4.33 ^{ab} (2.04±0.53)	2.67 ^{ab} (1.55±0.63)	1.67 ^b (1.27±0.24)
<i>K. varicola</i>	2.00(1.41±0.00)	5.00 ^a (2.23±0.23)	7.67 ^a (2.75±0.37)	5.00 ^{ab} (2.21±0.46)	2.33 ^{ab} (1.49±0.42)	2.00 ^{abc} (1.41±0.00)
<i>B. methylotrophicus</i>	1.67(1.24±0.42)	1.33 ^{bc} (0.94±0.81)	2.00 ^{bc} (1.41±0.00)	2.33 ^{ab} (1.47±0.50)	1.67 ^c (1.24±0.42)	2.67 ^{abc} (1.58±0.52)
<i>E. faecalis</i>	2.00(1.38±0.37)	3.67 ^{ab} (1.91±0.16)	4.33 ^{abc} (2.04±0.53)	2.33 ^{ab} (1.52±0.19)	1.33 ^c (1.14±0.24)	3.67 ^{abc} (1.86±0.53)
<i>S. marcescens</i>	2.33(1.52±0.19)	1.67 ^{abc} (1.27±0.24)	2.33 ^{abc} (1.52±0.18)	1.67 ^{ab} (1.24±0.42)	3.00 ^{ab} (1.62±0.75)	2.67 ^{abc} (1.62±0.19)
<i>B. pumilus</i>	2.00(1.38±0.37)	2.33 ^{abc} (1.47±0.50)	1.67 ^c (1.27±0.24)	1.67 ^c (1.05±0.92)	2.67 ^{ab} (1.62±0.19)	2.33 ^{abc} (1.52±0.19)
Media	0.89(0.76±0.68)	1.00 ^{bc} (1.00±0.00)	1.00 ^c (1.00±0.00)	3.00 ^{ab} (1.62±0.75)	1.67 ^c (1.27±0.24)	1.33 ^c (1.14±0.24)
Protein	2.22(1.44±0.46)	2.00 ^{abc} (1.38±0.37)	2.33 ^{abc} (1.52±0.18)	7.67 ^a (2.72±0.67)	6.67 ^a (2.56±0.39)	5.00 ^{ab} (2.19±0.57)
Sugar	1.33(1.14±0.24)	0.33 ^c (0.33±0.58)	1.00 ^c (1.00±0.00)	1.00 ^c (0.80±0.73)	1.33 ^c (1.14±0.24)	1.33 ^c (1.14±0.24)

Means followed by the same letters do not differ significantly at $p \leq 0.05$. * Non-significant. The values in parentheses square root transformed values. The values mean of 3 replications for 6 hr observations.

Table 3. Attraction of *B. cucurbitae* to various bacterial supernatants isolated from wild flies along with other control treatments

Bacteria species	Protein fed flies			Protein starved flies		
	0-2 days	6-8 days	20-30 days	0-2 days	6-8 days	20-30 days
<i>E. faecalis</i>	2.33 ^b (1.52±0.19)	3.67 ^b (1.91±0.16)	4.00 ^{abc} (1.97±0.42)	2.67 ^{ab} (1.62±0.19)	1.67 ^a (1.27±0.24)	3.00 ^{bc} (1.71±0.30)
<i>K. pneumoniae</i>	7.33 ^a (2.70±0.28)	8.67 ^a (2.91±0.51)	9.67 ^a (3.08±0.55)	9.00 ^{ab} (2.96±0.62)	9.33 ^a (3.04±0.41)	10.67 ^a (3.26±0.24)
<i>E. meningoseptica</i>	2.00 ^c (1.38±0.37)	2.00 ^{bc} (1.41±0.00)	1.00 ^c (0.80±0.73)	2.67 ^{ab} (1.58±0.52)	2.33 ^c (1.47±0.50)	3.33 ^{bc} (1.82±0.16)
<i>K. oxytoca</i>	2.33 ^b (1.52±0.19)	2.00 ^{bc} (1.38±0.37)	1.67 ^a (1.27±0.24)	2.33 ^a (1.15±1.23)	3.33 ^{bc} (1.80±0.34)	2.67 ^{bc} (1.62±0.19)
<i>B. amyloliquefaciens</i>	2.67 ^{abc} (1.62±0.19)	2.33 ^b (1.52±0.19)	2.67 ^{bc} (1.62±0.19)	3.33 ^{ab} (1.69±0.86)	3.33 ^a (1.72±0.73)	3.00 ^{bc} (1.69±0.48)
<i>R. ornithinolytica</i>	1.67 ^a (1.05±0.92)	2.67 ^b (1.62±0.19)	3.00 ^{bc} (1.71±0.30)	2.33 ^{ab} (1.49±0.42)	2.67 ^a (1.55±0.63)	4.00 ^b (1.99±0.26)
<i>E. cloacae</i>	6.67 ^{ab} (2.58±0.22)	8.33 ^a (2.87±0.39)	7.67 ^{ab} (2.72±0.67)	9.00 ^{ab} (2.98±0.46)	9.00 ^{ab} (3.00±0.17)	10.67 ^a (3.27±0.09)
Media	1.22 ^c (1.10±0.17)	1.33 ^b (1.14±0.24)	1.00 ^c (1.00±0.00)	0.67 ^a (0.67±0.58)	1.00 ^{cd} (1.00±0.00)	1.00 ^{cd} (1.00±0.00)
Protein	2.00 ^c (1.38±0.37)	1.33 ^{bc} (1.14±0.24)	1.67 ^a (1.27±0.24)	1.67 ^{ab} (1.27±0.24)	1.67 ^a (1.24±0.42)	1.67 ^{bcd} (1.27±0.24)
Sugar	1.33 ^c (1.14±0.24)	0.67 ^a (0.67±0.58)	1.00 ^c (1.00±0.00)	1.33 ^c (1.14±0.24)	0.00 ^d (0.00±0.00)	0.67 ^d (0.67±0.58)

Means followed by the same letters do not differ significantly at p≤0.05. The values in parentheses square root transformed values. The values mean of 3 replications for 6 hr observations.

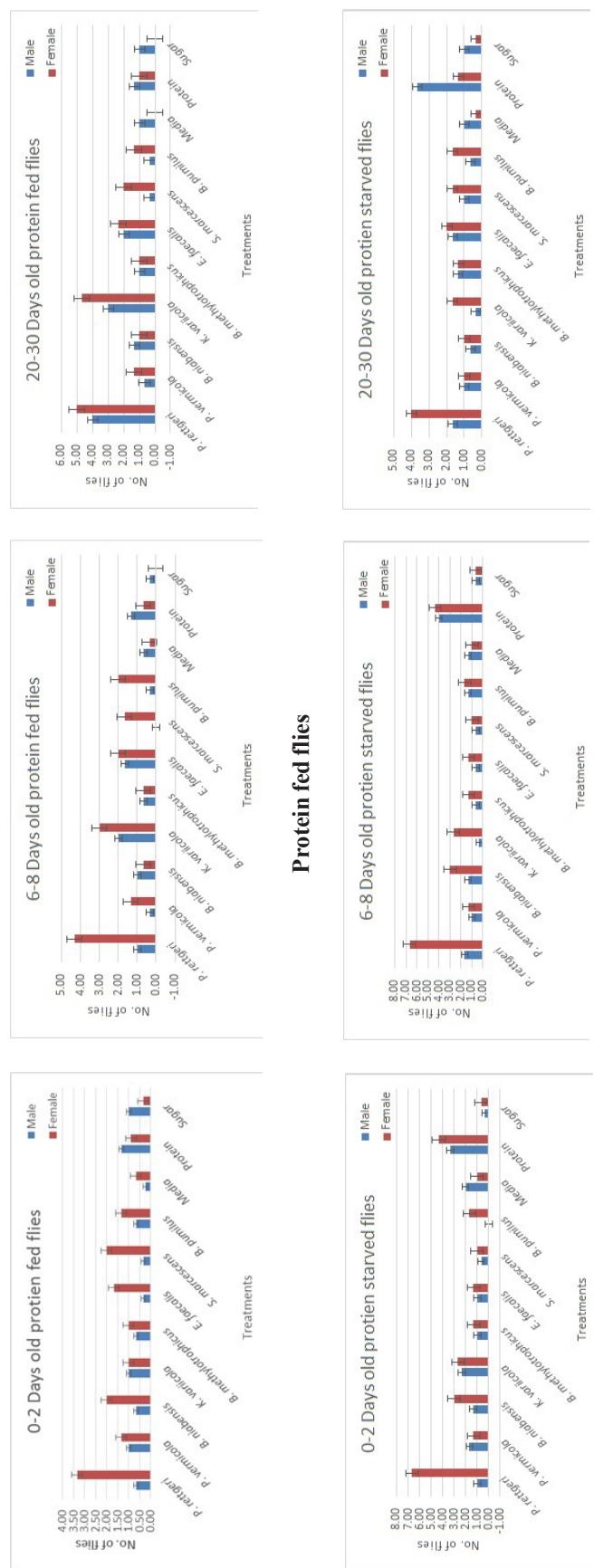


Fig. 1. Attractancy of different sex and age groups of melon fruit flies to bacterial cultures isolated from laboratory reared population of *B. cucurbitae*

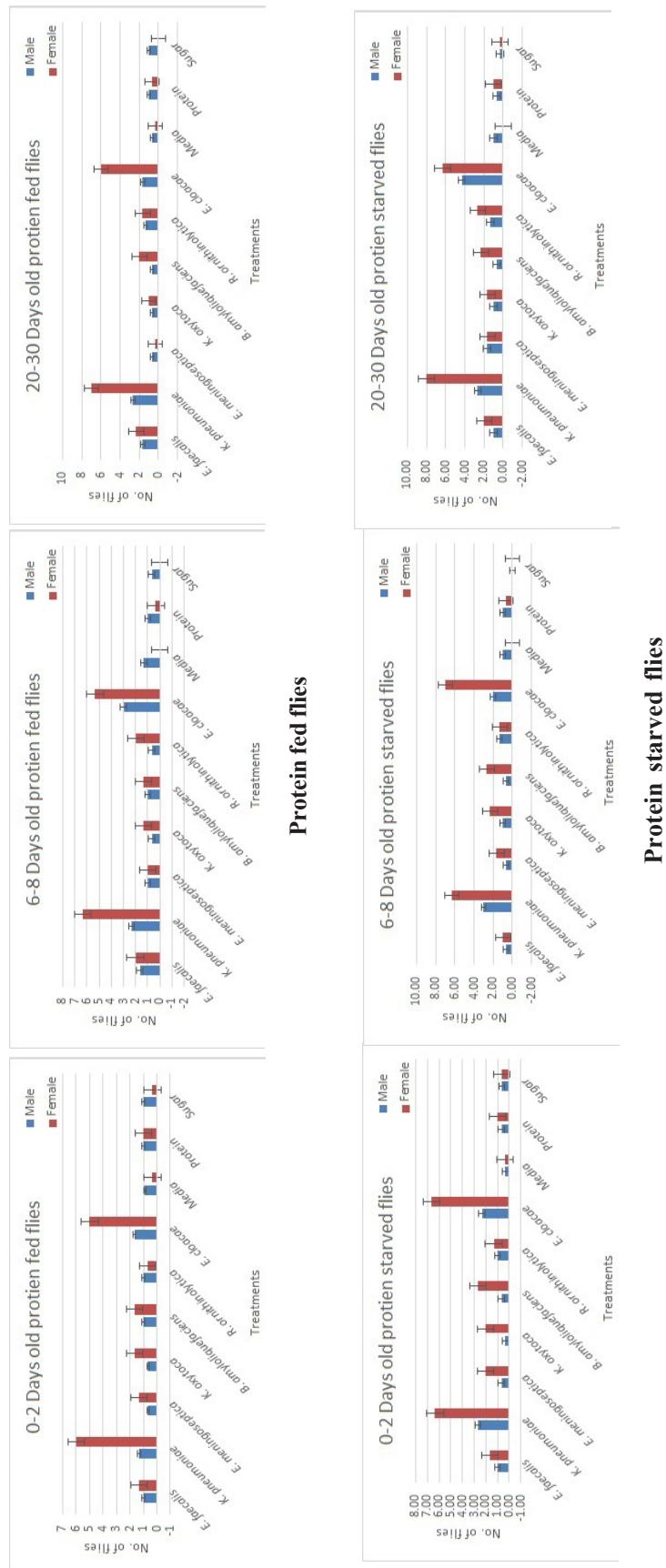


Fig. 2. Attractancy of different sex and age groups of melon fruit flies to bacterial cultures isolated from wild population of *B. cucurbitae*

pneumoniae were attractive to the flies. Jang and Nishijima (1990) found that *B. dorsalis* were attracted towards bacteria cultures compared to saline solution and water. These findings support the result obtained in the current study. Protein starved 0-2 days old flies were more attracted towards *E. cloacae* and *K. pneumoniae* (9.00 flies each) ($F=4.52$; $df = 9, 29$; $P \leq 0.05$).

All other bacterial cultures except *K. oxytoca* (2.33 flies) showed superior results compared to sugar (1.33 flies) and blank media (0.67 flies). Protein starved 6-8 days old flies were highly attracted towards *K. pneumoniae* (9.33 flies) followed by *E. cloacae* (9.00 flies) which differed significantly from all other treatments ($F=14.04$; $df = 9, 29$; $P \leq 0.05$). The 20-30 days old protein starved adults were more attracted towards *E. cloacae* (10.67 flies) and *K. pneumoniae* (10.67 flies) followed by *R. ornithinolytica* (4.00 flies) (Table 3). They showed higher attractancy towards protein and other bacterial cultures compared to blank media and sugar solution ($F=24.48$; $df = 9, 29$; $P \leq 0.05$). Robacker and Garcia (1993) and Thaochan and Chinajariyawong (2011) studied attractancy of protein fed and protein starved flies and reported that sugar-fed, protein-deprived Mexican fruit flies were more attracted towards the bacterial odour. The bacterial odour was found to be attractive to protein fed flies also in the experiment.

In this study it was observed that the protein starved flies were more attracted towards the bacterial supernatant compared to protein fed flies. Reddy et al. (2014) reported a significant difference in the attractancy towards bacteria between the protein fed and protein starved flies of different age groups. He also reported that all age groups of *B. zonata* flies showed higher attraction towards *E. cloacae* and *K. pneumoniae* in laboratory conditions (Reddy et al., 2013), supporting the results obtained in the current study. Hadapad et al. (2016) found that adults of *B. cucurbitae* and *B. dorsalis* were attracted towards the whole cell cultures and the supernatants of different species of bacteria. Sood et al. (2010) studied washed and fermented bacterial preparation of two predominant *B. tau* symbionts, *K. oxytoca* and *Pantoea agglomerans* for their attractancy to *B. cucurbitae* and *B. tau*. All the combinations of washed bacteria proved superior to control (sugar alone) in terms of attractancy for both the species.

As fermented bacterial preparation, *K. oxytoca* in combination with jaggery attracted maximum fruit flies of both the species when applied on potted cucumber plants. In present study, all bacteria cultures attracted more number of female compared to male flies in the laboratory cage bioassay. Whereas more male flies were attracted towards blank media and other control treatments (Figs. 1, 2). Martinez et al. (1994) tested attractancy of *Anastrepha ludens* towards 19 species of bacteria and found that comparatively double number of female flies were attracted to the bacterial odour than male flies. Hadapad et al. (2016) reported that *B. cereus*, *Klebsiella*, *Enterobacter*, *Providencia* and *Citrobacter* species were able to attract male and female adults of *B. cucurbitae* but significantly higher numbers of females were attracted towards supernatants of *Klebsiella*, *Citrobacter* and *Providencia* species. These results are in accordance with the result obtained in present study.

The attractancy of fruit flies towards the bacterial species may be due to the secondary metabolites produced by the bacteria. Different bacteria produce a wide range of volatile organic compounds as secondary metabolites or by products of different metabolic activities (Tait et al., 2013). Volatile materials had been reported from various bacteria and many of them were found to attract adults of fruit fly. By identifying the key volatile compounds having attractancy to fruit flies, there is a possibility of the bacteria to be utilized as a female targeted management strategy.

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