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ATTRACTION OF MELON FRUIT FLIES, *Bactrocera cucurbitae* (DIPTERA: TEPHRITIDAE) TO VARIOUS PROTEIN AND AMMONIA SOURCES UNDER LABORATORY AND FIELD CONDITIONS

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Present research work was carried out to access the attractancy of melon fruit flies towards different protein and ammonia sources under laboratory and field conditions. In the first experiment, different lure mixtures (LMs) comprised of ammonia and protein sources mixed with sugar, honey and vinegar in comparison with GF-120 (standard) were assessed for their attractive potential against *Bactrocera cucurbitae* under laboratory and field conditions. Out of all lure-mixtures, LM-9 (protein-hydrolysate based lure-mixture), LM-5 (Yeast-extract based lure-mixture) and LM-1 (Ammonium-acetate based lure-mixture) proved more attractive LMs to *B. cucurbitae* under field conditions. These three LMs (LM-9, LM-5 and LM-1) were selected to admix with juices/pulp of different fruits for preparation of eight FALMs [(Fruit juice/pulps Admixed with Lure-Mixtures (LM))] were assessed for their attraction against melon fruit flies under laboratory and field conditions in the second experiment. Out of all FALMs, FALM-1 (cucumber based FALM) proved strongly attractive FALM toward melon fruit flies in olfactometer and field studies. However, FALM-3 (banana based FALM), FALM-4 (pumpkin based FALM), FALM-5 (grapes based FALM) and FALM-7 (watermelon based FALM) proved strongly attractive FALMs to melon fruit flies in field studies. As FALM-1 proved strongly attractive FALM to both males and females of *B. cucurbitae* in olfactometer and field studies, so it can be used for IPM program of *B. cucurbitae* in cucurbit cropping system.
keywords: *Bactrocera cucurbitae*, lures, food attractants, attractiveness, cucurbits, bitter gourd.

INTRODUCTION

Tephritidae fruit flies comprise 400 species and 500 genera. Among 400 fruit fly species, approximately 250 species are considered major pests of different fruits and vegetable crops (White and Elson-Harris, 1996). About 12 fruit flies species are prominent in Pakistan but three of them [*Bactrocera zonata* (Saunders), *B. cucurbitae* (Coquillett) and *B. dorsalis* (Hendel)] are serious threats to fruit and vegetable crops (Panhwar, 2005). *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), also known as melon fruit fly, has been reported to cause severe economic loss to cucurbit crops (Gogi *et al*., 2009; Akram *et al.*, 2010). Losses to different crops up to 30-100% were reported by the different researchers (Dhillon *et al.*, 2005a, b, c; Shooker *et al.*, 2006). Bitter gourd is considered as the main host of melon fruit fly where 28.56% loss (Singh *et al*., 2000) and 16-75% infestation was reported (Gogi *et al*., 2009) due to melon fruit fly. *Bactrocera cucurbitae* causes direct loss due to its infestation on fruits; while it causes indirect loss due to rejection of food commodities by quarantine department (Chen and Ye, 2007; Kong *et al.*, 2008; Sapkota *et al.*, 2010; Li *et al.*, 2013).
Different control methods used for management of fruit flies include cultural practices (Gogi *et al.*, 2007; Gogi, 2009), pheromones (Shelly *et al.*, 2004; Panhwar, 2005), lures mixtures (Vargas *et al.*, 2008, 2010), biological control (Drew *et al.*, 2003) and hot water treatment (Panhwar, 2005; Gogi *et al.*, 2010). Application of insecticides is least effective because larvae develop and feed inside the fruit, covered by fruit pulp and not exposed to insecticides directly (Yee *et al.*, 2007; Gogi, *et al.*, 2009; Sapkota, 2010). Application of insecticides not only imposes harmful effects on beneficial arthropods but also contaminates the environment as well as raises MRLs issues (Tahir *et al.*, 2009; Gogi *et al.*, 2010; Kakakhel, 2012). Different fruits and vegetable consignments from KPK (Khan *et al.*, 2005), Punjab (Tahir *et al.*, 2009) and Sindh (Parveen *et al.*, 2011) were rejected due to detection of pesticides residues in fruits.
Attraction of melon fruit flies toward different food sources has been reported by different scientists. Strong attraction to cucumber by melon fruit fly was reported under field conditions (Miller *et al.*, 2004; Pinero *et al.*, 2006). Mass trapping of melon fruit fly in California was done by yeast in the field (Gilbert *et al*., 2010). Hydrolyzed animal protein proved to be highly effective for mass trapping of *Anastrepha* species (Lasa and Cruz, 2014; Herrera *et al*., 2015). Deferent lures like methyl eugenol and butanone acetate are available in the market only for attraction and management of male fruit flies. There are limited food lures for the attraction of female

fruit flies. Fruits and vegetables are also the most promising sources for the attraction of female flies. Since tephritid females prefer host fruit volatiles when searching for oviposition sites, so juices/pulps of such fruits can be assessed for attraction and finally for bait preparation (Alagarmalai *et al.*, 2009).

The ultimate goal of the present study was to develop a highly attractive food lure for both male and female melon fruit.

MATERIALS AND METHODS

Mass rearing of melon fruit fly: The infested bitter gourd and cucumber fruits were collected from the field, brought into the laboratory and kept in the card boxes already half-filled with sieved and sterilized sand. After a week, pupae were recovered by using a fine-mesh sieve. The pupae were placed in the dome-shaped rearing cages until the adult emergence. The spongy strips were suspended in cages after soaking in an adult diet containing honey, protein and sugar-water solution. The fresh, properly cleaned and washed fruits of bitter gourd and cucumber, as well as egg-receptacles, were suspended inside the rearing cage for egg collection. The fruits after three days were transferred to card boxes containing sterilized sand for obtaining the next progeny. This procedure was used for the mass culture of melon fruit fly. The culture of the melon fruit fly was sustained separately inside the laboratory/growth-chamber maintained at 30±5 ºC, 70±5% RH and 12:12 (D: L) photoperiod (Thomas and Mangan, 2005).

Screening of highly attractive proteins and ammonia source: In this experiment, seven protein sources (Beef-extract, Fish-extract, Yeast-extract, Starch, Casein, Rose-oil and Protein-hydrolysate) and two ammonia sources (Ammonium acetate and Acetic acid) were admixed with sugar (molasses), honey and vinegar (used as constant in all combinations) as per rational composition (by weight/volume) given in Table 1 to prepare nine phagostimulant/lure-mixtures (LMs). The protein and ammonia sources based LMs being attracted by fruits fly in high number was considered as the most attractive. The experiment was conducted in laboratory and field conditions as described below.

Screening of highly attractive proteins and ammonia source based lure-mixtures with olfactometer under laboratory condition: Nine phagostimulants/lures including ammonium acetate, acetic acid, beef extract, fish extract, yeast extract, starch, casein, rose oil and protein hydrolysate based LMs (LM-1, LM-2, LM-3, LM-4, LM-5, LM-6, LM-7, LM-8 and LM-9) were used to evaluate the attractiveness of *B. curcubitae*. GF-120 was used as a standard for comparison. This experiment was conducted using a free-choice method under laboratory conditions through specially developed 12-chambered olfactometer. Protein hydrolysate, yeast extract, beef extract, fish extract, starch and casein were purchased from market in powdered form whereas rose oil and acetic acid were purchased in liquid form. An adequate quantity of water was added to protein hydrolysate, yeast extract, beef extract, fish extract, starch and casein to make it into a paste. Cubes of sponge (7.5×7.5×7.5 cm) were cut and dipped in semiliquid-paste/solution of each LM till spongy-cubes get saturated. These LM saturated spongy-cubes were placed in the small chambers. Then a mixed population of fifty individuals of unsatiated *B. cucurbitae* adults of both sexes (25 males and 25 females) was released in the central large chamber of olfactometer and all the lure-saturated cubes were placed in small chambers of olfactometer, separately. Numbers of fruit flies visiting each LM inside the jar were counted after every sixty minutes (1 hour) till the total experimental period of 24 hours. The experiment was designed in CRD with three repeats.

Screening of highly attractive proteins and ammonia source based lure mixtures in bitter gourd field: For the conduct of this experiment, the free-choice method in RCBD with three replicates was used. LMs saturated spong-cubs and pastes were prepared as described above for olfactometer studies in the laboratory and were placed separately at the bottom of

Table 1. Rational composition (by weight/volume) of different lures for preparation of required different Lure-mixtures (LMs).

Lure Mixtures (LMs)	Major ingredients and their quantity in term of parts by weight/volume		Ingredients admixed in all majors ingredients and their quantity in term of parts by weight/volume		
			Molasses	Honey	Vinegar
LM-1 (Ammonium acetate based Lure Mixture)	Ammonium acetate	4	2	1	1
LM-2 (Acetic acid based Lure Mixture)	Acetic acid	4	2	1	1
LM-3 (Beef-extract based Lure Mixture)	Beef-extract	4	2	1	1
LM-4 (Fish-extract based Lure Mixture)	Fish-extract	4	2	1	1
LM-5 (Yeast-extract based Lure Mixture)	Yeast-extract	4	2	1	1
LM-6 (Starch-based Lure Mixture)	Starch	4	2	1	1
LM-7 (Casein based Lure Mixture)	Casein	4	2	1	1
LM-8 (Rose oil based Lure Mixture)	Rose oil	4	2	1	1
LM-9 (Protein hydrolysate based Lure Mixture)	Protein hydrolysate	4	2	1	1

Table 2. Rational composition of ingredients (by weight/volume) in different FALMs (Treatments)

FALMs (Treatments)	Ingredients and their ratio by parts (by weight/volume)				
	Fruit Extracts(Pulp and Juice)		LM-5	LM-9	LM-1
FALM-1	Cucumber	4	2	2	2
FALM-2	Eggplant	4	2	2	2
FALM-3	Banana	4	2	2	2
FALM-4	Pumpkin	4	2	2	2
FALM-5	Grapes	4	2	2	2
FALM-6	Tomato	4	2	2	2
FALM-7	Watermelon	4	2	2	2
FALM-8	Pineapple	4	2	2	2
Standard (GF-120)	-				

FALMs = [(Fruit juices/pulps Admixed with Lure Mixtures (LMs)]

separate bottle-traps. The traps consisting of LMs were hung on small bamboo-pole randomly in the bitter gourd field at equidistance from each other (5 ft) and their positions were changed on a weekly basis. The traps were also refreshed weekly. These practices were carried out from the flowering stage until the first picking of bitter gourd.The traps having a higher collection of *B. cucurbitae* adults were considered as highly effective LMs. The *B. cucurbitae* adults from traps were identified using key characters described by White and Elson-Harris (1996) and separated. Thus collected *B. cucurbitae* adults were then separated into sexes under the microscope in IPM laboratory using key characters of their male and female individuals as described by White and Elson-Harris (1996). The numbers of *B. cucurbitae* adults visiting per trap were counted weekly.

Screening of different FALMs [(Fruit juices/pulps Admixed with Lure Mixtures (LMs))] for their attractiveness to Bactrocera cucurbitae: The three highly attractive lure mixtures (Experiment 1) i.e., LM-5 (Yeast-extract based Lure Mixture), LM-9 (Protein hydrolysate based Lure Mixture)and LM-1 (Ammonium acetate based Lure Mixture) were co-administrated with different fruit pulps/juices as per rational composition (by weight/volume) for preparation of eight FALMs = [(Fruit juices/pulps Admixed with Lure Mixtures (LMs)] given in Table 2. Experimentation was done in two separate experiments i.e., field and laboratory experimentation.

Screening of FALMs for their attractiveness to Bactrocera cucurbitae adults under laboratory conditions by olfactometer: This experiment was conducted using a free choice method under laboratory conditions through specially developed olfactometer. Cubes of spong (7.5×7.5×7.5 cm) were cut and dipped in each of eight FALMs till spongy-cubes get saturated. These FALMs saturated spongy-cubes were placed separately in the small chambers. Then a mixed population of fifty individuals of unsatiated *B. cucurbitae* adults of both sexes (25 males and 25 females) were released in the central large chamber of olfactometer. Numbers of fruit flies visiting each FALM inside the jar were counted after every sixty minutes (1 hour) till the total experimental period of 24 hours. The experiment was designed in CRD with three repeats.

Screening of FALMs for their attractiveness to Bactrocera cucurbitae adults under field conditions**:** For the conduct of this experiment, free-choice method in RCBD with three replicates was used. FALMs saturated spong-cubs were prepared as described above for olfactometer studies in the laboratory and were placed separately at the bottom of separate bottle-traps. The traps consisting of FALMs were hung on small bamboo-pole randomly in the bitter gourd field at equidistance from each other (5 ft) and their positions were changed on a weekly basis. The traps were also refreshed weekly. These practices were carried out from the flowering stage until the first picking of bitter gourd. The traps having a higher collection of *B. cucurbitae* adults were considered as highly attractive and effective FALMs. The *B. cucurbitae* adults from traps were identified using key characters described by White and Elson-Harris (1996) and separated. Thus collected *B. cucurbitae* adults were then separated into sexes under microscope in IPM laboratory using key characters of their male and female individuals as described by White and Elson-Harris (1996). The numbers of *B. cucurbitae* adults visiting per trap were counted weekly.

Attractancy index**:** The attractancy rating/index of each LM and FALM was also calculated using following formula as described by Beroza and Green (1963).

$$\text{Attractancy Index} = \frac{\text{Insects attracted by candidate PHS} - \text{Insects attracted by standard PHS}}{\text{Total insect attracted (candidate PHS + standard PHS)}} \times 100$$

The LMs, as well as FALMs, were then classified into different attractancy classes on the basis of attractancy indices as described by Beroza and Green (1963) (Table 3).

Table 3. Ranges of attractancy indices and their respective attractancy-class for male and female *Bactrocera cucurbitae* as described by Beroza and Green (1963).

Class	Attractancy-index	
	Male	Female
I (Non or little attractive)	Less than 11	Less than 6
II (Moderately attractive)	11-50	6-50
III (Strongly attractive)	Greater than 50	Greater than 50

***Statistical analysis*:** The data regarding a number of fruit flies visiting different LMs and FALMs were subjected to ANOVA technique to determine the parameters of significance and mean values for different treatments which were compared with Tukey's honestly significant difference test, as performed by Danho *et al.* (2002) using statistical software of STATISTICA-6.

RESULTS

***Attractiveness of different lure-mixtures (LM) to adult fruit flies of B. cucurbitae under laboratory and field conditions*:** Olfactometer study revealed that different LMs had significant effects on the attractiveness of adults males ($F_{9/20}$ = 16.88, $P \leq 0.05$) and females ($F_{9/20}$ = 46.98, $P \leq 0.05$) of *B. cucurbitae* under laboratory conditions. Maximum number of *B. cucurbitae* males (9.67 male flies) was attracted to GF-120 followed by starch (3.25 male flies), ammonium acetate (2.50 male flies) and acetic acid (2.00 male flies). While beef-extract (1.41 male flies), fish-extract (1.01 male flies), yeast-extract (1.25 male flies), casein (1.45 flies), rose oil (1.21 female lies) and protein-hydrolysate (0.45 male flies) attracted less than 2 male flies and proved least attractive LMs to male *B. cucurbitae*. Unlikely, protein hydrolysate attracted maximum number of *B. cucurbitae* females (12.02 female flies) followed by ammonium acetate (2.87 female flies) and yeast-extract (2.46 female flies) and later two LMs were found statistically similar with each other. Beef-extract, acetic acid and starch captured 1.76, 1.27 and 1.19 female flies of *B. cucurbitae*, respectively and were found statistically similar to one another. Fish-extract, casein and rose oil captured least but statistically similar flies (0.86, 0.31 and 0.00 female flies, respectively). Results showed that protein hydrolysate, ammonium acetate and yeast-extract proved more attractive for female fruit flies under laboratory conditions. Field study indicated that number of male ($F_{9/20}$ = 39.845; P = 0.0000) and female ($F_{9/20}$ = 43.712; P = 0.0000) flies of *B. cucurbitae* attracted to different LMs varied significantly for different LMs. Protein hydrolysate captured maximum *B. cucurbitae* males (56.26 male-flies/trap/week) followed by yeast-extract (43.29 male-flies/trap/week), starch (31.75 male-flies/trap/week), ammonium acetate (24.58 male-flies/tap/week), beef-extract (17.87 male-flies/trap/week), acetic acid (15.69 male-flies/trap/week), casein (16.32 male-flies/trap/week) and fish-extract (13.73 flies/trap/week). Rose oil captured the least number of *B. cucurbitae* males (11.54 flies/trap/week). Maximum female fruit flies density was observed on protein hydrolysate trap (81.25 female-flies/trap/week) followed by yeast-extract, ammonium acetate, beef-extract, acetic acid, starch, rose oil and casein which captured 40.61, 36.52, 33.25, 30.75, 27.86, 18.57 and 13.63 female-flies/trap/week, respectively (Table 4).

The results of attractancy-Index (AI) for olfactometeric study under laboratory conditions indicate that all the tested lure mixtures proved non or little attractive (class-I with AI < 11%) to male *B. cucurbitae*. Lure mixtures used in this study i.e., LM-1, (Ammonium acetate based Lure Mixture), LM-2, (Acetic acid based Lure Mixture), LM-3, (Beef-extract based Lure Mixture), LM-4, (Fish-extract based Lure Mixture), LM-5, (Yeast-extract based Lure Mixture), LM-6,(Starch based Lure Mixture), LM-7, (Casein based Lure Mixture), LM-8, (Rose oil based Lure Mixture) and LM-9 (Protein hydrolysate based Lure Mixture) exhibited -75.00%, -55.56%, -55.62%, -75.00%, -75.00%, -55.75%, -55.43%, -55.51% and -16.28% AI, respectively for *B. cucurbitae* males. These results also explained that all aforementioned LMs proved 16.28-75.0% less attractive to *B. cucurbitae* males as compared to GF-120 (standard). The AI of aforementioned lure-mixtures was found comparatively higher for female *B. cucurbitae* in some extents under laboratory conditions. Protein-hydrolysate based LM (LM-9) exhibited highest attractancy-index (62.50%) and was demonstrated as strongly

Table 4. Density of adult fruit flies of *Bactrocera cucurbitae* (Means ± S.E.) attracted and captured to different phagostimulants/lure-mixtures (LMs) under laboratory and field conditions.

Phagostimulants	***B. cucurbitae* captures (Means ± S.E.)**			
	Laboratory studies		**Field studies**	
	Male	**Female**	**Male**	**Female**
LM-1 (Ammonium acetate based Lure Mixture)	2.50±0.50bc	2.87±0.88b	24.58±0.78d	36.52±1.21c
LM-2 (Acetic acid based Lure Mixture)	2.00±0.57c	1.27±0.41c	15.69±0.23ef	30.75±1.04e
LM-3 (Beef-extract based Lure Mixture)	1.41±0.33c	1.76±0.36c	17.87±0.52e	33.35±1.09d
LM-4 (Fish-extract based Lure Mixture)	1.01±0.56c	0.86±0.26d	13.73±0.31f	18.43±0.98g
LM-5 (Yeast-extract based Lure Mixture)	1.24±0.88c	2.46±0.54b	43.29±1.02b	40.61±1.25b
LM-6 (Starch based Lure Mixture)	3.25±1.20b	1.19±0.24c	31.75±0.81c	27.86±1.02f
LM-7 (Casein based Lure Mixture)	1.45±0.01c	0.31±0.15d	16.32±0.23ef	13.63±0.69h
LM-8 (Rose oil based Lure Mixture)	1.21±0.32c	0.00±0.65d	11.54±0.36g	18.57±0.87g
LM-9 (Protein hydrolysate based Lure Mixture)	0.45±0.31d	12.02±0.58a	56.26±1.53a	81.25±2.01a
GF-120 (Standard)	9.67±1.01a	2.85±0.54b	18.78±0.70e	28.74±0.23ef

Values in same column containing different letters of same format are significantly different from each other at probability level of 5% according to Tukey`s Honestly Significant Difference (HSD) test.

attractive LM (class-III with AI > 50%) for female flies of *B. cucurbitae*. Aammonium acetate based LM (LM-1) exhibited 14.29% AI and proved moderately attractive LM (AI = 6-50% and class-II) for female *B. cucurbitae*. However, the rest of the Lure-mixtures including LM-2, LM-3, LM-4, LM-5, LM-6, LM-7 and LM-8 exhibited -50.00%, -50.00%, -100.00%, -20.00%, -50.00%, -100.00% and -100.00% AI, respectively and proved non/little attractive (AI = < 6% & Class-I) to adult female flies of *B. cucurbitae*. These LMs were found 20-100% less attractive to *B. cucurbitae* females as compared to GF-120 (standard).The results of field study exhibit that LM-9 exhibited the highest AI for male melon fruit flies, *B. cucurbitae* (51.40%) and proved strongly attractive LM (class-III with AI > 50%). LM-5, LM-6 and LM-1 demonstrated 41.0%, 26.50% and 14.30% AI, respectively and proved moderately attractive (AI = 11-50% and class-II) for *B. cucurbitae* males. However, rest of the lure-mixtures including LM-3 (AI = -2.90%) LM-2 (AI= -9.10%), LM-4 (AI= -16.10%), LM-7 (AI= -5.90%) and LM-8 (AI= -24.10%) proved non/less attractive LMs (AI<11% & Class-I) and exhibited 2.90%, 9.10%, 16.10%, 5.90% and 24.10% less attractancy to *B. cucurbitae* males than that of standard (GF-120), respectively. Their negative AI values demonstrated that their attractancy was lower than that of standard (GF-120). The attractancy indices of aforementioned lure-mixtures were found comparatively higher for female than male *B. cucurbitae* under field conditions. LM-9 exhibited the highest attractancy-index (53.54%) and proved strongly attractive LM (class-III with AI >50%) to *B. cucurbitae* females followed by LM-5, LM-1 and LM-3. LM-5 (AI= 20.69%), LM-1 (AI= 14.81%) and LM-3 (AI= 9.80%) proved moderately attractive LMs (class-II with AI = 6-50%) and demonstrated 20.69%, 14.81% and 9.80% higher attractiveness to *B. cucurbitae* females than GF-120, respectively. LM-2 (AI= -4.16%), LM-4 (AI= -27.78%), LM-6 (AI= -2.22%), LM-7 (AI= -48.39%) and LM-8 (AI= -27.87%) proved non/little attractive LMs (Class-I with AI < 6%). These results also explain that LM-2, LM-4, LM-6, LM-7 and LM-8 demonstrated 4.16%, 27.78%, 2.22%, 48.39% and 27.87% less attractiveness to *B. cucurbitae* females than that of standard (GF-120) (Table 5).

***Screening of different FALMs for their attractiveness to Bactrocera cucurbitae under laboratory and field conditions*:** Attractiveness of FALMs to *B. cucurbitae* varied significantly ($F_{8,36}$ = 90.726; $P \leq 0.05$). Unlikely, sexual attraction of both male and female *B. cucurbitae* explained non-significant variations for FALMs ($F_{1,36}$ = 0.048; P = 0.828). First level interaction between *B. cucurbitae* sex and FALMs also exhibited significant variation in attractiveness ($F_{8,36}$ = 18.155; $P \leq 0.05$). The maximum *B. cucurbitae* males (7.29 male flies) were attracted to FALM-3 (banana based FALM) followed by FALM-1, FALM-7, FALM-5, FALM-8 and standard treatment (GF-120) which attracted 5.45, 4.71, 4.59, 0.56 and 0.56 *B. cucurbitae* males, respectively. FALM-2 and FALM-6 did not attract any *B. cucurbitae* male (0.00 male fly). The highest number of *B. cucurbitae* females were attracted to FALM-1 (7.32 female flies) followed by FALM-3, FALM-8, FALM-5, FALM-7, standard treatment (GF-120) and FALM-4 which attracted 5.41, 4.78, 2.63, 2.19, 2.12 and 0.38 female flies, respectively. However, no *B. cucurbitae* female was attracted to FALM-2 and FALM-6. Attractancy-indices of FALMs for *B. cucurbitae* females in olfactometer revealed that FALM-1 demonstrated highest attractancy-index (55.56%) and proved strongly attractive FALM (Class III with AI > 50%) to *B. cucurbitae* females followed by FALM-3 (AI= 42.86%), FALM-5 (AI= 20%), FALM-7 (AI= 20%) and FALM-8 (AI= 33.33%) which were found moderately attractive (Class II with AI = 6-50) and proved 42.86%, 20%, 20% and 33.33% more attractive than GF-120, respectively. FALM-2 (AI= -100%), FALM-4 (AI= -33.33%) and FALM-6 (AI= -100%) exhibited least attractancy-index, proved non/little attractive FALMs (Class I with AI = < 6%)

Table 5. Attractancy-indices and attractancy-classes of different lure-mixtures (LMs) for adult fruit flies of *B. cucurbitae* under laboratory and field conditions (AI of GF-120 is zero because it was used as standard).

LURE-MIXTURES (LM)	Attractancy-Indices (%) (Attractancy Classes)			
	Laboratory studies		Field studies	
	Male	Female	Male	Female
LM-1 (Ammonium acetate based Lure Mixture)	-75.00 (I)	14.29 (II)	14.30 (II)	14.81 (II)
LM-2 (Acetic acid based Lure Mixture)	-55.56 (I)	-50.00 (I)	-9.10 (I)	-4.16 (I)
LM-3 (Beef-extract based Lure Mixture)	-55.62 (I)	-50.00 (I)	-2.90 (I)	9.80 (II)
LM-4 (Fish-extract based Lure Mixture)	-75.00 (I)	-100.00 (I)	-16.10 (I)	-27.78 (I)
LM-5 (Yeast-extract based Lure Mixture)	-75.00 (I)	-20.00 (I)	41.00 (II)	20.69 (II)
LM-6 (Starch based Lure Mixture)	-55.75 (I)	-50.00 (I)	26.50 (II)	-2.22 (I)
LM-7 (Casein based Lure Mixture)	-55.43 (I)	-100.00 (I)	-5.90 (I)	-48.39 (I)
LM-8 (Rose oil based Lure Mixture)	-55.51(I)	-100.00 (I)	-24.10 (I)	-27.87 (I)
LM-9 (Protein hydrolysate based Lure Mixture)	-16.28 (I)	62.50 (III)	51.40 (III)	53.54 (III)
GF-120 (Standard)	0.00	0.00	0.00	0.00

Roman values in parentheses indicate the attactancy-classes of phagostimulants/LMs categorized on the basis of their attractancy indices according to scale of attractancy as described by Beroza and Green (1963) for fruit flies.

(-100%) and demonstrated 33.33-100% less attractiveness than GF-120 for *B. cucurbitae* females. ANOVA parameters explain that significant differences existed in attractiveness of FALMs to *B. cucurbitae* under field conditions ($F_{8,36}$ = 239.393; $P \leq 0.05$). Similarly, sexual attraction of both males and females *B. cucurbitae* explained non-significant variations for FALMs ($F_{1,36}$ = 25.960; $P \leq 0.05$). First level interaction between *B. cucurbitae* sex and FALMs also exhibited significant variation in attractiveness ($F_{8,36}$ = 3.277; P = 0.0066). The maximum *B. cucurbitae* males were captured on FALM-1 (89.52 male-flies/trap/week) followed by FALM-4, FALM-7, FALM-3, FALM-5, FALM-2, FALM-8 and FALM-6 which attracted and captured 84.64, 78.65, 71.34, 61.45, 32.75, 22.44 and 13.43 male-flies/trap/week, respectively. GF-120 captured least *B. cucurbitae* males (12.54 male-flies/trap/week). Similarly, FALM-1 attracted and captured the highest *B. cucurbitae* females (91.24 female-flies/trap/week) followed by FALM-3, FALM-6, FALM-4, FALM-5, FALM-2, FALM-8 and FALM-6 which captured 88.42, 83.93, 78.18, 68.91, 35.63, 31.58 and 19.07 female flies/trap/week, respectively. GF-120 captured least *B. cucurbitae* females (17.32 flies/trap/week) and was found statistically similar to FALM-6 (Table 6).

The attractancy-indices of FALMs calculated in olfactometer study demonstrate that FALM-1 (cucumber based FALM), FALM-3 (banana based FALM), FALM-5 (grapes based FALM) and FALM-7 (watermelon based FALM) demonstrated 66.67%, 75.00%, 60.00% and 66.67% AI for *B. cucurbitae* males, respectively and proved strongly attractive FALMs (Class-III with AI > 50%). FALM-4 (pumpkin based FALM) exhibited 33.33% AI and proved moderately attractive FALM (Class II with AI = 11-50%) for *B. cucurbitae* males. These results also reveal that FALM-1, FALM-5, FALM-3, FALM-7 and FALM-4 demonstrated 66.67%, 75.00%, 60.00%, 66.67% and 33.33% higher attractiveness to *B. cucurbitae* males, respectively than GF-120 (standard). However, FALM-2 (eggplant based FALM), FALM-6 (tomato based FALM) and FALM-8 (pineapple based FALM) explained -100%, -100% and 0.00% AI and proved non/least attractive FALMs (Class I with AI < 11%) to *B. cucurbitae* males. These results also explain that FALM-2 and FALM-6 were 100% less attractive than GF-120 while

Table 6. Density of adult fruit flies of *Bactrocera cucurbitae* (Means ± S.E.) attracted and captured to different FALMs [(Fruit juice/pulps Admixed with Lure-Mixtures (LM))] under laboratory and field conditions.

PHS-Admixture	*B. cucurbitae* captures (Means ± S.E.)			
	Laboratory studies		Field studies	
	Male	Female	Male	Female
FALM-1	5.45±0.87ab	7.32±0.31a	89.52±2.27ab	91.24±2.14a
FALM-2	0.00±0.00d	0.00±0.00d	32.75±2.10e	35.63±1.54e
FALM-3	7.29±0.69a	5.41±0.11ab	71.34±2.08c	88.42±1.99ab
FALM-4	1.28±0.05c	0.38±0.05cd	84.64±2.21ab	78.18±1.53b
FALM-5	4.59±0.31b	2.63±0.19c	61.45±2.11e	68.91±2.31d
FALM-6	0.00±0.00d	0.00±0.00d	13.43±1.52h	19.07±1.24g
FALM-7	4.71±0.16b	2.19±0.27c	78.65±2.41ab	83.93±2.54ab
FALM-8	0.56±0.02cd	4.78±0.21b	22.44±1.94f	31.58±2.04e
Standard (GF-120)	0.56±0.03cd	2.12±0.15c	12.54±1.32h	17.32±2.09g

Values in column containing different letters of same format are significantly different from each other at probability level of 5% according to Tukey`s Honestly Significant Difference (HSD) test.

Table 7. Attractancy-indices and attractancy-classes of different FALMs [(Fruit juice/pulps Admixed with Lure-Mixtures (LM))] for *Bactrocera cucurbitae* under laboratory and field conditions (AI of GF-120 is zero because it was used as standard).

Phagostimulants	Attractive Indices (%) (attractancy classes)			
	Laboratory studies		Field studies	
	Male	Female	Male	Female
FALM-1	66.67 (III)	55.56 (III)	76.24 (III)	68.52 (III)
FALM-2	-100.00 (I)	-100.00 (II)	45.00 (II)	35.00 (II)
FALM-3	75.00 (III)	42.86 (II)	71.08 (III)	67.62 (III)
FALM-4	33.33 (II)	-33.33 (I)	75.00 (III)	64.21 (III)
FALM-5	60.00 (III)	20.00 (II)	67.12 (III)	60.00 (III)
FALM-6	-100.00 (I)	-100.00 (I)	4.00 (I)	6.00 (II)
FALM-7	66.67 (III)	20.00 (II)	69.62 (III)	66.00 (III)
FALM-8	0.00 (I)	33.33 (II)	29.41 (II)	30.61 (II)
Standard (GF-120)	0.00	0.00	0.00	0.00

Roman values in parentheses indicate the attractancy-classes of FALMs categorized on the basis of their Attractancy-indices according to scale of attractancy as described by Beroza and Green (1963) for fruit flies.

FALM-8 proved attractive equally to GF-120 in case *B. cucurbitae* males. Results of attractancy of field-bioassay for *B. cucurbitae* males reveal that FALM-1, FALM-3, FALM-4, FALM-5 and FALM-7 demonstrated 76.24%, 71.08%, 75%, 67.12% and 69.62% AI, respectively and proved strong attractive FALMs (Class III with AI > 50%) to *B. cucurbitae* males. These five FALMs proved 76.24%, 71.08%, 75.00%, 67.12% and 69.62% more attractive than GF-120. FALM-2 and FALM-8 demonstrated 45.00% and 29.41% AI, respectively and were found moderate attractive FALMs (Class II with AI = 11-50%) to *B. cucurbitae* males. FALM-6 exhibited least attractancy-index (4%) and proved non/little attractive FALM (Class I with AI < 11%) to *B. cucurbitae* males. Similarly, results of attractancy field-bioassay for *B. cucurbitae* females reveal that FALM-1, FALM-3, FALM-4, FALM-5 and FALM-7 demonstrated 68.52%, 67.62%, 64.21%, 60.00% and 66.00% AI, respectively and proved strongly attractive FALMs (Class III with AI > 50%) to *B. cucurbitae* females under field conditions. However, FALM-2, FALM-6 and FALM-8 exhibited 35.00%, 6.00% and 30.61% AI and were found moderately attractive FALMs (Class II with AI = 6-50%) (Table 7) to *B. cucurbitae* females under field conditions. FALM-1, FALM-3, FALM-4, FALM-5, FALM-7, FALM-2, FALM-6 and FALM-8 demonstrated 68.52%, 67.62%, 64.21%, 60.00%, 66.00%, 35.00%, 6.00% and 30.61% more attractiveness to *B. cucurbitae* females under field conditions than GF-120 (standard) (Table 7).

DISCUSSION

Tephritid flies continuously pursuit for protein sources like honeydews, yeast, plant juice and animal excreta for survival and reproduction (Prokopy and Roitberg, 1989). Different proteins, minerals, carbohydrates and amino acid along with amino acetate have been used as phagostimulants for attraction of different fruit fly species (Tsitsipis, 1989; Manrakhan and Lux, 2008). Different sources of protein including protein hydrolysate, beef-extract, starch and fish-extract are used for capturing fruit flies (Putruele *et al.*, 1993; Pinero *et al.* 2009, Pinero *et al.* 2011). Similarly, different fruit juice like banana, pineapple, grapes, guava and bread are used as source of carbohydrate to stimulate attraction of fruit flies (Miller *et al.* 2004; Pinero *et al.* 2011; Balagawi *et al.* 2012). Use of such compounds as fruit flies attractants is an effective management tool. In the present study, different experiments were conducted to screen out different protein and ammonia sources for attractiveness to *B. cucurbitae* under laboratory and field conditions. Findings of these experiments revealed that protein hydrolysate, yeast-and ammonium acetate-based lure-mixtures (LMs) captured comparatively more number of *B. cucurbitae* adults in field as well as in laboratory studies. These results are in accordance with the results of Cornelius *et al.* (2000) who reported that pretentious bait captured more oriental fruit fly. In different studies, Satpathy and Samarjit (2002) and Fabre *et al.* (2003) also revealed that *B. cucurbitae* was significantly attracted to protein sources. Fabre *et al.* (2003) also documented that solution of protein hydrolysate effectively captured female melon fruit flies over a week. Rajitha (2004) revealed that protein, yeast, and soybean were most attractive for capturing fruit flies. The results of all aforementioned researchers are highly in agreement with the results of present studies on protein hydrolysate attraction to *B. cucurbitae.* The results of present studies about the attractiveness of FALMs exhibit that mixing of cucumber, banana, grapes and watermelon in ammonia-yeast-protein based lure-mixtures (LMs) enhanced their attractiveness significantly to both male and female flies of *B. cucurbitae*. These results are highly confirmatory with those of Ravikumar (2005) who reported that mixing of banana proportionally with ammonia acetate increased attractiveness of bait and more fruit flies were captured in such bait (Ravikumar, 2005). Similarly, Ravikumar and Viraktamath (2007) documented that mixing of proteinex with ammonium acetate proved more effective for trapping *B. cucurbitae.* These results of Ravikumar and Viraktamath (2007) are also in confirmation with the results of present studies. The results of present experiments on more attractiveness of fruit-ammonia-yeast-protein based FALM to *B. cucurbitae* are highly in confirmation with the results of Pandey *et al.* (2010) who documented that mango pulp mixed with yeast and protein hydrolysate increased the number of melon fruit flies and oriented fruit fly captured. The LMs and FALMs developed and evaluated in present research were found more attractive to female fruit flies than male fruit flies of *B. cucurbitae.* Lower population of male fruit flies on protein sources may be attributed to fact that male flies need protein in lower quantity while female flies need significantly more protein. This fact is also supported by McInnis *et al.* (2004) who, in his finding, reported that *B. cucurbitae* males need protein in lower amount. Protein is very vital requirement for female fruit flies during sex maturation, copulation, ovigenesis and egg-maturation. Shelly *et al.* (2004) and Perez-Staples *et al.* (2007) also supported this theory in cases of *B. dorsalis* and *B. tryoni* (Froggatt). All these facts support the results of present research on more attractiveness of female flies to LMs and FALMs compared to male flies of *B. cucurbitae*. Mixing of two attractive compounds proved more effective for tephritids fruit flies capturing instead of applying single compound (Ripley and Hepburn, 1929). For example, mixing ethyl alcohol with acetic acid often has positive effect on capturing tephritids flies (Barrows, 1907). Gow (1954) and Morton and Bateman (1981) reported that *B. cucurbitae* had positive responsive to sources of attraction comprising beer and vinegar. The results of present studies also reveal that two and three level mixtures of more than one attractants in form of LMs and FALMs were found more attractive as compared to LMs and fruits alone.

Conclusion: LM-9 (protein-hydrolysate based lure-mixture), LM-5 (Yeast-extract based lure-mixture) and LM-1 (Ammonium-acetate based lure-mixture) proved more attractive LMs to *B. cucurbitae* under field conditions and were selected to admix with juices/pulps of different fruits for preparation of FALMs [(Fruit juice/pulps Admixed with Lure-Mixtures (LM))] in another experiment. FALM-1 (cucumber based FALM) proved strongly attractive FALM to both males and females of *B. cucurbitae* in olfactometer and field studies. However, FALM-3 (banana-based FALM), FALM-4 (pumpkin-based FALM), FALM-5 (grapes based FALM) and FALM-7 (watermelon based FALM) proved strongly attractive FALMs to both males and females of *B. cucurbitae* only in field studies. FALM-1 proved strongly attractive FALM to both males and females of *B. cucurbitae* in olfactometer and field studies, so it can be used for IPM program of *B. cucurbitae* in cucurbit cropping system.

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