RESEARCH ARTICLE

Behavioural Response of Female Melon Fruit Fly, *Zeugodacus cucurbitae* (Coquillett) (Diptera: Tephritidae) of Bitter Gourd Accessions/Variety

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ABSTRACT

The biochemical factors can provide a source of resistance and chemical stimuli play a vital role in host plant selection by feeding and oviposition. The development of genotypes/varieties resistant to melon fruit fly is an essential component of Integrated Pest Management. Field experiments were conducted during 2018-19 in farmer's field at Ellamanam village and laboratory studies were conducted at the Department of Plant Protection, Anbil Dharmalingam Agricultural College and Research Institute, Tamil Nadu Agricultural University, Tiruchirappalli District. A screening of 12 accessions/varieties (2 resistant, 6 moderately resistant, 3 susceptible, and 1 highly susceptible) was selected for y- tube olfactometer studies to confirm resistance. The behavioral response of fruit fly, Z. *cucurbitae*, to kairomone compound emitted from bitter gourd leaves and fruits were extracted using different solvents viz., hexane, dichloromethane, and distilled water. The result concluded that hexane and dichloromethane leaf and fruit extract of TCR 393 showed high duration for fruit fly attraction and lower attractancy, followed by Musiri local-1, MC-10, Ucha small, Bikner -2 , Musiri local-2, and CO-1 and if identified kairomone compound may be useful for monitoring and managing of Z. *cucurbitae*.

Keywords: *Bitter gourd; Melon fruit fly; Kairomone; Y- tube olfactometer*

INTRODUCTION

Bitter gourd (*Momordica charantia* L.) (Cucurbitaceae) is the most important tropical and sub-tropical vegetable which occupies a predominant position in Indian vegetables (Rai *et al*., 2008). Bitter gourd is being attacked by insect pests, viz. aphids, melon fruit fly, hadda beetle, pumpkin caterpillar, leafhopper, leaf miner, and pumpkin beetle during different growth stages. Among them, fruit fly is playing major role in causing yield loss from 30 to 100 per cent, based on crop growth stages and season (Dhillon *et al*., 2005b). The general management practices that are used to manage fruit flies are bagging of fruits, food lures attractants, parapheromone traps and spraying insecticides (Sapkota *et al*., 2010). Due to repeated usage of toxic insecticides, the fruit fly has gained resistance and resurgence against new insecticides (Wang *et al*., 2015). This increased cost of production by up to 25 per cent (Nasiruddin *et al*., 2004). Therefore, integrated pest management practices like trapping methods are one of the alternatives to synthetic chemical pesticides for pest management (El-Wakeil, 2013). Melon fruit fly behaviour, such as host-searching, mating, and oviposition are mediated by semiochemicals. Most adult fruit flies can detect volatiles of host fruits from some distance through olfaction and orient upwind towards the fruiting regions of a host (Kimbokota *et al*., 2013). With this information, the research was made to screen the bitter gourd accessions/ genotypes for resistance to fruit fly species and to identify the kairomone compounds from plant parts. Because, those compounds are dissimilar in resistance and susceptible genotypes. Hence, identifying susceptible genotypes attracting compounds, as it is used for trapping methods and host plant resistance. Among the commercially available synthetic cue lures, 4-P-acetoxyphenyl-2-butanone was the widely used for monitoring and mass trapping of melon fruit flies (Vargas *et al.*, 1989) but that is unlikely to attract only male fruit flies and not female fruit flies, therefore identification of the kairomonal compound from bitter gourd plant, that attracts both male and female melon fruit flies is requisite. In the present study, the behavioural responses of female Z. *cucurbitae* to kairomone from fruits and leaves plants at different genotypes were compared using y- tube olfactometer.

MATERIAL AND METHODS

Accessions/variety/local types screening

A preliminary screening was carried out with 50 bitter gourd accessions (wild types and commercial cultivars). Among these, 12 accessions (2 resistant, 6 moderately resistant, 3 susceptible, and 1 highly susceptible) were selected to study the influence of biochemical and morphological traits on larval density and reaction to melon fruit fly. The bitter gourd accessions were raised in a plot of 3.0 m ×1.5 m with 0.5 m (plant to plant) and 2.5 m (row to row) spacing from August to November in a farmer's field at Ellamanam village, Tiruchirappalli District. Each accession was replicated thrice with four plants in each replication using Randomized Block Design (RBD) and recommended package of practices was followed according to TNAU crop production guide except for plant protection measures.

Mass culturing of melon fruit fly

The infested bitter gourd fruits were collected from the bitter gourd field and kept in 20cm × 20cm × 8 cm plastic trays in a layer of sieved moist sand to facilitate pupation. After 3-4 days, sand was sieved and the pupae were collected and kept in 10 cm (diameter) Petri dishes with moisture paper. The pupae were placed inside the rearing cages of 35cm × 30cm × 35 cm. Each rearing cage had wire mesh on 3 sides, glass on top, and a wooden door on one side with a round trap door to facilitate the collection of adult flies for experimental purposes and also to provide food and water. Glucose solution (10 % W/V) was kept in 50 mL beaker and a water-soaked cotton swab was laid in such a way that half of the cotton was immersed in a glucose solution and half stayed above the rim of the beaker, slices of bitter gourd were kept inside the breeding cages for oviposition. These slices were replaced daily to avoid decay. The entire culture was maintained at a temperature and relative humidity of 26 ± 2 0C and 65 ± 5 %, respectively. The collected eggs were placed in a Petri dish with moist filter paper. After hatching, the fresh bitter gourd slices were kept in Petri dish for feeding the young larvae. The slices were again replaced daily and this procedure was repeated until the death of the females.

Sample preparation

The kairomone compounds present in the bitter gourd leaves and fruits were extracted using different solvents viz., hexane, dichloromethane, and distilled water. The bitter gourd leaf and fruit samples (50 g) were taken and macerated with 50 mL of specific solvents using a pestle and mortar. The extract was filtered through a glass funnel using Whatman No. 42 filter paper. A total volume of 100 mL of filtrate from each solvent was collected in 250 mL reagent bottle. The solvent extracts were further dried using a rotary evaporator and the kairomone thus obtained using different solvent systems were stored in airtight glass vials at -20 0C for further use.

Y – Tube olfactometer bioassay

The behavioral response of fruit flies, Z. *cucurbitae* to kairomone compound emitted from bitter gourd leaves and fruits was estimated using y- a tube olfactometer as per the procedure described by Sulaeha et al. (2017). The air source was from 115 V and 1.5 A air pump. The air was passed through a charcoal filter tube (3 cm dia., 15 cm length) and airflow hose was connected to the air flow meter for air flow adjustment. The flow rate in the tube was 12-15 m/sec. Then the tube was connected to Y -the tube olfactometer, which had a 35 cm length of Y-Tube with 750 branching angles and a 65 cm length of connected tube. The 0.1 ml of the extract was dropped on a 4 × 3 cm piece of Whatman no. 42 filter paper. Then whatman paper was inserted into y-tube right side chamber and control (pure solvent hexane or dichloromethane) was placed in the left side chamber. It was ensured that female fruit flies were starved before releasing in the olfactometer. A female fruit fly was released into olfactometer and the time duration taken by fruit fly to reach the extract sources was measured. Each treatment was replicated ten times with new female fruit flies. After each treatment, the odour chamber was replaced with a new one. The olfactometer used was deodorized before every experiment by rinsing with hexane or dichloromethane.

RESULTS AND DISCUSSION

Screening of bitter gourd accessions for the resistance to melon fruit fly *Z. cucurbitae*

The results of fruit damage in different genotypes and variety/local types are presented in (Table 1). The maximum number of fruits was recorded in variety CO-1 (31.67 no. /plant) and minimum in accession/variety/local types viz, TCR 393 and Ucha small (22.33 no. /plant). These was followed by Musiri local-1 (25.00 no. /plant), MC-10 (24.67 no. / plant) and Paravai local (27.33 no. /plant). The fruit damage was maximum in susceptible genotypes of MC-41 (21.00 no. /plant) and minimum in TCR-393 (4.00 no. /plant). The maggot population was lowest in TCR-393 (6.33 no. /fruit) followed by Musiri local-1 (6.50 no. /fruit), CO-1 (9.33 no. /fruit), Paravai local (11.87 no. /fruit) and MC-41 (13.89 no. /fruit). The fruit infestation was minimum in resistant accession/ variety/local types viz, TCR-393 (17.90 %) and Musiri local-1 (20.00 %) followed by MC-10 (23.00 %), CO-1 (49.50 %), Paravai local (62.20 %) and MC-41 (77.79 %). Screening of bitter gourd accession/ germplasm against melon fruit fly by earlier workers has reported Short Green Kerali (Lall and Sinha, 1974), IC -213311, IC - 248282, IC- 248281, IC-256110, IC- 68314(b) lines were showing resistance to this pest (Dhillon et al., 2005a). The bitter gourd genotypes Hisar II, ACC-3, ACC - 23 and 33, Kerala Collection 1, and Faizabad Collection-17 have earlier been reported to be resistant to melon fruit fly (Tewatia et al., 1997; Srinivasan, 1991). Panday et al. (2012) result trial revealed that IC 248282 with (13.64%) fruit infestation was found the least susceptible to the attack of melon fruit fly followed by Pusa Do Mausami with 57% fruit infestation. From the selected accession/genotypes, none was found to be high resistant. Out of 74 genotypes, five were found resistant, 61 were moderately resistance, five were susceptible and three were highly susceptible. Our experimental results eventually fit into earlier works of bitter gourd genotypes screening.

Behavioural response of female melon fruit fly, *Z. cucurbitae* to hexane and dichloromethane extract of bitter gourd accessions/ variety/ local types

The data revealed that hexane leaf extract attractancy was maximum and minimum from 65.67 to 23.64 per cent (Table 2 and Fig. 1). The hexane leaf extract of TCR 393 showed high fruit fly attracted time (15.48 min.) and lower attractancy (23.64 %) followed by Musiri local-1 (14.00 min. and 26.85 %), MC-10 (13.32 min. and 30.40 %), Ucha small (12.33 min. and 34.51 %), Bikner -2 (11.32 min. and 40.85 %) respectively. The hexane fruit extract of TCR 393 showed a high attractants time (12.24 min.) with less attractancy (32.07 %) followed by musiri local-1 (12.36 min. and 31.41 %), MC-10 (11.22 min. and 37.73 %), Ucha small (11.00 min. and 38.96 %), Bikner -2 (10.29 min. and 42.90 %) and Musiri local-2 (10.04 min. and 44.28 %). The dichloromethane leaves extract of TCR 393 had a maximum attractance time (12.38 min.) with minimum attractancy (11.94 %) followed by Musiri local-1 (12.32 min. and 12.37 %), MC-10 (11.34 min. and 19.34 %), Ucha small (10.36 min. and 26.31 %), Bikner -2 (11.28 min. and 19.77 %) and Musiri local-2 (10.16 min. and 27.73 %) (Table 3). The dichloromethane fruit extract of TCR 393 had higher fruit fly attraction duration (10.14 min.) and lower attractancy (22.83 %) followed by Musiri local-1 (9.25 min. and 29.60 %), MC-10 (9.28 min. and 29.37 %), Ucha small (7.30 min. and 44.44 %), Bikner -2 (8.31 min. and 36.76 %), Musiri local-2 (7.36 min. and 43.99 %) and PKM local (8.03 min. and 38.89 %).

The plant volatile compounds play a pivotal role for insect pests in searching for their hosts and detecting unsuitable hosts (Gerofotis *et al*., 2016). The insects have shown excellent response to olfactory favourable signals and specificity for certain volatiles from a suitable host (De Bruyne and Baker, 2008). For this reason, odor quality and titer will depend on the combination of volatile compounds from host plants. The volatile organic compounds (VOC) are easily evaporated or released from bitter gourd, which is extracted by using hexane and dichloromethane solvents. Among the solvents, hexane (nonpolar) extract was more responsive than dichloromethane (polar). Since, the solvent n-hexane belongs to terpenoid group and has a low level of polarity (Harborne, 1984) attracted the gravid female fruit fly more than the newly emerged fruit fly (Allwood, 1997). Based on experimental results isolated terpenoid content from leaves and fruits showed maximum attraction of female fruit flies. Results concluded that fruit extractions were found to be a more attractive percentage compared to leaf extractions. In contrast, Smart *et al.* (2014) reported that cucurbits leave attracted more fruit flies because of their high phytochemical content (alkaloid, saponin, flavonoid, and steroid).

The Y-tube olfactometer results revealed that hexane, dichloromethane leaf, and fruit extract took longer attracting time while less time for resistant accessions TCR-393. The less duration and maximum attractancy were observed in susceptible accessions of MC-41. These are in accordance with Sulaeha *et al*., (2017) who showed n-hexane extract of bitter gourd leaf and fruit favored higher response in melon fruit fly in Y-tube olfactometer. Female fruit flies were found to flutter their wings and rub their antenna with posterior legs depending on the type of compound mixture their olfactory system can detect it. Light and Jang (1987) conducted experiments on Gas chromatography electroantenogram (GC-EAD) with leaves and fruits of bitter gourd and several components (-1-octen-3-ol, (Z)-6-nonenal, Z-13-octadecenal, fernesyl acetone, pentadecanone, linalool, (E)-2-hexanal, acetic acid, hexadecanoic acid, and methyl-1-inolenate) were found to induce response in fruit fly. Among these components, ketone (6,10,14-trimethyl-2-pentadecanon) and aldehyde (3,7,11,15-trimethyl-2-hexadecan-1-ol) were more responsible for host preference and were confirmed by the studies of Siderhurst and Jang (2010) by using GC-EAD. Hence, these compounds can be used to develop a new variety and new synthetic lures for attracting male and female fruit flies for Integrated Pest Management (IPM).

Table 1. Screening of bitter gourd accessions for resistance to melon fruit fly Z. cucurbitae

S. No.	Bitter gourd accessions/variety/ local types	Biological attributes			Fruit fly infestation* (%)	Resistance Index
		Total fruits* (no. /plant)	Damaged fruit* (no. /plant)	Maggots/ fruit* (no.)		
1	TCR-393	22.33	4.00	6.33	17.90	Resistant
2	Musiri local-1	25.00	5.00	6.50	20.00	Resistant
3	MC-10	24.67	5.67	7.25	23.00	Moderately Resistant
4	Ucha small	22.33	6.67	7.40	29.80	Moderately Resistant
5	Bikaner-2	30.33	11.67	7.80	38.50	Moderately Resistant
6	Musiri local-2	28.33	12.33	8.17	43.50	Moderately Resistant
7	Pkm local	24.33	11.67	9.16	47.90	Moderately Resistant
8	Co-1	31.67	15.67	9.33	49.50	Moderately Resistant
9	MC-39	23.67	15.67	10.10	66.20	Susceptible
10	MC-105	25.67	15.33	10.67	59.70	Susceptible
11	Paravai local	27.33	17.00	11.87	62.20	Susceptible
12	MC-41	27.00	21.00	13.89	77.79	Highly Susceptible

*Mean of ten observations

Table 2. Behavioural response of female melon fruit fly, Z. *cucurbitae* to hexane extract of bitter gourd accessions/ variety/local types

S. No.	Bitter gourd accessions/ variety/ local types	Female fruit flies attracted hexane extract*			
		Leaf extract (no./min.)	Attractancy (%)	Fruit extract (no./min.)	Attractancy (%)
1	TCR-393	15.48	23.64	12.24	32.07
2	Musiri local-1	14.00	26.85	12.36	31.41
3	MC-10	13.32	30.40	11.22	37.73
4	Ucha small	12.33	34.51	11.00	38.96
5	Bikaner-2	11.32	40.85	10.29	42.90
6	Musiri local-2	10.39	45.10	10.04	44.28
7	Pkm local	11.15	41.74	9.30	48.39
8	Co-1	9.26	51.62	9.27	48.56

9	MC-39	8.44	55.90	7.42	58.82
10	MC-105	8.21	57.10	8.18	54.60
11	Paravai local	7.50	60.51	6.23	65.43
12	MC-41	6.57	65.67	5.35	70.31
13	Untreated control	19.14		18.02	

*Mean of ten observations

Table 3. Behavioural response of female melon fruit fly, *Z. cucurbitae* to dichloromethane extract of bitter gourd accessions/variety/local types

S. No.	Bitter gourd accessions/ variety/ local types	Female fruit flies attracted dichloromethane Extract*			
		Leaf extract (no./min.)	Attractancy (%)	Fruit extract (no./min.)	Attractancy (%)
1	TCR-393	12.38	11.94	10.14	22.83
2	Musiri local-1	12.32	12.37	9.25	29.60
3	MC-10	11.34	19.34	9.28	29.37
4	Ucha small	10.36	26.31	7.30	44.44
5	Bikaner-2	11.28	19.77	8.31	36.76
6	Musiri local-2	10.16	27.73	7.36	43.99
7	Pkm local	9.35	33.50	8.03	38.89
8	Co-1	9.31	33.78	6.32	51.90
9	MC-39	8.30	40.97	6.18	52.97
10	MC-105	7.34	47.79	5.32	59.51
11	Paravai local	6.30	55.19	5.34	59.36
12	MC-41	5.30	62.30	4.57	65.22
13	Untreated control	14.06		13.14	

*Mean of ten observations

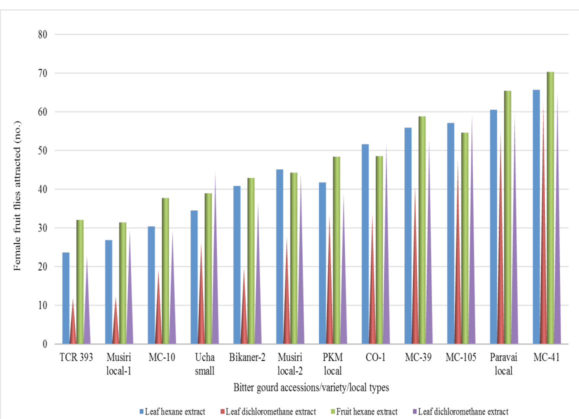

Figure 1. Behavioural response of female melon fruit fly, *Z. cucurbitae* to hexane and dichloromethane extract of bitter gourd accessions/variety/local types

CONCLUSION

The findings from these investigations indicate that the kairomone of fruits and leaves of the TCR 393 and Musiri local-1 were less attractive to female *Z. cucurbitae* followed by moderately resistant (MC-10, Ucha small, Bikner-2, PKM local, and CO-1), susceptible (MC-105, Paravai local) and highly susceptible (MC-41) genotypes.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Originality and plagiarism

The authors declare that the work carried out in this research paper is the original work and has not been published earlier or sent for publication to other research journals.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There were no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required. If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail; mawthammm1996@gmail.com

Author contributions

Idea conceptualization- CG, SS, MMM, Experiments- MMM ,Guidance - CG, SS, Writing original draft - MMM, Writing- reviewing &editing - CG, SS

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