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Electroantennographic Responses of Female Fruit Piercing Moth, *Eudocima materna* Linnaeus (Lepidoptera : Erebidae) to Fruit Extracts of Host Plants

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ABSTRACT

To identify an effective attractant or repellent host-fruit volatile, antennal responses of fruit piercing moth, *Eudocima materna* adult females to fruit extracts were screened and assessed using Electroantennography (EAG). Volatiles from pomegranate and papaya fruits elicited higher responses from the female moths. Wind tunnel bioassay of pomegranate and papaya fruit volatiles showed 'took flight' (TF), 'upwind flight' (UF) and 'source approach' (SA) responses but not 'source contact' (SC). These fruit kairamones may be useful as attractants for monitoring and mass trapping fruit piercing moths in pomegranate orchards.

Keywords: Behavioural responses, Electroantennography, *Eudocima materna*, Female fruit piercing moth

FRUIT piercing moth, *Eudocima materna* (L.) (Lepidoptera : Erebidae) attained importance as major pest on fruit crops in several countries (Jayanthi *et al*., 2015). According to the development and degree of introduced fruit cultivation, particularly of citrus and pomegranate, this insect has become the major pest in recent years. The adults of fruit sucking moths damage fruits and vegetables. Mote *et al*. (1991) estimated the fruit piercing moth infestation level upto 57 per cent on pomegranate in Rahuri and Maharashtra. Insecticides are often ineffective and uneconomical against *E. materna* due to typical feeding habits of moths and use of chemical insecticides resulting in adverse effects on the export of fruits. This is due to high residue levels in harvested fruits. Low level of parasitism by natural enemies due to difficulty in reaching the host is yet another important reason for the fruit piercing moth infestation (Bhumannavar *et al*., 2000).

The fruit maturity and ripening stage of Mrig bahar season (June-November) crop is the most preferred phenological stage for feeding and perpetuation of *E. materna*. Concerted efforts to suppress *E. materna* over the years have utilized both insecticidal and non-insecticidal tools. However, as female moths are attracted to the host fruits for juice sucking, this as the basis for developing a management strategy can be exploited. Hence, semiochemical based management forms one of the viable technologies to combat fruit sucking moths in pomegranate. Papaya, banana and guava contains papain, gallic acid and quercetin respectively that are attractive to moths.

An adult moth lives upto 25-30 days and can lay 200-400 eggs. Spherical eggs laid singly on the climbers like *Cocculus pendulus*, *Cocculus hirsutus* and *Tinospora cardifolia*, which grow along fence or hedges around the orchards or away from it. Incubation period for egg is 8-10 days. The larval stage passes through five instars and takes 14-28 days. Full-grown caterpillars are 50-60 mm in length, bright coloured with orange, yellow and blue spots on speckled body and are semiloopers. Pupation takes place in a transparent pale whitish silken cover in soil, which last for 13 days (Suroshe *et al*., 2013).

The duration of egg to egg-laying adult female is about 35 to 49 days. Moths of genus *Eudocima* have a highly specialized proboscis with hard spines capable of piercing hard, unripe fruits such as green citrus and tender pomegranates. Adult males and females of moth penetrates the fruits at night and damaged fruits become soft owing to secondary infections from different fungi and bacteria. Punctured holes on fruits with oozing fruit juice is often noticed. Hence, the identification of host kairomone plays a crucial role in the management of *E. materna* (Felipe *et al*., 2018).

Material and Methods

In the present study, Electroantennography (EAG) technique was used to screen the fruit volatiles of pomegranate [*Punica granatum* (L.)]; guava [*Psidium guajava* (L.)]; papaya [*Carica papaya* (L.)] and banana [*Musa acuminata* (Colla)] for identifying volatiles with possible semiochemical properties. *Eudocima materna* insect culture was established in the laboratory rearing cages at National Research Center on Pomegranate, Solapur, Maharashtra and at rearing facility of Biocontrol Research Laboratories (BCRL), Pest Control (India) Pvt. Ltd. (PCI), Sriramanahalli, Bengaluru.

i) Maintenance of adult moth culture

The culturing was initiated with field collected adults from fruit bearing orchards at ICAR-NRCP Solapur. Insect colonies were maintained in a 12 light (L): 12 dark (D) regime with room temperature of 26±2 °C and relative humidity of 70±10 per cent. Honey (10%) has been provided in the form of cotton swabs dipped in 5 per cent honey for moths.

ii) Extraction of fruit volatiles from pomegranate, banana, papaya and guava

Solvent extraction method was adopted for collection of volatiles from fruits. From each host, approximately 200-300 g whole fruits were collected and immersed in 500 ml Hexane (C_6H_{14}) and Dichloromethane (DCM) (CH_2Cl_2) (HPLC grade) at room temperature (25±2 °C) for 1 hour. The solvents were filtered and concentrated under a gentle stream of nitrogen. The concentrated extracts were used for the bioassays.

Electroantennography (EAG) (Syntech, The Netherlands) bioassay was carried out at BCRL to assess the antennal receptivity of adult females of *E. materna to* different fruit extracts. 10µl of aliquot was placed on a filter paper strip (60 mm long, 5 mm wide Whatman No. 1) inside a glass pasteur pipette (Dimensions-5.75, Length- Overall 145.0 mm; tip 47.0 mm) was used for stimulus delivery. This was connected to the stimulus controller by silicone rubber tubing. After 10 seconds, the solvent was blown out with first puff. Another 60 seconds later, the stimulus was puffed on to the excised antenna by injecting the vapour phase of the chemical stimuli through a polystyrene tube along with a continuous air stream (pulse rate 0.5 s, continuous flow 25 ml s^{-1}, pulse flow 21 ml s^{-1}) to the antenna. Each recording was replicated five times with one antenna per replication. Hexane and dichloromethane were used as control.

iii) Bioassay of fruit volatiles through electroantennography

Best performed fruit extract after EAG screening was further tested to determine the behavioral responses in a wind tunnel at Insect Behaviour Testing Lab (IBTL) of BCRL. A wind tunnel of 80x30x30 cm was calibrated to 25 cm s^{-1}, 5 lux (light intensity), 24±1^{0}C and 65±5 per cent RH in a room separated from the *E. materna* culture, was used.

iv) Bioassay through wind tunnel

Bioassays were carried out from four hours after the beginning of scotophase, which corresponded with the peak mating behaviour of *E. materna.* Test sources positioned 18 cm from the upwind end of the wind tunnel. The control or blank (DCM/Hexane) treatment tested first to check that the wind tunnel was not contaminated. Assays were conducted following Ohmasa *et al.* (1991) procedure. The responses of females to the sources scored as follows: NR: no response, moths did not respond (did not leave the release box); TF: moth took flight but did not fly upwind; UF: upwind flight by moth, but did not reach within 20 cm of the source; SA: source approach, moth clearly followed plume and hovered in front of the source within 20cm, but failed to contact the source; SC: source contacted, made contact with or landed on the source.

Results and Discussion

All the four different fruit extracts elicited significantly ($F_{5, 24}$= 9.216; P=0.002) higher antennal response than control (Hexane). However, pomegranate fruit extracts elicited significantly higher EAG response 2.51±0.27 (Mean±SE). Nevertheless, extracts of banana, guava and papaya were found on par with each other (Table 1). Similarly in case of DCM extracts, all the fruit extracts were significantly

TABLE 1

EAG responses of female *Eudocima materna* to Hexane extract of fruit volatiles

Source	*EAG Response (mV) (mean±SE)
Pomegranate	2.51±0.27 [a]
Banana	2.36±0.70 [ab]
Papaya	2.32±0.27 [ab]
Guava	2.01±0.18 [ab]
Honey	1.397±0.16 [bc]
Hexane	0.592±0.9 [c]
Df	**5, 24**
F	**9.216**
P	**0.002**

Note : Figures within a column followed by a common letter are not significantly different by Tukey's HSD test ($P<0.05$)

TABLE 2

EAG responses of female *Eudocima materna* to DCM extract of fruit volatiles

Source	*EAG Response (mV) (mean±SE)
Pomegranate	1.421±0.26 [ab]
Banana	1.231±0.34 [ab]
Papaya	1.901±0.23 [a]
Guava	0.912±0.11 [ab]
Honey	0.456±0.13 [ab]
DCM	0.342±0.12 [b]
Df	**5, 24**
F	**3.562**
P	**0.027**

Note : Figures within a column followed by a common letter are not significantly different by Tukey's HSD test ($P<0.05$)

($F_{5, 24}$=3.562; P=0.027) different from control dichloromethane (DCM). Papaya fruit extracts elicited significantly the maximum EAG response of 1.901±0.23 (Mean ± SE) (Table 2).

Pomegranate and papaya fruit extracts, which recorded the highest response in EAG studies, were further subjected to behavioral assays in wind tunnel. Both the extracts elicited 'took flight' (TF), 'upwind flight' (UF) and 'source approach' (SA) responses except 'source contact' (SC). In the behavioral category of 'TF'to either stimulus did not differ between host plants compared with the control (χ^2=4.652; P=0.100). In other categories like 'UF' and 'SA' significantly more females flew upwind (χ^2=16.348; P=0.001) and approached the source (χ^2=11.457; P=0.004) in the stimuli presented than control (Table 3). However, none of the moths contacted the source (SC).

In the present study, it was observed that female *E. materna* responded to fruit derived volatile constituents, used by the female to identify and locate the hosts for feeding. The extracts from pomegranate fruit and papaya have shown higher antennal and taxis response. Papaya and guava fruits principally contain papain and quercetin compounds which are attractive to moths supportive information on principal compunds of fruits viz., pomegranate, papaya, banana and guava tested for response of fruit piercing moths is furnished

TABLE 3

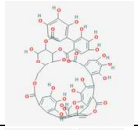
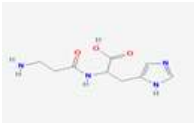
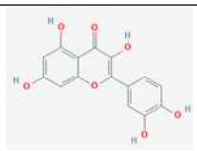
Taxis response of *E. materna* female moths to pomegranate and papaya fruit extract in wind tunnel

Source	No. of Replications	Took flight (TF)	Upwind flight (UF)	Source approach (SA)	Source contact (SC)
Pomegranate	15	17 [b]	14 [a]	10 [a]	0
Papaya	15	19 [a]	16 [a]	13 [a]	0
Control (DCM)	15	11 [b]	4 [b]	3 [b]	0
Chi-Square value		**4.652**	**16.348**	**11.457**	**-**
P value		**0.100**	**0.001**	**0.004**	**-**
Df		**2**	**2**	**2**	**-**

Figures within a column followed by a common letter were not significantly different (Beasley and Schumaker, 1995; post-hoc test). TF- Took flight; UF- Upwind flight; SA- Source approach and SC- Source contact.

TABLE 4

Principle compounds of fruits tested for response of fruit sucking moths

Name of the fruit	Principle moiety	Mol. Structure	Category of the compound
Pomegranate	Punicalagin		Anthocyanins
Banana	Gallic acid		Phenolic acid
Papaya	Papain		Proteolytic enzyme
Guava	Quercetin		Flavonoid

for reference in Table 4. Felipe *et al*. (2018) suggested that attraction to suitable host plants in part was mediated by similar olfactory cues from different host plants.

The fruit volatiles can be used as attractant to trap the moths for monitoring and management of the pest. Major volatiles released by the host plants, might be useful in exploring monitoring and or management strategies by host plant based attractants for semiochemical based pest management of *E. materna.*

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