Oviposition response of melon fruit fly, *Bactrocera cucurbitae* (Coquillett) to different phenolic compounds

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ABSTRACT

The oviposition deterrent effects of four phenolic compounds (quercetin, rutin, gallic acid and tannic acid) were investigated against the melon fruit fly, *Bactrocera cucurbitae*. All the phenolic compounds effectively reduced egg laying in choice and no-choice conditions except rutin. Also observations made for their effect on ovipunctures made by female melon fly showed a significant decrease in the mean numbers of ovipunctures made on the substrate treated with quercetin, gallic acid and tannic acid under choice and multiple-choice tests. Rutin had no effect on oviposition behaviour under no-choice condition. Under multiple choice conditions rutin significantly reduced mean number of ovipunctures. The results of our studies clearly demonstrated the oviposition deterrent activity of the phenolic compounds.

Keywords: oviposition deterrent, *Bactrocera cucurbitae*, quercetin, ovipunctures, gallic acid, tannic acid

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INTRODUCTION

Melon fruit fly, *Bactrocera cucurbitae* (Coquillett) is a major pest of cucurbit crops throughout the world. *B. cucurbitae* is known to attack more than 125 plants including commercially important crops such as pumpkin, watermelon, squash, gourd, cucumber, tomato, eggplant and beans (White and Elson-Harris, 1992). It can cause 30-100% damage to crop depending upon the agroclimatic season (Dhillon *et al.,* 2005). The commercial value of vegetables and fruits is drastically reduced even by slight damage which affects their export. Synthetic pesticides, no doubt have made a significant contribution in the control of insect pests, yet their harmful effects on human health and environment can no longer be ignored. In the search for new strategies for pest management, botanical pesticides being safe and biodegradable will have an important role as future pest control agent.

Plants produce large number of allelochemicals that play a major role in plant protection. More than 100,000 allelochemicals have been identified (Buckingham, 1993). Phenolic compounds which are characterized by the presence of hydroxyl group on aromatic ring are one of the major classes of allelochemicals (Appel, 1993). Phenolic compounds play important roles in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and precursors to toxic substances. They are also recognised for possessing anti-allergic, anti-inflammatory, anti-viral, anti-proliferative and anti-carcinogenic activities in animals (Daniel *et al.*, 1999; Parr and Bolwell, 2000; Hollman, 2001). These compounds have also been postulated to play a role in plant defence against insect pests (Appel, 1993; Eleftherianos *et al.*, 2006). Oviposition is an important part of insect life cycle. Insects lay eggs on different parts of plant which hatch into larvae. Larvae start feeding on plant material soon after hatching. Therefore, egg laying by insects pose serious threat for the plant. If oviposition is prevented, the insect life cycle is disrupted and insect population can be reduced. Therefore, the present study was envisaged to explore the oviposition deterrent activity of phenolic compounds against *B. cucurbitae*.

MATERIALS AND METHODS

Insect cultures

Flies were obtained from cultures of *B. cucurbitae* which were maintained on pumpkin pieces under controlled conditions of temperature (25±2°C), relative humidity (70–80%) and photoperiod (L10: D14), in wire mesh cages (L45 x B45 x H50cm) in the insect culture room.

Chemical used

Quercetin, rutin, gallic acid and tannic acid were purchased from Sisco Research Pvt. Limited (Mumbai), Himedia Laboratories Pvt. Limited (Mumbai), Loba Chemie Pvt. Limited (Mumbai) and S.D. fine-chem Limited (Mumbai) (with 99.0%, 90.0%, 99.5% and 90.0% purity).

Biassays

Cleaned pumpkin pieces (2.5cm^3) were given dipping treatment with phenolic compounds at different concentrations (control, 1ppm, 5ppm, 25ppm, 125ppm, 625ppm and 3125ppm) for one minute and allowed to dry at room temperature. Three pairs of twenty days old flies taken in the ratio of 1 male: 1 female were transferred to glass chimneys (9×14cm) containing pumpkin pieces and provided with cotton swabs dipped in sugar and protinex mixture hanged from roof of chimneys covered with muslin cloth with the help of paper clips. There were six replications for each concentration. Experiments were conducted for 24h under choice, multiple choice and no-choice conditions (Singh and Singh, 1998), after which fruits were removed and dissected under binocular microscope to count the number of eggs laid by three females in each replication. For choice test control pumpkin pieces were marked on pumpkin skin which was kept on lower side to distinguish between control and treated pumpkin pieces in same chimney. Effective repellency in case of choice test was calculated by using the formula (Rajkumar and Jebanesan, 2009):

$$\mathrm{ER}\ (\%) = \frac{\mathrm{NC} - \mathrm{NT}}{\mathrm{NC}} \times 100$$

where
ER = percent effective repellency
NC = number of eggs in control and
NT = number of eggs in treatment

Statistical Analysis

A statistical analysis on the data was performed using computer software (SPSS 16) to calculate one way analysis of variance (ANOVA). The latter was used to analyse the effect of phenolic compounds on oviposition of melon fruit fly, *B. cucurbitae*. The results were expressed as mean ± S.E. If the variable was significant, Tukey's multiple range test (Assistat version 7.6) was used for pair wise comparison of the difference between treatments for mean separation ($p<0.05$).

RESULTS

Oviposition under no-choice conditions

Treatment of pumpkin pieces with different phenolic compounds reduced egg laying by *B. cucurbitae* indicated by lower proportion of eggs laid on treated pumpkin pieces in comparison with controls (dipped in distilled water) (Table 1). Gallic acid showed maximum inhibition where oviposition decreased by 2.99% of the control at 3125ppm followed by tannic acid where the oviposition was reduced to 17.77% of the control.

Oviposition under choice conditions

Under choice conditions effective repellency of *B. cucurbitae* flies increased with gallic acid ($P_{0.01}$=22.27) followed by tannic acid ($P_{0.05}$=3.29) (Table 1). Effective repellency in case of rutin decreased with treatment but the decline was non-significant.

Ovipunctures made under no-choice condition

The mean number of ovipunctures made by *B. cucurbitae* flies on pumpkin pieces treated with phenolic compounds was the least with gallic acid (Table 2). Gallic acid reduced the number of ovipunctures to 20.95% of the control at 3125ppm. On the other hand the least number of ovipunctures by quercetin and rutin was observed at 125 ppm while that with tannic acid the number of ovipunctures was reduced to half of that in control at 625ppm.

Table 1. Effect of phenolic compounds on the oviposition by *B. cucurbitae* under no-choice and choice conditions conditions

Concentrations (ppm)	Number of eggs laid/female			
	Quercetin	**Rutin**	**Gallic acid**	**Tannic acid**
		No choice conditions		
Control	9.27±0.77[a]	26.00±1.51[a]	7.35±0.81[a]	8.10±0.70[a]
1	6.88±0.40[ab]	25.33±2.20[a]	5.94±0.29[a]	5.22±0.33[bc]
5	7.82±0.70[ab]	22.67±1.50[a]	2.72±0.20[b]	5.99±0.28[b]
25	7.44±0.59[ab]	23.17±1.97[a]	1.38±0.15[bc]	4.99±0.48[bc]
125	6.27±0.68[b]	20.83±1.11[a]	0.44±0.06[c]	3.60±0.51[cd]
625	6.99±0.66[ab]	23.17±1.60[a]	0.55±0.06[c]	2.88±0.20[de]
3125	7.05±0.26[ab]	20.50±1.23[a]	0.22±0.11[c]	1.44±0.29[e]
F-value (*df*=6)	2.48*	1.60[N.S.]	70.20**	25.40**
		Effective repellency % (Choice conditions)		
1	36.71±2.35ab	27.51±4.46[a]	27.69±3.99[c]	49.08±7.28[b]
5	35.32±4.52ab	11.59±2.50[a]	32.35±5.63[c]	60.56±3.87[ab]
25	42.03±4.53ab	15.14±5.22[a]	30.69±5.82[c]	69.81±5.64[ab]
125	28.79±5.59b	16.48±5.12[a]	46.80±3.34[bc]	77.25±4.97[a]
625	47.49±3.45a	15.73±3.91[a]	53.10±6.30[b]	70.29±9.01[ab]
3125	48.12±3.60a	14.52±7.34[a]	87.53±2.19[a]	80.62±6.12[a]
F-value (*df*=6)	3.32*	1.21[N.S.]	22.17**	3.29*

**=significant at 1%, *=significant at 5%, N.S. = Non significant
Means followed by the same letter within columns are not significantly different according to the Tukey test at P=0.05.

Ovipunctures made under choice condition

Under multiple choice conditions, gallic acid was again found to have the inhibitoriest effect as the number of ovipunctures made by *B. cucurbitae* flies was reduced to 19.42% of the control followed by tannic acid (24.79%) when observations were made at 3125ppm (Table3). It was also noticed that ovipunctures made at higher concentrations of gallic acid and tannic acid were devoid of eggs.

DISCUSSION

Chemical inhibitors play an important role in the inhibition of oviposition on the host plant, and, in turn, in insect larval growth and survival of progeny (Chapman, 1974; Stotz *et al*., 1999). Adult flies laid lesser number of eggs on pumpkin pieces treated with quercetin and tannic acid under no-choice test. In a previous study, Kaur *et al.* (2010) had also reported deterrent effect of polyphenolic rich extracts from the bark of *Acacia auriculiformis* on the oviposition of the melon fruit fly, *B. cucurbitae* (Kaur *et al*., 2010). Quercetin isolated from *Ricinus communis* had also shown a significant deterrent effect on ovipositional behaviour of pulse beetle, *Callosobruchus chinensis* L. (Upasani *et al.*, 2003). Salunke *et al*. (2005) had also reported

Table 2. Mean number of ovipunctures made by female *B. cucurbitae* on substrates (pumpkin pieces) treated with different concentrations of phenolic compounds under no-choice conditions

Concentrations (ppm)	Number of ovipunctures			
	Quercetin	**Rutin**	**Gallic acid**	**Tannic acid**
Control	16.00±2.05^{a}	14.50±1.73^{a}	7.16±0.30^{a}	9.16±0.70^{a}
1	9.83±1.38^{ab}	12.50±1.36^{a}	6.33±0.21^{a}	9.50±0.61^{a}
5	11.66±1.41^{ab}	11.33±0.55^{a}	3.66±0.66^{b}	7.50±0.71^{ab}
25	15.66±4.31^{a}	13.00±1.65^{a}	2.00±0.25^{bc}	6.50±0.80^{ab}
125	6.66±0.95^{b}	9.00±0.96^{a}	3.00±0.51^{bc}	5.16±0.65^{b}
625	8.16±0.94^{ab}	10.50±0.56^{a}	2.66±0.66^{bc}	4.66±0.42^{b}
3125	8.33±0.49^{ab}	10.00±1.59^{a}	1.50±0.34^{c}	6.83±1.05^{ab}
F-value (*df*=6)	3.35*	2.17$^{N.S.}$	22.27**	6.31**

**=significant at1%, *=significant at 5%, N.S. =Non significant;
Means followed by the same letter within columns are not significantly different according to the Tukey test at P=0.05

Table 3. Mean number of ovipunctures made by female *B. cucurbitae* on substrates (pumpkin pieces) treated with different concentrations of phenolic compounds under multiple-choice conditions

Concentrations (ppm)	Number of ovipunctures			
	Quercetin	**Rutin**	**Gallic acid**	**Tannic acid**
Control	8.91±0.49^{a}	12.49±0.91^{a}	6.85±0.40^{a}	6.05±0.20^{a}
1	3.83±0.54^{b}	12.00±1.41^{a}	5.33±0.49^{ab}	3.16±0.40^{b}
5	4.00±0.51^{b}	12.67±1.20^{a}	4.50±0.56^{ab}	2.50±0.42^{b}
25	4.33±0.33^{b}	8.66±0.66^{a}	4.50±0.76^{ab}	1.83±0.40^{b}
125	4.83±0.30^{b}	9.00±1.00^{a}	4.66±0.88^{ab}	1.83±0.47^{b}
625	4.50±0.84^{b}	9.16±0.70^{a}	3.16±0.65^{bc}	2.33±0.42^{b}
3125	4.83±0.70^{b}	8.50±0.76^{a}	1.33±0.21^{c}	1.50±0.22^{b}
F-value (*df*=6)	9.66**	3.79**	8.15**	16.92**

**=significant at 1%, *=significant at 5%, N.S.=Non significant
Means followed by the same letter within columns are not significantly different according to the Tukey test at P=0.05

oviposition deterrent activity of quercetin and rutin against chinese bean weevil, *Callosobruchus chinensis* (L.). In another study, lactones and flavonoids in ethanolic extract of *Andrographis paniculata* also showed oviposition deterrent activity against malarial vector, *Anopheles stephensi* Liston (Chenniappan and Kadarkarai, 2008). The addition of rutin at lower concentration decreased egg laying by zebra swallowtail, *Eurytides marcellus* (Cramer) while at higher concentrations the decrease was not significant (Haribal and Feeny, 2003). Tannin also decreased oviposition by western flower thrips, *Frankliniella occidentalis* (Pergande) (Whittaker and Kirk, 2004). Under choice-test all the phenolics except rutin effectively inhibited oviposition by adult flies. Four other flavonoid compounds, poncirin, rhoifolin, naringin and marmesin, from *Poncirus trifoliata* were also reported to have an oviposition deterrent activity against the yellow fever mosquito, *A. aegypti* (Rajkumar and Jebanesan, 2008). On the other hand, gallic acid in contrast to our present findings showed stimulatory effect on oviposition behaviour of spruce budworm, *Choristoneura fumiferana* (Clemens) (Grant and Langevin, 2002). Results also revealed lesser number of punctures on treated pumpkin pieces under no-choice and multiple choice tests. The role of flavonoids in modulating oviposition behaviour of insects has also been reported by Simmonds (2001). An insect before ovipositing probes the surface of the leaf or stem by using its antenna or walking which brings the contact sensilla located on the tarsi and antenna into close contact with compounds on the plant's surface. The present findings clearly suggest that the insect can perceive phenolic compounds on the substrate and can also differentiate between the various phenolic compounds. In an earlier study, Nair and Thomas (2001) had perceived that before oviposition the melon fruit flies thoroughly probed the substrate treated with extracts of *Acorus calamus* L. with the help of chemoreceptors present in their mouthparts and ovipositors. During such probing, they intercepted the extracts and avoided oviposition on the treated surface. The present findings clearly reveal phenolic compounds to be effective oviposition deterrents against *B. cucurbitae*.

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