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Bioefficacy of quercetin against melon fruit fly

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Abstract

Quercetin is one of the most abundant flavonoid in plants. Flavonoids are widely recognised for their diverse beneficial effects on health, such as anti-inflammatory, anti-oxidant and anti-cancer effects. They are also known to modulate the feeding and oviposition behaviour of some insects. In the present study the influence of quercetin was investigated against melon fruit fly, *Bactrocera cucurbitae* (Coquillett) by treating eggs and larvae with different concentrations (0, 1, 5, 25, 125, 625 and 3125 ppm) of quercetin. Quercetin adversely affected egg hatching which decreased with treatment. The larval period decreased significantly in the treatment of second and third instar. Inhibitory effects were observed on percentage pupation and percentage emergence which were significantly reduced. The food assimilated as well as larval and pupal weight decreased with treatment. The findings clearly revealed a toxic effect of quercetin on *B. cucurbitae.*

Key words: flavonoids, insecticidal, development, food assimilation, *Bactrocera cucurbitae*.

Introduction

In an endeavour to find alternatives to synthetic pesticides whose adverse secondary effects on environment and human health are increasingly becoming a matter of great concern, alternatives are being explored to find compounds which are eco-friendly and less toxic for human health. Secondary compounds in plants constitute an inbuilt chemical barrier to herbivorous insects which may protect plants against herbivores and pathogens. These secondary metabolites include mainly terpenoids, alkaloids, glucosinolates and phenolics (Freeman and Beattie, 2008).

Among the plant secondary metabolites, phenolic compounds are the most common in plants. They are characterized by the presence of aromatic ring bearing one or more hydroxyl groups. They provide mechanical support and inhibit pathogenic invasions in plants (Sadasivam and Thayumanavan, 2003). In insects, phenolics show a prooxidant activity as they are oxidized to hydrogen peroxide (H_2O_2) and organic hydroperoxides (ROOH) in lumen of the gut (Barbehenn *et al*., 2005). These reactive oxygen species can inhibit the absorption of ingested nutrients and cause oxidative damage to midgut cells (Bi and Felton, 1995). Flavonoids (C6-C3-C6 group) are low molecular weight compounds and constitute the largest group of plant phenolics. They possess a wide range of biological activities and they have been recognized largely for their antioxidant, anticarcinogenic and anti-cardiovascular disease capacity (Parr and Bolwell, 2000; Hollman, 2001). They are beneficial for the plant itself as physiological active compounds, as stress protecting agents, as attractants or as feeding deterrents, and, in general, by their significant role in plant resistance (Treutter, 2006). Their effect on behaviour, development, and growth of some insects has been reported (Green *et al*., 2003; Mallikarjuna *et al*., 2004). However most of the work on phenolics is confined to Lepidopteran insects and not much is known regarding their interaction with Dipteran insects. Substantial economic damage from *Bactrocera* fruit flies which belongs to order Diptera has been documented for various Asian countries (e.g., Vijaysegaran and Osman, 1991; Stonehouse *et al*., 1998; Orankanok *et al*., 2007; Hasyim *et al*., 2008). Melon fruit fly, *Bactrocera cucurbitae* (Coquillett) is one of major pest of cucurbit crops throughout the world. It can cause 30-100% damage to crop depending upon the agroclimatic season (Dhillon *et al*., 2005). Adult fly lives for 1-3 months (Weems *et al*., 2001). Females prefer to lay eggs in young and healthy fruits (Dhillon *et al*., 2005). Larvae emerge about 24 h later, where they feed and burrow into the pulp of the host, causing considerable damage (Shahjahan *et al*., 2000). There are three larval stages, which together last about one week (Hollingsworth and Allwood, 2000). The adult fly emerges from the puparium after about one week. The early stages being voracious feeders are more susceptible to organic control strategies. The purpose of this study was to evaluate the insecticidal potential of quercetin by studying its effect on various growth and development parameters of melon fruit fly, *B. cucurbitae*.

Materials and methods

Insect rearing

The cultures of *B. cucurbitae* were maintained on pumpkin in wire mesh cages kept in the insect culture room at 25 ± 2 °C, 70-80% R.H. and a photoperiod of 10:14 L:D. For experiments, larvae were reared on artificial diet as suggested by Srivastava (1975).

Chemical used

Quercetin was purchased from Sisco Research Pvt. Limited (Mumbai) with 99.0% purity.

Bioassays

Treatment of eggs

The 0-8 h old eggs were given dipping treatment with various concentrations (0, 1, 5, 25, 125, 625 and 3125 ppm) of quercetin for 1 min. Distilled water was used as

Table 1. Percentage egg hatching (means ± S.E.) of *B. cucurbitae* when 0-8 h old eggs were treated with different concentrations of quercetin.

Concentrations (ppm)	Percentage egg hatching
Control	87.0 ± 3.74 a
1	86.0 ± 2.45 ab
5	86.0 ± 1.87 ab
25	79.0 ± 1.87 ab
125	80.0 ± 2.24 ab
625	79.0 ± 2.92 ab
3125	75.0 ± 2.24 b
F (*df* = 6)	3.26*

Means followed by the same letter within columns are not significantly different according to the Tukey test at P = 0.05; * = significant difference at P ≤ 0.05.

the control. The eggs were then placed in a Petri dish containing moist filter paper and observed for hatching at intervals of 24 h. There were 20 eggs in each Petri dish with six replications for each concentration and control and the experiment was repeated twice.

Treatment of larvae

Fresh pumpkin pieces were kept in wire mesh cages with approximately 100 gravid females for 6-8 h. These egg laden pumpkin pieces were removed from the cages and dissected after 44 (for 1st instar), 64 (for 2nd instar) and 88 h (for 3rd instar) to harvest the larvae. The harvesting was done in saline water and the larvae were washed in distilled water before transferring them into culture vials (25 O.D. × 100 mm length) containing culture medium incorporated with various concentrations of quercetin and culture medium without quercetin (control). Observations were made daily for recording the time taken for pupae formation, number of pupae formed, time taken for emergence of flies and number of flies emerged. There were six replications with 15 larvae per treatment.

Separate experiments were carried out for determining larval weight and pupal weights of the larvae. The second instar larvae (64-72 h old) were chosen for these experiments as they are voracious feeders. Mean relative growth rate (MRGR) was determined by taking larval weight before and after two days of feeding interval. Food assimilated (FA) was determined through observations made from above experiment.

MRGR was calculated by following formula (Martinez and van Emden, 2001):

$$\text{MRGR}\left(\text{mg}/\frac{\text{mg}}{\text{day}}\right) = \frac{\text{logN final weight (mg)} - \text{logN initial weight (mg)}}{\text{time (in days)}}$$

food assimilated w.r.t. control was assessed by the formula (Khan and Saxena, 1985):

$$\text{Food assimilated} = \frac{ti \times (ci - cf)}{ci} + tf - ti$$

where *ci* is initial weight of control larvae, *cf* is final weight of control larvae, *ti* is initial weight of treated larvae and *tf* is final weight of treated larvae.

Statistical analysis

The data obtained from egg hatching, time taken for pupae formation, number of pupae formed, larval and pupal weight, time taken for emergence of flies, number of flies emerged, larval weight, pupal weight, mean relative growth rate and food assimilated was subjected to one-way ANOVA (Assistat, 2012). The means were compared by the Tukey honestly difference test (P < 0.05) (Assistat, 2012).

Results and discussion

Effect on egg hatching

Reduction in egg hatching was observed when 0-8 h old eggs of *B. cucurbitae* were treated with quercetin (table 1). Egg hatching declined almost in a consistent manner with increase in concentration. Maximum decline was observed at 3125 ppm where egg hatching was reduced to 86.20% of the control. The Dipteran egg shell is comprised of vitelline membrane, waxy layer and chorion (Margaritis, 1985a). The chorion consists of several proteins which are crosslinked *via* di- and trityrosine bonds and provide the egg shell with the required hardness and elasticity. In a study conducted by Mindrinos *et al*., (1980), Margaritis (1985a; 1985b; 1985c); Keramaris *et al*. (1991), Phloroglucinol, a phenolic compound produced during the breakdown of

Table 2. Larval period and total development period (means ± S.E.) of *B. cucurbitae* when the larvae (44-48 h-old, 64-72 h-old, 88-96 h-old) were treated with different concentrations of quercetin.

Concentrations	Larval period (in days)			Total development period (in days)		
(ppm)	44-48 h old	64-72 h old	88-96 h old	44-48 h old	64-72 h old	88-96 h old
Control	8.2 ± 0.13 a	9.0 ± 0.12 ab	5.4 ± 0.30 ab	16.8 ± 0.52 a	18.1 ± 0.21 a	13.2 ± 0.21 abc
1	8.5 ± 0.13 a	8.8 ± 0.12 ab	4.3 ± 0.32 c	17.4 ± 0.41 a	18.7 ± 0.16 a	13.1 ± 0.21 bc
5	8.7 ± 0.17 a	8.4 ± 0.13 b	5.0 ± 0.22 abc	17.2 ± 0.20 a	18.0 ± 0.12 a	14.2 ± 0.23 ab
25	8.6 ± 0.25 a	8.7 ± 0.09 ab	4.3 ± 0.22 bc	17.6 ± 0.30 a	18.4 ± 0.21 a	12.7 ± 0.36 c
125	8.4 ± 0.20 a	8.8 ± 0.08 ab	4.8 ± 0.23 abc	17.7 ± 0.15 a	18.6 ± 0.19 a	13.1 ± 0.54 abc
625	8.6 ± 0.19 a	8.6 ± 0.17 b	5.6 ± 0.29 a	17.1 ± 0.19 a	18.9 ± 0.29 a	13.8 ± 0.22 abc
3125	8.5 ± 0.29 a	9.4 ± 0.26 a	5.8 ± 0.19 a	17.6 ± 0.28 a	18.2 ± 0.50 a	14.5 ± 0.16 a
F (*df* = 6)	0.63 N.S.	3.94**	6.08**	1.01 N.S.	1.63 N.S.	4.56**

Means followed by the same letter within columns are not significantly different according to the Tukey test at P = 0.05; ** = significant difference at P ≤ 0.01; N.S. = not significant.

Table 3. Larval weight, pupal weight, mean relative growth rate (MRGR) and food assimilated (FA) (means ± S.E.) of *B. cucurbitae* when the larvae (64-72 h-old) were treated with different concentrations of quercetin.

Concentrations (ppm)	Larval weight (mg)	Pupal weight (mg)	MRGR (mg/mg/days)	FA w.r.t. control (mg)
Control	7.2 ± 0.10 a	11.4 ± 0.70 a	0.4 ± 0.00 ab	-
1	7.0 ± 0.17 a	10.6 ± 0.51 ab	0.4 ± 0.00 a	8.2 ± 0.25 a
5	6.9 ± 0.21 a	10.8 ± 0.40 ab	0.4 ± 0.01 a	8.1 ± 0.23 a
25	7.0 ± 0.14 a	11.0 ± 0.25 a	0.4 ± 0.01 ab	8.2 ± 0.16 a
125	6.0 ± 0.25 b	10.7 ± 0.29 ab	0.3 ± 0.01 bc	7.1 ± 0.36 ab
625	5.3 ± 0.18 b	9.9 ± 0.16 ab	0.3 ± 0.01 c	6.5 ± 0.24 b
3125	5.4 ± 0.21 b	8.8 ± 0.41 b	0.3 ± 0.02 c	6.6 ± 0.28 b
F (*df* = 6)	18.11**	4.05**	15.71**	9.27**

Means followed by the same letter within columns are not significantly different according to the Tukey test at P = 0.05; ** = significant difference at P ≤ 0.01.

Table 4. Percentage pupation and percentage emergence (means ± S.E.) of *B. cucurbitae* when the larvae (44-48 h-old, 64-72 h-old, 88-96 h-old) were treated with different concentrations of quercetin.

Concentrations	Percentage pupation			Percentage emergence		
(ppm)	44-48 h old	64-72 h old	88-96 h old	44-48 h old	64-72 h old	88-96 h old
Control	73.3 ± 3.44 a	76.6 ± 2.85 a	77.7 ± 2.81 a	62.2 ± 3.30 a	71.1 ± 2.22 a	60.0 ± 2.98 a
1	71.1 ± 3.30 a	71.1 ± 2.22 ab	65.5 ± 2.05 ab	55.5 ± 3.72 ab	64.4 ± 1.40 ab	51.1 ± 2.22 ab
5	63.3 ± 4.13 a	66.6 ± 1.72 abc	63.3 ± 5.09 abc	50.0 ± 4.47 ab	56.6 ± 2.28 bc	41.1 ± 2.05 bcd
25	63.3 ± 2.28 a	66.6 ± 2.43 abc	61.1 ± 5.28 abc	45.5 ± 3.18 bc	52.2 ± 3.19 cd	43.3 ± 3.75 bc
125	60.0 ± 2.43 ab	60.0 ± 2.92 bcd	51.1 ± 2.81 bc	42.2 ± 2.22 bc	42.2 ± 3.30 d	33.3 ± 1.72 cde
625	45.5 ± 5.28 b	57.7 ± 2.22 cd	61.1 ± 2.05 abc	33.3 ± 3.44 c	35.5 ± 1.41 ef	30.0 ± 3.33 de
3125	45.5 ± 4.36 b	50.0 ± 3.75 d	47.7 ± 4.69 c	31.1 ± 4.10 c	27.7 ± 2.05 f	25.5 ± 3.18 e
F (*df* = 6)	8.83**	11.13**	6.77**	10.12**	43.56**	18.36**

Means followed by the same letter within columns are not significantly different according to the Tukey test at P = 0.05; ** = significant difference at P ≤ 0.01.

plant polyphenols inhibited the enzyme, Peroxidase which is a functional and structural component of the chorion involved in the hardening process. In the present study decreased egg hatching observed could be due to interference of quercetin in the protein synthesis or cross linking of proteins. Ovicidal effects of flavonoids have also been reported in eggs of the bruchid beetle (Salnuke *et al*., 2005).

Effects on development

The larval period was significantly shortened when the second and third instar larvae were treated with various concentrations of quercetin (table 2). However, at the highest concentration of 3125 ppm, a slight prolongation in larval period was observed which was delayed by only 0.4 days. In an earlier study, Saric *et al*. (2007) had perceived a decrease in the duration of the larval period of *Drosophila melanogaster* Meigen flies raised on 1.75% quercetin diet. They found that quercetin fed larvae entered metamorphosis sooner than the control ones. No significant effect of quercetin was observed in the treatment of the first instar larvae of *B. cucurbitae*. On the other hand, the total development period was delayed in all the larval instars fed on quercetin incorporated diet but the delay was significant only in the third instar larvae (88-96 h old) (table 2). Growth inhibitory and toxic effects of quercetin have also been reported in the mosquito, *Aedes aegypti* (L.) at concentrations above7 µg/ml and in mealworm beetle, *Tenebrio molitor* L. larvae (Gikonyo *et al*., 1998; Sosa *et al*., 2000). Inhibitory effects of quercetin on moulting in insects have been observed by Oberdorster *et al*. (2001). They reported that quercetin acts by significantly inhibiting ecdysone receptor (EcR) dependent gene expression. However, contrary to the present findings, quercetin was found to have no influence on the development of southern armyworm, *Spodoptera eridania* (Cramer) (Lindroth and Peterson, 1988). Nevetheless other flavonoids like flavones have been reported to delay the development in bertha armyworm*, Mamestra configurata* Walker (Onyilagha *et al*., 2004).

A significant decrease in larval weight and pupal weight was observed when the second instar larvae of *B. cucurbitae* were treated with different concentrations of quercetin (table 3). In contrast quercetin was found to have no effect on larval weight of another cucurbitaceae pest, *Epilachna paenulata* Mulsant (Diaz Napal *et al*., 2010) but inhibited the larval weight of tobacco cutworm, *Spodoptera litura* (F.) (Stevenson *et al*., 1993; Beninger and Abou-Zaid, 1997). Similar reduction in larval weights was observed in western spruce budworm, *Choristoneura occidentalis* Freeman, cabbage looper, *Trichoplusia ni* (Hubner) and crucifer pest, *M. configurata* treated with flavonoids (Zou and Cates, 1997; Hoffman-Campo, 2001; Onyilagha *et al*., 2004).

Quercetin adversely affected the percentage pupation and percentage emergence as it declined in all the trea-

ted larval instars (table 4). However a dose dependent decline in pupation and emergence was observed only in first and second instar larvae. Maximum inhibitory effect of quercetin was noticed in the third instar larvae where the pupation was inhibited by 38.57%. On the other hand, maximum decrease in percentage emergence was perceived in the treatment of second instar larvae where the emergence was inhibited by 60.95% as compared to control. Our findings get support from the work of Atyyat *et al*. (2012) who had reported an increase in mortality of the nymphs of the woolly apple aphid, *Eriosoma lanigerum* (Hausmann) with increase in the concentration of the flavonoid, quercetin. Peric-Mataruga *et al*. (2001) had also demonstrated a high mortality of the first instar caterpillars of gypsy moth, *Lymantria dispar* (L.) from oak forest reared on 1% and 1.5% quercetin.

Effect on nutritional indices

Quercetin treatment caused a significant reduction in MRGR in 64-72 h hold larvae which decreased to 75% of the control at the highest concentration of 3125 ppm (table 3). The observations made for food assimilated showed that larvae fed on diet incorporated with quercetin were not able to assimilate food in a manner as larvae fed on control diet as a consistent decline with increase in concentration was observed. The oxidation of quercetin in insect body generates reactive oxygen species (ROS) such as superoxide anion, hydrogen peroxide and free radicals (e.g. •OH and semiquinone) (Ochiai *et al*., 1984, Hodnick *et al*., 1986). These reactive oxygen species can degrade the nutritional quality of food present in gut lumen of insect (Barbehenn *et al.,* 2005) which could have decreased the food assimilation in quercetin treated larvae and adversely affected the growth of the melon fruit fly.

Conclusions

The deterrent effects of quercetin on various growth parameters of *B. cucurbitae* reveal the potential of quercetin for use as a biopesticide against insect pests.

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