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Bioefficacy of rutin against *Bactrocera cucurbitae* (Diptera: Tephritidae), an economically important pest of cucurbits

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Abstract

Diurnal *Bactrocera cucurbitae* (Coquillett) is a major pest of cucurbits and other vegetable crops. In an endeavour to find eco-friendly methods to prevent damage by this pest, laboratory studies were conducted to determine the effects of rutin, a flavonoid on *B. cucurbitae.* Rutin significantly decreased the mean relative growth rate and food assimilation as compared to control larvae. The larvae on treated diet gained significantly less weight as compared to those fed on control diet. The number of first and second instar and the emergence of adults, from larvae fed on treated diet, significantly decreased and that decrease correlated directly with increasing concentration of the rutin. Abnormalities, in the form of malformed adults with crumpled wings were also observed. The findings revealed a possible toxic effect of rutin on *B. cucurbitae.*

Keywords: abnormalities, cucurbitaceae, flavonoid, food assimilation, growth rate

Introduction

Inorganic pesticides pose severe human health problems and other hazards to environment. Consequently, there has been a growing interest in seeking alternative pest management strategies which are safe and less toxic to humans. Plants, being stationary organisms, have evolved a wide array of secondary metabolites which may act as plant's primary defense against herbivory (Harborne, 1994). Plant phenolics are a large group of secondary metabolites in plants that affect the larval growth and development of herbivores either by feeding inhibition, or in post ingestion phenomena (Treutter, 2006). Among the phenolic compounds, flavonoids occur widely in plants and possess wide range of biological activities. In plants, they are important for growth and development, attraction of pollinator animals, nitrogen fixation in leguminous plants, and for protection against damage by herbivores, microbes, UV and reactive oxygen species (Ndakidemi and Dakora, 2003; Matkowski and Wolniak, 2005; Treutter 2006; Makoi and Ndakidemi, 2007). In insects, their antibiotic and antifeedant effect has been demonstrated in a number of studies where they delayed the development and reduced the survivorship of herbivorous insects (Duffey *et al.*, 1986; Stamp, 1990; Jadhav *et al.*, 2012). Flavonoids affect the growth of insects by undergoing chemical alteration in the gut of the insect body (Elliger *et al.*, 1980). Rutin, a prominent flavonoid in plants, has been shown to have growth inhibitory effects on a number of agricultural pests (Shaver and Lukefahr, 1969; Elliger *et al.*,1981; Isman and Duffey, 1982; Salvador *et al.*, 2010; Jadhav *et al.*,2012). In the insect gut the glycosidic bonds in rutin are hydrolysed releasing the flavonoid quercetin. Once quercetin is liberated in the gut, it could inhibit the cytochrome P_{450} dependent ecdysone 20 Mono-oxygenase activity (Mitchell *et al.*, 1993). Quercetin has also been reported to inhibit the activity of mitochondrial ATPase (Lang and Racker, 1974) and phosphodiesterase (Beretz *et al.*, 1978).

The genus *Bactrocera* contains approximately 440 species distributed primarily in Southeast Asia, the South Pacific, and Australia (White and Elson-Harris, 1992). The melon fly, *Bactrocera cucurbitae* (Coquillett) is a major pest of cucurbitaceous vegetables and fruits. It can cause 30–100% damage to crop depending upon the agroclimatic season (Dhillon *et al.,* 2005). In India, this species generally infests a large number of melons and wild cucurbits to a relatively lesser extent though, sometimes serious damage is reported (Bhatia and Mahto, 1968; Viraktamath *et al.*, 2003). Keeping in mind the antibiosis effect of rutin on some insect pests, the present study was envisaged to explore the effect of the flavonoid on the growth and development of *B. cucurbitae.*

Methods

Rearing of insects

The melon fruit fly was procured from the infested bitter gourd, *Momordica charantia* L. collected from the vegetable market of Amritsar and kitchen gardens of Guru Nanak Dev University Campus, Amritsar. The flies were identified on the basis of taxonomic characters given by Kapoor (1993) and reared according to the procedure described by Gupta et al. (1978). Adult flies were kept in wire mesh cages (Rescholar equipment; L45× B45× H50cm) and were

provided with proteinex (Pfizer India) and 20% sugar solution as food. The pumpkin fruit, *Cucurbitae moschata* Dusch was placed in the
cages for oviposition and also served as natural food for larvae. The cultures of *B. cucurbitae* were maintained in insect culture room under controlled conditions with temperature maintained at 25±2°C, 70–80% R.H. and L10: D14 photoperiod. For experiments, larvae were reared on artificial diet as suggested by Srivastava (1975).

Treatment of larvae

In order to obtain larvae of the same age, medium sized 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 h (for 1st instar), 64 h (for 2nd instar) and 88 h (for 3rd instar) to harvest the larvae. The harvesting was done in saline water (4ml) 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 (1ppm, 5ppm, 25ppm, 125ppm, 625ppm, 3125ppm) of rutin and culture medium without rutin (control). Rutin was purchased from Himedia Laboratories Pvt. Limited (Mumbai) with 90.0% purity. Observations were made daily for recording the number of pupae formed, number of flies emerged and abnormalities (such as malformed adults with distorted or crumpled wings or half emerged adults) in pupae and adults. There were six replications with 15 larvae per treatment.

In another experiment the larval weight and the weight of the pupae formed were determined by feeding second instar larvae (64–72h old) in diet incorporated with different concentrations of rutin. 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 Emden, 2001):

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

Food assimilated with respect to 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 was subjected to one-way ANOVA with the help of SPSS version 16.0 (I.B.M. Corporation, Chicago). The means were compared by the Tukey honestly significant difference ($P < 0.05$) using Assistat (7.6) (Brazil).

Results and Discussion

Growth and development of *B. cucurbitae* was significantly affected with rutin incorporated in artificial diet. Rutin significantly affected the survival of the first (44–48h old) and second instar larvae (64–72h old) as both percentage pupation and adult emergence decreased significantly ($P<0.01$) in a concentration dependent manner (Table 1, 2). The inhibitory effect of rutin was more in the second instar larvae (64–72h old) where the pupation was inhibited by 57.57% at the highest concentration of 3125ppm. In an earlier study, Shaver and Lukefahr (1969) had reported reduced pupation in tobacco budworm, *Heliothis virescens* (F.), neonate bollworm, *Heliothis zea* (Boddie) and pink bollworm, *Pectinophora gossypiella* (Saunders) after the larvae were fed on rutin incorporated diet. Lindroth and Peterson (1988) had also observed 50% mortality in the fifth instar larvae of southern armyworm *Spodoptera eridania* (Cramer) fed on concentrations of rutin higher than 2.2 %. However, the pupation and emergence of the third instar larvae of *B. cucurbitae* was not affected by rutin treatment. This could be a stage specific response of the insect to rutin.

Table 1. Percentage pupation (means ± S.E.) of *Bactrocera cucurbitae* when the larvae were treated with different concentrations of rutin

Concentrations (ppm)	Age of larvae		
	44–48h old	64–72h old	88–96h old
Control	78.89±2.05^{a}	85.55±4.36^{a}	80.00±1.72^{a}
1	78.89±2.68^{a}	81.11±3.18^{a}	81.11±4.01^{a}
5	74.44±2.05^{ab}	81.11±3.18^{a}	80.00±3.85^{a}
25	66.66±2.43^{bc}	78.89±6.30^{ab}	80.00±6.21^{a}
125	65.55±2.05^{bc}	72.22±4.69^{ab}	78.89±2.05^{a}
625	57.77±3.30^{cd}	72.22±5.28^{ab}	78.88±4.69^{a}
3125	47.77±2.05^{d}	60.00±4.55^{b}	77.77±4.77^{a}
F(*df*=6)	22.76**	3.40**	0.07$^{N.S.}$

** = significant at 1%, 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

MRGR, food assimilation and weight gain by the second instar larvae were adversely affected with treatment (Table 3). The larvae (64–72h old) of *B. cucurbitae* were unable to grow on diet containing different concentrations of rutin. This was evident from the decrease in MRGR which reduced to a maximum of 48.67% at 625ppm.

Table 2. Percentage emergence (means ± S.E.) of *Bactrocera cucurbitae* when the larvae were treated with different concentrations of rutin

Concentrations (ppm)	Age of larvae		
	44–48h old	64–72h old	88–96h old
Control	67.77±2.05[a]	73.33±3.85[a]	66.66±2.98[a]
1	65.55±3.62[a]	70.00±3.75[ab]	62.22±3.72[a]
5	64.44±1.40[a]	70.00±1.49[ab]	64.44±1.40[a]
25	53.33±2.44[b]	65.55±4.69[ab]	66.66±2.98[a]
125	51.11±2.22[b]	56.66±2.85[b]	64.44±4.45[a]
625	43.33±1.49[bc]	36.66±3.33[c]	58.89±3.18[a]
3125	38.89±2.05[c]	31.11±2.81[c]	66.66±2.98[a]
F(*df*=6)	24.82**	25.58**	0.81[N.S.]

**=significant at1%, 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

Similar findings have been reported in the penultimate instar of *S. eridania* and the second instar of gypsy moth, *Lymantria dispar* L. where rutin significantly reduced the growth of larvae (Lindroth and Peterson, 1988; Beninger and Abou-Zaid, 1997). The reduced growth of *S. eridania* was attributed to reduced food uptake and food digestibility. Rutin also reduced the growth of *H. zea* and tobacco leaf eating caterpillar, *Spodoptera litura* (F.) neonates but not that of the third or fifth instar. (Elliger *et al.*, 1980; Ghumare *et al.*, 1989). Also, the 64–72h old larvae of *B. cucurbitae* fed on rutin incorporated diet gained less weight as compared to those fed on control diet. The decrease in larval weight could be due to less assimilation of diet containing rutin as the food assimilated by the larvae decreased with treatment. Hoffman-Campo *et al.* (2001) had also observed a decrease in the weight of the cabbage looper, *Trichoplusia ni* (Hübner) larvae when fed on higher concentrations of rutin (1% and 2%). The inhibitory effect of rutin on weight gain was also observed in the third instar larvae of *S. litura* when they were fed on diet containing rutin at or up to 2.5 mol/m^3 (Stevenson *et al.*, 1993). The toxic effect of rutin was further evident from the abnormalities observed in the emerged adults when the larvae of different age groups were treated and were in the form of crumpled wings. The abnormalities were found to increase at higher concentrations and were significantly more ($p<0.01$) in the first instar larvae (44–48h old) (Table 4, Figure 1a, b, c).

Flavonoids have been found to alter moulting in insects, causing death (Stamp and Yang, 1996). Most of the flavonoids either act as anti-estrogen or inhibit cytochromeP450 isozyme expression and activity (Mitchell et al., 1993; Tsyrlov *et al.*, 1994). The inhibition of P_{450} dependent mixed function oxidases (MFO) is damaging to early instars since MFO activity

levels are low in early instars (Ahmad, 1986) but are essential to detoxify flavonoids as well as other allelochemicals. Simmonds (2001) suggested that this inhibitory activity could be the reason for the detrimental effects on the development of the early stadia larvae of *H. zea* (Isman and Duffey, 1982), *S. litura* (Stevenson *et al*., 1993) and *L. dispar* (Beninger and Abou-Zaid, 1997) whereas the later and larger stadia of these species consume rutin enriched diets with no adverse effects.

Table 3. Mean relative growth rate (MRGR), food assimilated (FA) w.r.t. control and larval weight (means ± S.E.) of *Bactrocera cucurbitae* when 64–72h old larvae were treated with different concentrations of rutin.

Concentrations (ppm)	MRGR (mg/mg/days)	FA w.r.t. control (mg)	Final larval weight (mg)
Control	0.35±0.04^{a}	-	7.84±0.24^{a}
1	0.25±0.03^{ab}	5.71±0.29^{a}	6.32±0.35^{b}
5	0.20±0.02^{ab}	4.36±0.37^{abc}	4.87±0.33^{cd}
25	0.19±0.02^{b}	4.34±0.27^{bc}	4.85±0.27^{cd}
125	0.30±0.03^{ab}	5.45±0.30^{ab}	5.96±0.35^{bc}
625	0.17±0.04^{b}	3.89±0.43^{c}	4.39±0.35^{d}
3125	0.21±0.01^{ab}	4.44±0.165^{abc}	4.95±0.14^{cd}
F(*df*=6)	3.75**	5.03**	15.64**

**= significant at 1%. Means followed by the same letter within columns are not significantly different according to the Tukey test at P = 0.05; w.r.t. = with respect to control

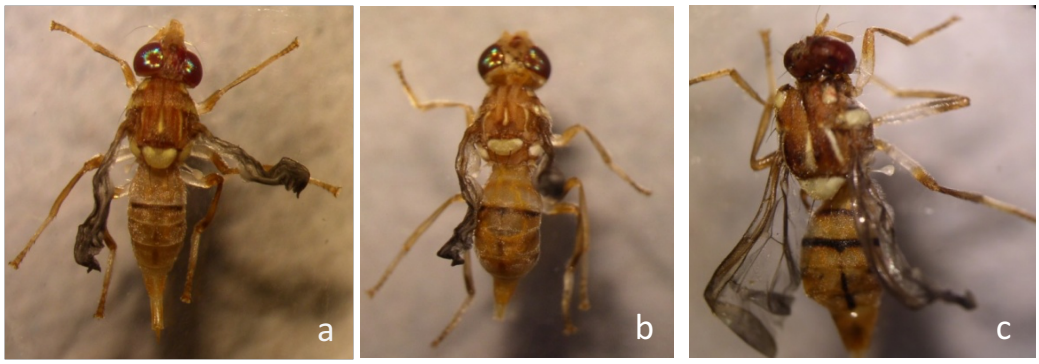

Figure 1. a, b, c. Abnormalities (in the form of crumpled wings) in flies of *B. cucurbitae* emerged after treatment of the larvae of different age groups with rutin (Magnification10X×4X)

Table 4. Percentage abnormalities (means ± S.E.) of *Bactrocera cucurbitae* when the larvae were treated with different concentrations of rutin.

Concentrations (ppm)	Age of larvae		
	44–48h old	64–72h old	88–96h old
Control	4.68±3.14^{c}	3.24±2.08^{a}	5.18±2.32^{b}
1	6.50±3.18^{bc}	3.51±2.23^{a}	8.73±3.38^{ab}
5	5.18±2.32^{c}	3.18±2.02^{a}	10.71±4.04^{ab}
25	8.16±2.63^{abc}	6.46±2.09^{a}	10.09±0.41^{ab}
125	11.30±2.29^{abc}	7.45±2.38^{a}	8.93±1.83^{ab}
625	21.02±5.37^{a}	13.21±4.40^{a}	21.09±5.36^{a}
3125	20.15±2.78^{ab}	17.50±6.29^{a}	18.71±2.70^{ab}
F(*df*=6)	4.50**	2.63*	3.20*

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

The MFO activity levels increase as the age of the larvae and instar increases (Ahmad, 1986). In the present findings also, the adverse effects of rutin were more evident in the treatment of the first and second instar larvae of *B. cucurbitae* while the third instar larvae of *B. cucurbitae* was not affected. This could be due to decreased detoxification capacity of the early instars whereas the third instar larvae might have had enough MFO to detoxify the phenolic compound. The present findings thus reveal the potential of rutin to inhibit the development of *B. cucurbitae*.

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