Development inhibitory effect of *Acacia auriculiformis* extracts on *Bactrocera cucurbitae* (Coquillett) (Diptera:Tephritidae)

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ABSTRACT

The effect of acetone and water extracts of *Acacia auriculiformis* A. Cunn. bark was investigated on biology of *Bactrocera cucurbitae* (Coquillett). The eggs, larvae and adults were treated with different concentrations (1, 5, 25, 125 and 625 ppm) of the bark extracts. Both the extracts significantly prolonged the larval period and total developmental period. Percentage pupation, percentage emergence, oviposition and egg hatching decreased in a dose dependent manner. The LC_{50} values were lower for the acetone extract as compared to water extract indicating that acetone extract was more toxic than water extract. The present findings demonstrated the potential of the plant extracts in controlling melon fruit fly.

Keywords: *Acacia auriculiformis*, plant extracts, *Bactrocera cucurbitae*, development.

INTRODUCTION

Plants contain thousands of compounds which are virtually an untapped reservoir of pesticides that can be used directly or as templates for synthetic pesticides. India being a tropical country has a vast resource of plants, a large number of which have not been screened for their pesticidal properties. *Acacia auriculiformis* A. Cunn (Fabaceae) is an important medicinal plant. It has a rich source of tannins and terpenoids along with polyphenols (Singh and Sehgal, 2001). It's antimutagenic, cytotoxic and antioxidant activities have been reported (Singh *et al*., 2007a, 2007b, 2007c). However, it has not been explored for its pesticidal potential.

Bactrocera cucurbitae is distributed widely in temperate, tropical and sub-tropical regions of the world but India is considered as its native home (Dhillon *et al.*, 2005). It damages over 81 plant species and plants belonging to the family Cucurbitaceae are preferred most which include fruits of bitter gourd (*Momordica charantia*), muskmelon (*Cucumis melo*), snap melon (*Cucumis melo* var. *momordica*) and snake gourd (*Trichosanthes anguina* and *T. cucumeria*) (Sapkota *et al*. 2010). Plant products (Shivayya and Kumar, 2008), *Arisema* sp. (Kaur 2009) Imapact of methoprene (Ihsan ul Hag *et al.* 2010) on this pest was reported. Therefore, in the present study it was envisaged to investi gate the effect of the extracts of the plant on the develop ment of the melon fruit fly, *Bactrocera cucurbitae* (Coquillett).

MATERIALS AND METHODS

Plant material and extract preparation

A.auriculiformis procured in the month of September was identified by comparing it with the specimen available in the herbarium (Voucher No. 6422) of the Guru Nanak Dev University. The fresh bark of *A. auriculiformis* was selected, washed and dried in oven at 30^0C overnight and ground to fine powder with grinder. The extracts of the bark powder were prepared by dissolving the bark powder in acetone and water and these were concentrated in rotary vacuum evaporator. The dried extracts were weighed and preserved in sealed bottles in a refrigerator after making stock solutions of the required concentrations. The desired amount of stock solution required for making various test concentrations for the experiment was calculated as given below: Amount of stock solution required (ml) = Concentration required/concentration of the stock solution X Amount of diet (ml).

Insect

The infested bitter gourd, *Momordica charantia* (L.) and guava, *Psidium guajava* L. were collected from the vegetable market and scanned for melon fruit fly, *B. cucurbitae*. The flies were identified on the basis of criteria given in the taxonomic keys (Kapoor,1993; White and Elson-Harris, 1992). The cultures of *B. cucurbitae* were maintained in insect culture room under a constant photoperiod (10L: 14D), temperature (28±20°C) and relative humidity (70-80%). They were fed on a diet of sugar solution and protinex incorporated with vitamin E.

Experiments with eggs

About 100 gravid females were released for 6-8 h in a wire mesh cage, in which 3-4 pumpkin pieces were placed for oviposition. The pumpkin pieces were removed and the top layer of the pumpkin flesh was teased with the help of

scalpel and a needle in the saline water. The eggs were collected from water with the help of camel hair brush, washed in distilled water and placed on the sterilized filter paper to soak the extra water. These eggs were dipped in 5ml solution of various test concentrations of bark extracts for one minute. The eggs were dried on filter paper and transferred to petridishes with moist filter paper. These Petri dishes were kept in B.O.D. incubator and observed at a dial periodicity for egg hatching. All the five concentrations of bark extracts had six replications with 20 eggs in each replication.

Experiments with larvae

The first instar, second instar and third instar larvae were collected after 48-56 hours, 64-72hours and 88-96 hours respectively and used for the experiments. The larvae of same age were procured by releasing around 100 gravid females in a cage for oviposition on pumpkin pieces for an interval of 6 to 8 hours. The larvae were transferred to artificial diet containing different concentrations of the plant extracts along with control. There were six replications for each concentration of all the extracts, each having 15 larvae for each experiment. Observations were made for larval period, pupal period, total developmental period, percentage pupation and percentage of adult emergence.

Experiments with adults

Pumpkin pieces (2.5½×1.5½×1.25½) cleaned and incubated for 48h at 27±1°C and 65±5% R. H. were dipped in different concentrations of extracts for 30 seconds and allowed to dry at room temperature. Five pairs of 20 days old flies were transferred to small wire cages (15×15×16 cm). These flies were provided with protinex and sugar solution as their food and plant extract impregnated pumpkin pieces for oviposition. Water treated fruit served as control. Three replications were provided for each treatment including control. Pumpkin pieces were removed after 6 hours and dissected under a binocular microscope to count the number of eggs laid by the female flies.

Statistical analysis

The data was subjected to one way analysis of variance (ANOVA), using SPSS (10.0 for window) computer software programme to assess the significance of observations on the development period, pupation and emergence of *B. cucurbitae*. The LC_{50} value was calculated for adult emer gence through probit analysis (Finney, 1971) and the means were separated using Tukey HSD test (Assistat v7.5beta).

RESULTS AND DISCUSSION

In tropical countries having a rich biodiversity of plants, botanical pesticides are fast emerging as a viable component of integrated pest management. The toxic effect of plant extracts is due to the synergistic effect of secondary compounds which are postulated to have evolved for the plant's defense. These compounds confer protection to crops through reduction of fitness to insect herbivores. Although the plant extracts may be less toxic than the conventional pesticides (Koul *et al.*, 2008) but they are relatively safe and ecofriendly.

Eggs

From the present study it was evident that extracts of *A. auriculiformis* had an adverse effect on the development of *B. cucurbitae*. A concentration dependent decreased egg hatching was observed when melon fly eggs were treated with different concentrations of *A. auriculiformis* acetone and water extracts. Between the two extracts, acetone highly reduced the egg hatching (45.75%) than water extract (49.15%) at 625ppm (Figure 1). Reduced egg hatching in *B. cucurbitae* has also observed by Nair and Thomas (2001) after treatment of eggs with different concentrations of *Acorus calamus* L. (Acoraceae) extracts. Similar impact was reported in peach fruit fly, *Bactrocera zonata* (Saunders) (Mahmood and Shoeib, 2008) and western cherry fruit fly (Renden *et al.*, 1998). The egg shell in Diptera is comprised of vitelline membrane, waxy layer and chorion (Margaritis, 1985a). The chorion consists of several proteins which are crosslinked *via* di- and tri-tyrosine bonds and provide the egg shell with the required hardness and elasticity. The enzyme, Peroxidase which is a functional and structural component of the chorion involved in the hardening process is inhibited by Phloroglucinol, a phenolic compound produced during the breakdown of plant polyphenols (Mindrinos *et al*., 1980; Margaritis, 1985a, 1985b, 1985c; Keramaris *et al*., 1991). Decreased egg hatching observed in the present study could be attributed to the secondary compounds particularly polyphenols present in the bark extracts of *A. auriculiformis* which might have interfered in the protein synthesis or cross linking of proteins.

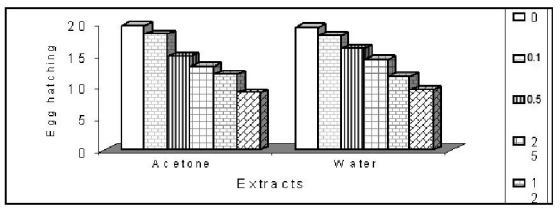

Figure 1. Effect of extracts of *A. auriculiformis* on egg hatching of *B. cucurbitae*

Larvae

The larval period and total developmental period of the insect prolonged in the first instar and second instar

Table 1. Larval Period of *B. cucurbitae* when first instar, second instar and third instar larvae were treated with different extracts of *A. auriculiformis*.

Concentrations (ppm)	Remaining Larval Period when different age groups were treated					
	First instar		Second instar		Third instar	
	acetone extract	water extract	acetone extract	water extract	acetone extract	water extract
00	20.25±0.66^{b}	20.36±0.39^{d}	6.58±0.33^{e}	6.99±0.22^{b}	3.55±0.14^{a}	3.37±0.11^{a}
01	22.91±0.25^{c}	21.38±0.25^{c}	8.63±0.35^{d}	7.01±0.14^{b}	2.66±0.14^{b}	3.15±0.04^{b}
05	24.38±0.28^{b}	22.23±0.18^{b}	9.66±0.26^{c}	7.21±0.20^{b}	2.53±0.09^{bc}	3.07±0.07^{b}
25	25.50±0.47^{a}	22.37±0.23^{ab}	10.64±0.25^{b}	7.55±0.24^{ab}	2.42±0.06^{bc}	2.86±0.05^{c}
125	24.92±0.24^{ab}	22.97±0.24^{a}	11.03±0.18^{b}	7.82±0.18^{a}	2.27±0.07^{cd}	2.75±0.04^{c}
625	0.00±0.00	0.00±0.00	11.86±0.18^{a}	8.08±0.25^{a}	2.02±0.07^{d}	2.28±0.08^{d}
F-value	24.48**	14.88**	44.99**	4.12**	25.38**	30.64**

** Significant at 1% level ; Means within a column followed by the same letter are not significantly different according to the Tukey test (P=0.05).

larvae, however it was reduced in the third instar larvae (Tables 2 and 3). This could be a stage specific response of the insect to the plant extracts. All first instar larvae were dead at 625 ppm concentration of both the extracts. As observed in eggs, acetone extract has more impact than water extract on larvae too. In a study conducted by Mustafa and Al-Khazraji (2008), delayed larval development of the mosquito, *Culex pipiensmolestus* Forskal was observed with the extracts of *Azadirachta excelsa* Jack. Previously, 12 different New Zealand gymnosperm species extracts were tested against larvae of Australian sheep blowfly, *Lucilia cuprina* (Wiedemann) and results showed that extracts significantly reduced larval and developmental period (Gerard *et al*., 1997).

Pupa formation and emergence was adversely affected in the larvae of the melon fly treated with bark extracts of *A. auriculiformis* (Tables 4 and 5). A very few number of larvae pupated after ingesting the treated food. Statistically significant effects were observed in all the treated instars with both acetone and water extract. The LC_{50} values calculated on the basis of adult emergence was lower in all the three instars with acetone extract as compared to water extract indicating that acetone extract was more toxic to the larvae of the melon fruit fly. Toxic effects of other species of *Acacia* has been reported against *Aedes aegypti* (L.) and *Culex quinquefasciatus* (Say) mosquito larvae (Tauro *et al*., 2004; Chaubal *et al*., 2005), *C. quinquefasciatus, A. stephensi* and *A. aegypti* (Sakthivadivel and Daniel, 2008). Larvicidal effects of *Pavonia zeylanica* and *Acacia ferruginea* have been reported in the late third instar larvae of *C. quinquefasciatus* (Vahitha *et al.*, 2002). Other plant extracts such as *Parthenium hysterophorus, Peganum harmala* L., *Sawsswera lappa* C. B. Klarke and *Valariana*

Table 2. Total development period of *B. cucurbitae* when first instar, second instar and third instar larvae were treated with different extracts of *A. auriculiformis*.

Concentrations (ppm)	Total development period when different age groups were treated					
	First instar		Second instar		Third instar	
	acetone extract	water extract	acetone extract	water extract	acetone extract	water extract
00	26.59±0.54^{d}	27.47±0.46^{e}	14.89±0.24^{e}	14.42±0.19^{e}	13.27±0.08^{a}	11.75±0.04^{a}
01	30.11±0.29^{c}	28.67±0.19^{d}	18.09±0.36^{d}	15.55±0.32^{d}	12.88±0.12^{b}	11.45±0.10^{a}
05	32.33±0.26^{b}	30.56±0.38^{c}	19.32±0.25^{c}	15.88±0.28^{cd}	12.59±0.07^{b}	11.07±0.07^{b}
25	33.14±0.20^{ab}	32.25±0.31^{b}	20.24±0.31^{b}	16.36±0.28^{bc}	12.16±0.11^{c}	10.81±0.11^{bc}
125	33.75±0.17^{aa}	33.50±0.37^{a}	21.09±0.20^{ab}	17.05±0.16^{b}	11.72±0.11^{d}	10.71±0.10^{c}
625	0.00±0.00	0.00±0.00	21.87±0.37^{a}	17.93±0.22^{a}	11.28±0.11^{e}	10.29±0.19^{d}
F-value	84.92**	79.33**	103.33**	27.13**	47.39**	24.93**

** Significant at 1% level ; Means within a column followed by the same letter are not significantly different according to the Tukey test (P=0.05).

Table 3. Percentage pupation of *B. cucurbitae* when first instar, second instar and third instar larvae were treated with different extracts of *A. auriculiformis*.

Concentrations (ppm)	Percentage Pupation					
	First instar		Second instar		Third instar	
	acetone extract	water extract	acetone extract	water extract	acetone extract	water extract
00	41.67±3.07[a]	35.00±2.24[a]	83.33±5.58[a]	85.00±2.24[a]	86.67±2.11[a]	90.0±2.58[a]
01	36.67±3.33[a]	30.0±2.58[ab]	73.33±2.11[b]	78.33±3.07[ab]	76.67±2.11[b]	78.33±3.07[b]
05	35.00±2.24[a]	28.33±3.07[ab]	66.67±3.33[b]	71.67±3.07[bc]	73.33±3.33[b]	68.33±3.07[c]
25	25.00±2.24[b]	25.00±2.24[bc]	56.67±3.33[c]	66.67±3.33[cd]	51.67±1.67[c]	56.67±2.11[d]
125	16.67±2.11[c]	20.00±2.58[c]	53.33±2.11[c]	60.00±2.58[d]	45.00±2.24[c]	53.33±2.11[de]
625	0.00±0.00	0.00±0.00	48.33±3.07[c]	46.67±2.11[e]	45.00±2.24[c]	48.33±1.67[e]
F-value	14.76**	4.06**	15.51**	22.57**	52.24**	40.48**

officianalis L.were also found to have an inhibitory effect on percentage pupation and emergence of *B. cucurbitae*, *B. zonata*, western cherry fruit fly, *Rhagoletic indifferens* Curran (VanRenden and Roiteberg, 1998; Sohal *et al.*, 2003; Jilani *et al*., 2006; Khattak *et al*., 2006). The diethyl ether extract obtained from fresh lemon peel was reported to cause 98.8% larval mortality in the larvae of mediter ranean fruit fly, *Ceratitis capitata* (Salvatore *et al*., 2004). It is apparent from the present study that the *A. auriculi formis* bark extracts possess potent alleloch emicals which could be exploited for management of fruit flies.

Adults

The extracts from the bark of *A. auriculiformis* had a deterrent effect on the oviposition of the melon fruit fly, *B. cucurbitae*. The extracts significantly reduced the number of eggs laid by the adult flies (Figure 2). The effect was more severe in the flies treated with the acetone extract where no egg laying was observed at the highest concentration of 625ppm. Singh and Singh (1998) had observed deterrent effects of neem seed kernel extracts and Azadirachtin against *B. cucurbitae* and Oriental fruit fly, *Bactrocera dorsalis* Hendel. Oviposition deterrent effect of neem extracts on *D. cucurbitae* has also been observed by Singh and Srivastva (1983). Similar findings have been reported in *B. dorsalis* after the insect was treated with neem extracts (Areekul *et al*., 1988; Chen *et al*., 1996). Mohmood and Shoeib (2008) revealed a high effect of neem formulation in repelling oviposition in *B. zonata*.

Table 4. Percentage emergence of *B. cucurbitae* when first instar, second instar and third instar larvae were treated with different extracts of *A. auriculiformis*.

Concentrations (ppm)	Percentage emergence					
	First instar		Second instar		Third instar	
	acetone extract	water extract	acetone extract	water extract	acetone extract	water extract
00	38.33±3.07[a]	30.0±2.58[a]	71.67±4.77[a]	73.33±2.11[a]	76.67±3.33[a]	80.0±2.58[a]
01	26.67±3.33[b]	25.0±2.24[ab]	51.67±3.07[b]	60.00±2.58[b]	70.0±2.58[ab]	71.67±1.67[b]
05	25.0±2.24[b]	23.33±2.11[bc]	45.00±2.24[bc]	58.33±3.07[bc]	66.67±4.22[b]	63.33±2.11[c]
25	16.67±3.33[c]	18.33±1.67[cd]	43.33±2.11[bc]	51.67±3.07[cd]	48.33±3.07[c]	51.67±3.07[d]
125	11.67±1.67[c]	15.0±2.24[d]	38.33±3.07[cd]	46.67±2.11[d]	36.67±2.11[d]	45.0±2.24[d]
625	0.00±0.00	0.00±0.00	33.33±4.22[d]	35.00±2.24[e]	36.67±2.11[d]	33.33[e]±2.11[e]
F-value	13.56**	6.21**	14.79**	29.66**	31.74**	49.14**
LC50(ppm)	19.5	57.02	251.2	354.8	100	229.1

.** Significant at 1% level ; Means within a column followed by the same letter are not significantly different according to the Tukey test (P=0.05).

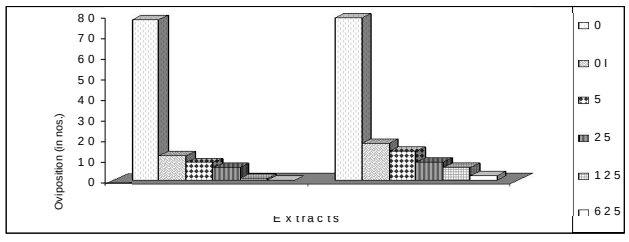

Figure 2. Effect of extracts of *A. auriculiformis* on the oviposition of *B. cucurbitae*.

The present study clearly revealed the susceptibility of *B. cucurbitae* to both the extracts of *A. auriculiformis* as indicated by their deleterious effects on growth and development of melon fruit fly. Further studies are being undertaken to isolate and identify the bioactive compounds from the most potent extract and evaluate its effect on the melon fruit fly.

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