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EFFICACY OF SOME BOTANICALS AS INSECTICIDES ON THE MELON FLY, *BACTROCERA CUCURBITAE* (COQUILLETT) (DIPTERA : TEPHRITIDAE)

S. Sultana, A.F.M. Aslam*, F.A. Ahmed[1] and A.J. Howlader

Department of Zoology, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka-1342, Bangladesh.

Abstract: Efficacy of methanolic extracts of different indigenous plant parts, such as the leaves and barks of neem (*Azadirachta indica*); leaves of basil (*Ocimum basilicum),* mahua (*Madhuca indica*), lantana (*Lantana camara*), eucalyptus *(Eucalyptus globulus)*, surjokonna (*Spilanthes acmella)* and flowers of golden shower (*Cassia fistula*), rosy periwinkle (*Catharanthus roseus*) and orchid (*Mokara charkuan)* were tested against the oviposition behavior of the melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera : Tephritidae). Most effective botanicals as attractants were observed in *C. fistula* and *M. indica.* In choice test, the percentage of *B. cucurbitae* pupae emerged were 0.0 in the leaves and barks of *A. indica,* 0.22 in flowers of *M. charkuan,* 1.43 in leaves of *O. basilicum,* 2.68 in leaves of *L. camara,* 6.83 in flowers of *C. roseus,* 5.51 in leaves of *S. acmella*, 13.27 in leaves of *E. globules*, 19.99 in flowers of *C. fistula* and 14.09 in leaves of *M. indica.* In no-choice test, the respective percentages of *B. cucurbitae* pupae emerged were 0.0, 2.19, 2.29, 11.68, 10.65, 12.34, 7.73, 6.76, 13.66 and 15.16 for the leaves of *A. indica,* bark of *A. indica,* in flowers of *M. charkuan,* in leaves of *O. basilicum,* in flowers of *L. camara*, in flowers of *C. roseus,* in leaves of *S. acmella*, in leaves of *E. globules*, in flowers of *C. fistula* and in leaves of *M. indica.* Phytochemical screening of the methanolic crude extracts of the botanicals showed that *M. indica* was strongly positive for steroid; *C. fistula* were strongly positive for alkaloid, flavonoids and glycoside and *M. charkuan* was weakly positive for carbohydrates, alkaloid and steroid.

Key words: Efficacy, insecticidal properties, botanicals, *Bactrocera cucurbitae.*

INTRODUCTION

The melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is regarded as one of the most important global pest of quarantine importance. It is distributed widely in temperate, tropical, and sub-tropical regions of the world (Dhillon *et al.* 2005). More than 125 species of fruits and vegetables mostly belonging to the family Cucurbitaceae and Solanaceae have been recorded as hosts of *B. cucurbitae.* The extent of losses varies between 30% to 100%, depending on the cucurbit species and the season (Sapkota *et al.* 2010). Generally, the females prefer to lay eggs in soft tender fruit tissues by piercing

*Author for correspondence. [1]Department of Botany, Faculty of Biological Sciences, Jahangirnagar University, Savar, Dhaka – 1342, Bangladesh.

piercing them with the ovipositor. Larval feeding in the fruits is the most damaging, as a result, young fruits become distorted and usually dropped. The larval tunnels provide entry points for bacteria and fungi that cause the fruit to rot. Because of the high egg laying capacity and mobility, each female is capable of destroying large numbers of fruit in her lifetime. In Bangladesh, *B. cucurbitae* represents 74% of the total number of flies infesting different vegetables growing areas (Akhtaruzzaman *et al.* 1999). The melon fly damages 10-30% mangoes, star fruits and guava, and average 30-40% vegetables in Bangladesh every year (Wadud *et al.* 2005).

At present, the main aspect of the fly control program is based on the use of chemical insecticides. Traditional control measures using chemical insecticides experience disadvantages, such as pest resistance, residues in food, environmental contamination, outbreaks of secondary pests, reductions in populations of beneficial insects and inability of insecticides to penetrate infested fruits to kill larvae. Plant extracts are one of several non- chemical control alternatives that inspiring great interest due to their availability, reduced human and mammalian toxicity and friendliness to the environment (Aqil *et al.* 2010). There is no extensive report on the management of the melon fly, *B. cucurbitae* by using plant extracts. Neem (*Azadirachta indica*) is a key ingredient in non-pesticidal management (NPM), providing a natural alternative to synthetic pesticides. It acts as an anti-feedant, repellent, and egg-laying deterrent, protecting the crop from damage. Essential oil of basil *(Ocimum basilicum)* showed antifungal and insect-repelling properties (Dube 1989). Extracts of lantana (*Lantana camara)* may be used for the protection of vegetables against various insect pests (Shreth *et al.* 2009). The leaf extracts of eucalyptus *(Eucalyptus globulus)* shows efficacy against *B. cucurbitae* (Ali *et al.* 2011). Extracts from rosy periwinkle (*Catharanthus roseus*) have been used against numerous diseases and various insect pests (Toki *et al.* 2008). Therefore, present study was undertaken to assess the bioefficacy of the above plant extracts against the fly aiming to develop an ecofriendly and sustainable management system of the pest species.

MATERIAL AND METHODS

Preparation of plant extracts: Ten different botanicals, such as leaves and barks of neem (*Azadirachta indica*); leaves of basil (*Ocimum basilicum),* mahua (*Madhuca indica*), lantana (*Lantana camara*), eucalyptus *(Eucalyptus globulus)*, surjokonna (*Spilanthes acmella)* and flowers of golden shower (*Cassia fistula*), rosy periwinkle (*Catharanthus roseus*) and orchid (*Mokara charkuan)* were collected from Jahangirnager University campus. Different parts of the plants were dried in an oven at 60-70^{0}C for four hours and grinded separately using mortar and pastel. Each of the plant part was extracted for 8 hours successively with analytical grade methanol in a Soxhlet apparatus at 60°-70°C. The extracts obtained were then stored at 4^{0}C keeping each in a separate conical flask. One gram of each crude extract was dissolved in 10 ml methanol. Thus, 100 g crude extracts of each plant part were used for preparing 100 ml stock solution.

Oviposition preference test: B. cucurbitae deposit their eggs inside the host tissue and the emerging larvae remain inside. It is difficult to count the number of larvae inside the food medium, therefore, the oviposition preference was calculated by counting the number of pupae emerged. Pieces of sweet gourds (*Cucurbita maxima)* were used as oviposition medium. Oviposition preference of the melon fly, *B. cucurbitae* was tested against the ten different types of plant extracts performing two types of tests- choice test and nonchoice test. The experiments were conducted in the laboratory of Entomology, Department of Zoology, Jahangirnagar University from October 2012 to December 2012.

Choice test: A whole sweet gourd was collected from the local market and cut into pieces each weighing about 200 g. A total of 12 pieces of sweet gourds were prepared at a time. One piece of the sweet gourd was soaked with 5 ml of a particular type of plant extract. Thus, there were 10 pieces of sweet gourd treated with the 10 different types of plant extracts. A piece of the sweet gourd was treated with 5 ml of pure methanol and a piece was kept as untreated control. Each piece of the sweet gourd was then placed on a small petri dish. The pieces of the sweet gourd treated with different types of the plant extract, only methanol and fresh were randomly arranged inside the rearing cage (76.2 × 66 × 76.2 cm). Then 400 pairs of gravid adults were released into the rearing cage. After 24 hours of egg deposition, the pieces of sweet gourds along with the petri dishes were collected and then placed on a layer of saw dust (pupation media) inside a plastic bowl which was covered with thin clothes to permit pupation. After 2 to 3 days, plastic bowls were checked regularly for drop out of the rotting host-juice from the petri-dish to avoid the unnatural death of larvae. After 6-8 days, pupae were collected by sieving the saw dust. The same experiment was replicated three times. The total number of pupae produced from each type of sweet gourd was counted, recorded and compared with each other.

Nonchoice test: The oviposition preference tests in non-choice condition with each type of plant extract was conducted with three pieces (each weighing 200 g) of sweet gourd. Each piece of the sweet gourd was treated with 5ml of a particular plant extract individually. Each piece of the sweet gourd was then placed on a small petri dish. All petri dishes along with pieces of sweet gourd were then distributed into small (31x12x31 cm) rearing cage. Then 25 pairs of gravid melon fly were released into each cage and allowed for deposition of eggs for 24 hours. A similar set of experiment was conducted using fresh piece of sweet gourds as untreated control. To find out the effect of solvent (if any) on the oviposition of the fly, a separate set of treated control experiments was conducted with the same volume of solvent only. The number of pupae produced from each piece of sweet gourd was counted and recorded following the same procedure as mentioned above in case of choice tests.

Phytochemical screening: The crude plant extracts were subjected to different qualitative tests to find out the presence of following chemical constituents: carbohydrates, alkaloids, glycerides, flavonoids, phenoles, terpenoids, steroids and saponins. These were identified by characteristic color changes using standard tests following Trease and Evans (1989), Sofowora (1993) and Evans (2002).

Statistical analysis: Data obtained from the experiments were statistically analyzed through ANOVA at RCBD (1 factor) design. Since F-test was significant, means were separated using Duncan's Multiple comparison Range Tests at 0.05% level of significance using MSTAT-C statistical program.

RESULTS AND DISCUSSION

Oviposition preference test: In choice test (Table 1), pupal yield of *B. cucurbitae* was higher from *C. fistula* (2982) treated host followed by methanol (2742), control (2624), *M. indica* (2102), *E. globulus* (1979), *C. roseus* (1018), *S. acmella* (822), *L. camara* (399), *O. basilicum* (214), and *M. charkuan* (33). It was zero (0) in case of *A. indica* leaf and bark extracts. Significantly ($p < 0.01$) higher pupal production was recorded in case of *C. fistula.* Whereas, *C. fistula* was found to be most preferred/susceptible boatanical for *B. cucurbitae* while considering mean pupal production from the natural host treated with different botanicals under controlled laboratory conditions. *A. indica* extracts treated hosts appeared as least preferred.

In nonchoice test (Table 1), the highest (1019) number of *B. cucurbitae* pupa were produced from only methanol treated hosts. *M. indica* (881) showed second highest susceptible botanical followed by control (797), *C. fistula (*794), *C. roseus* (717), *O. basilicum (*679), *L. camara* (619), *S. acmella* (449), *E. globulus* (393), *M. charkuan* (133) and *A. indica* bark (127) in terms of pupal yield. Significantly ($P < 0.05$) higher pupal production was recorded in case of *M. indica.* Pupal production was also zero (0) in case of *A. indica* leaf extract treated hosts.

Table 1. Number of *Bactrocera cucurbitae* pupae emerged from fresh, methanol treated and methanol extracts of different botanicals treated hosts in choice and non-choice tests

Treatments	Choice test		Non-choice test	
	Total pupae produced	Mean ± SE	Total pupae produced	Mean ± SE
Fresh	2624	874 ± 85.16 [a]	797	265.67 ± 10.97 [a]
Methanol	2742	914 ± 314.21 [a]	1019	339.67 ± 7.22 [ab]
M. indica	2102	700.67 ± 114.89 [a]	881	293.67 ± 7.80 [bc]
C. fistula	2982	994.00 ± 170.90 [ab]	794	264.67 ± 43.05 [bc]
E. globulus	1979	659.67 ± 12.99 [ab]	393	131.00 ± 32.33 [bc]
S. acmella	822	274.00 ± 71.01 [bc]	449	149.67 ± 48.21 [bc]
C. roseus	1018	339.33 ± 57.45 [c]	717	239.00 ± 17.62 [cd]
L. camara	399	133.00 ± 6.81 [c]	619	206.33 ± 15.60 [de]
O. basilicum	214	71.33 ± 1.76 [c]	679	226.33 ± 35.22 [e]
M. charkuan	33	11.00 ± 0.58 [c]	133	44.33 ± 23.45 [f]
A. indica (bark)	zero	zero	127	42.33 ± 2.60 [f]
A. indica (leaf)	zero	zero	zero	zero

Table 1 revealed that lowest number of *B. cucurbitae* pupae were produced from *A. indica* treated hosts. This finding has similarity to the result of Mahmoud and Shoeib (2008) who showed that low concentrations of neem can be applied effectively as sterilant and oviposition deterrent for the peach fruit fly populations. It is pertinent to mention here that the findings of Mahfuza *et al.* (2007) they reported that the Neem blocks the ovarian development and can be used as safe alternative of insecticides for the control of *Bactrocera* species. Orchid, *M. charkuan* treated host also produced least number of pupae in both choice and nonchoice tests and acted as oviposition deterrent for *B. cucurbitae*. The result conflict the findings of Nishida *et al.* (2004) in that the flowers of a Southeast Asian orchid, *Bulbophyllum cheiri*, attract males of *Bactrocera papayae* and other fruit fly species sensitive to methyl eugenol for pollination. Crude extracts of basil (*O. basilicum)* leaf also deterred the oviposition of female melon fly and pupal production was minimum. These result shows similarities with the findings of Chang *et al*. (2009) who reported that several varieties of *O. sanctum* and *O. basilicum* contain different amounts of methyl euginol in their essential oils and methyl eugenol is very toxic to *C. capitata* and *B. cucurbitae,* but less toxic to *B. dorsalis* to which methyl eugenol is a male lure. The toxic action of basil oil in *B. cucurbitae* occurred significantly faster than in *B. dorsalis*.

Phytochemical screening: Data presented in Table 2 represent the qualitative chemical analysis of methanolic crude extracts of leaf, bark and flowers of relatively effective botanicals, either as attractant or deterrent. Preliminary phytochemical screening showed that leaf extract of *M. indica* contained carbohydrate, alkaloid, flavonoid, phenolic compounds and steroid; extracts from the flower of *C. fistula* contained carbohydrate, alkaloid, flavonoid, glycoside, saponin and steroid; flower extracts of *M. charkuan* contained carbohydrate, alkaloid, glycoside and steroid. All these compounds (viz. carbohydrates, glycoside, steroids, tannins, alkaloids and flavonoids) are known to be biologically active and therefore aid in insecticidal activities. These secondary metabolites exert insecticidal activity through different mechanisms. Shimada (2006) reported tannins to form irreversible complexes with proline rich protein resulting in the inhibition of cell protein synthesis. Steroidal compounds are of importance and interest due to their relationship with various anabolic hormones including sex hormones (Okwu 2001). Different parts of plants contain the toxic alkaloid esters which prevent insects from feeding on these. Hence, the presence of these compounds in the said botanicals corroborates the insecticidal activities observed.

Table 2. Qualitative chemical examination of the methanolic crude extracts of different plants showing the preferences to the *Bactrocera cucurbitae*

Tests for	Specific test		Leaf of *M. indica*	Flower of *C. fistula*	Flower of *M. charkuan*
Carbohydrate	Molish reagent test		-	+	+
	Fehling's reagent test		+	+	+
Alkaloid	Hager's reagent.		-	+	+
	Wagner's reagent.		+	+++	+
	Mayer's reagent.		+	+	+
	Dragendorff's reagent.		++	+	-
	Tannic acid (10%)		++	+	+
Flavonoid	Ferric Chloride($FeCl_3$) test		+	+	-
	Lead Acetate test		+	+	-
	Alkali test		+	-	-
	Con. H_2SO_4 treatment		++	+++	+
Glycoside	Common test	Ferric Chloride($FeCl_3$) test	-	+	+++
	Anthraquinon Glycosides test	Borntragers test	-	-	++
	Cardiac Glycosides test	Keller killiani test	+	+	-
phenolic compounds & tannins	Ferric Chloride($FeCl_3$) test		+	+	-
	Lead Acetate test		+	-	-
	HNO_3 test		-	-	-
	Amonia (NH_3) test		++	-	+
Saponin		Foam test	-	-	-
Terpenoid	Common test	Con. H_2SO_4 test	-	-	++
	Triterpenoids test	Acetic anhydride test	-	-	+++
Phytosterol/ Steroid	Salkowski test		+++	++	+

+++ = Highly preferred, ++ = Moderately preferred, + = Least preferred, - = No preference.

However, from the overall findings, we may conclude that *M. indica and C. fistula* were the most preferred botanicals to be considered for the control of the melon fly. As far as the production of pupae is concerned, the leaf and bark of *A. indica,* leaf of basil *O. basilicum* and flowers of *M. charkuan* extracts have high oviposition deterrent effect. Experimental results might be useful in developing phytochemical bait to attract and killing technique to combat against the melon flies. Besides traditional ways, it assumes that the further advancement of this work will be most promising to us that may lead to open new door for the future control strategies to solve the melon fly problem.

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