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Repellent and oviposition deterrent activity of leaf extracts of *Azadirachta indica* A. Juss., *Persicaria hydropiper* (L.) Spach. and *Vitex negundo* Linn. against the melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

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Abstract
The present study was carried out to investigate the repellent activity and ovicidal deterrence effect of methanolic (CH_3OH) extracts of leaves of *Azadirachta indica* A. Juss., *Persicaria hydropiper* (Linn.) Spach and *Vitex negundo* Linn. against the melon fruit fly, *Bactrocera cucurbitae* (Coquillett) in the Crop Protection and Toxicology Laboratory, University of Rajshahi, Bangladesh, during November 2016 to October 2017. In repellent activity test, only the leaf extract of *A. indica* offered significant repellency at 5% level of significance (P<0.05); however, the extracts of *P. hydropiper* and *V. negundo* did not show any significant repellency. In case of oviposition deterrent activity test, the highest oviposition deterrence of 61.15% was observed in treated sweet guard with the extract of *P. hydropiper* while the lowest oviposition deterrence of 22.28% was found in treated sweet guard with the extract of *A. indica*.

Keywords: Repellency, Oviposition deterrence, Azadirachta, Persicaria, Vitx, Bactrocera

1. Introduction
Tephritidae, a large dipteran family of insects, commonly known as “fruit flies”, made up of about 4000 species under 500 genera and is found in nearly every zoogeographic i.e. temperate, tropical, subtropical regions, etc. of the province, occupying a wide variety of habitats [1, 2]. These flies are the most significant horticultural pest among the economical insect pests that attack different fruits [3, 4]. *Bactrocera cucurbitae* (Coquillett) universally well-known as melon fruit fly is one of the leading cucurbits pests and is a cosmopolitan and economically important fruit fly species. The first report on melon fruit flies was published by Bezzi [5], who listed 39 species from India. The fly seems to show a preference for attacking the different fruits such as melon, cucumber, watermelon, cantaloupe, pumpkin, angled luffa, bitter gourd, mango, guava, peach; also attack, but less frequently, other species of vegetables and fruits, such as papaya, *Carica papaya* L [6-8]. It may cause a loss ranged from 30% to 100% to the crop yield to nearly 70 host plants all over the year, depending on the cucurbit species and the season [9-11]; damage caused as high as 31.27% on bitter gourd and 28.55% on Water melon [12]; pays to about 50% yield loss in the cucumber crop [13]; damage caused by the larvae feeding on the fruit can reach 90% of the crop yield [14]. Along with direct losses, fruit fly invasion can result in severe losses in trade value and export prospect due to strict quarantine protocols executed by most importing republics [15].
In Bangladesh, *B. cucurbitae* conveys three fourth of the total number of flies invading diverse vegetables growing regions [16]. The melon fly compensations 10-30% mangoes, guava and star fruits; average 30-40% vegetables in Bangladesh per annum [17]. As a consequence, the use of pesticides is frequently supposed to be the most effective practice to control the notorious pests. However, nonstop substantial use of many pesticides has generated serious problems arising from factors, such as, direct toxicity to parasites, predators, pollinators, fish and human [18, 19]. However, analyses have clearly specified that multiple resistance mechanisms are developed in insects to a wide range of pesticides [20, 21]. Therefore, presently special emphasize has been given to the possible use of plant products as auspicious replacements to chemical insecticides in controlling insect pests [22-24]. Locally available plants are presently in widespread use in several provinces of the world to protect different crops from

damage triggered by insect infestation [25-27]. In this study, three plants *Azadirachta indica* A. Juss., *Persicaria hydropiper* (Linn.) Spach and *Vitex negundo* Linn. have been selected to investigate their comparative bioactive properties for possible use to control the destructive pest, *B. cucurbitae*.

2. Materials and Methods

2.1 Collection and preparation of test materials

The fresh and green leaves of the tested plants were collected from different places of Rajshahi, Bangladesh in the month of November, 2016. Before extraction the identification of the plants was confirmed from the experts on plant taxonomy in the Department of Botany, University of Rajshahi. Collected leaves were chopped into small pieces and were spread out on wooden-tray (45×30 cm) to dry without accumulating the materials together. It was done under the shade avoiding direct sunshine in well-ventilated room. After that the leaves were kept in an incubator in stainless tray for 24 hours in a control temperature of <40°C for making them ready to grind. Then the leaves were powdered in a grinder machine avoiding additional heat during grinding. The grinded dried leaves were soaked with sufficient amount of methanol (CH_3OH) in proportion of 10:1 as solvents and plant dust materials and sealed in conical flask (250 ml) to keep on a shaker for 48 h. Extracts, thus obtained were filtered one after another into a conical flask with a funnel setting in stand and kept for evaporation. The same process was repeated thrice for each of the leave samples. The output extracts were removed to glass vials and well-kept with proper labeling. Lastly, the amount of extracts was recorded for each of the samples.

2.2 Collection and culture of test insect

The pupae of the test species were collected from the Insect Biotechnology Laboratory of the Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment (AERE), Savar, Dhaka, Bangladesh and reared as subcultures in the Crop Protection and Toxicology Laboratory, Department of Zoology, Rajshahi University. About 2000 adult flies were maintained in wooden framed cages (40×30×30 cm) covered with wired net. The front side of the cage has one hole covered with nylon mesh net to insert food, water and egging receptacles. The flies were supplied with protein based artificial diets viz., (i) baking yeast: sugar: water at 1: 3: 4 ratio, and (ii) casein: yeast extract: sugar at 1:1:2 ratio. Foods were replaced at few days interval to provide the fresh food to the flies. Water was supplied in a petridish socked with cotton ball. The temperature and the relative humidity of the rearing room maintained at 28 ºC±5 ºC and 75%±5%, and a photoperiod of L14-D10, with photo phase starting at 0600h. Light was provided by daylight fluorescent tubes and by natural light from two big windows. The intensity of light in the experimental room was 1000-1500 Lux.

2.3 Repellent activity and ovicidal deterrence effect tests against adult flies

The concentrations used to test the repellency and oviposition deterrent for these three plant leaves extracts were different from each other. *A. indica* doses were 0.1729, 0.0865, 0.0432, 0.0216 and 0.0108 mg/cm^2; it was 0.0786, 0.0393, 0.0197, 0.0098 and 0.0049 mg/cm^2 for *P. hydropiper*; and 0.2358, 0.1179, 0.0590, 0.0295, 0.0147 mg/cm^2 for *V. negundo*. For the preparation of certain targeted dose, each extract was weighed separately in a glass vial to which 10 ml of distilled water was added, and stirred constantly for 5 minutes with a glass rod to make identical thick paste. Following that, 1 ml solution of 5 different concentration made by serial dilution were poured into 5 Petridis with a radius of 4.5 cm. Then the cutting pieces of fresh sweet guard were dipping into the treated petridish and dried at room temperature for two hours. Same quantity of fresh sweet guard also kept in untreated petridish. Treated and untreated sweet guard were offered to 10 pairs of 15-16 days old gravid flies in wooden cages for 48 h in a free choice bioassay for settling and oviposition response [28].

A test cage (12 × 12 × 15 inch) was constructed with a wooden frame to make sanitization easier. All sides were covered with an observable white net to allow viewing. A netting cloth was added to the front side of the test cage to allow access by a human forearm. Then the box was divided into two equal halves. The dishes with treated and non-treated were kept into the two halves. The settled flies on each of treated and untreated area were counted after every 1 h interval for 10 hours (5 hours from 11:00 am to 4.00 pm in each of two days). After observing their repellence effect for 10 hours in two days the dishes were then removed from the cages and number of eggs lay in both treated and untreated dish were counted. In fact, it is not so easy to count the eggs in naked eyes, so the magnifying glasses helped lot at the time of counting eggs. The skin of the fruit was peeled off carefully for egg counting.

2.4 Statistical analysis

The repellence activity was calculated in percentages of repulsion, after converted into angular transformation the data were statistically analyzed through ANOVA at RCBD (single factor) design. Percentage of oviposition deterrence was calculated by the following formula [29]: Percent oviposition deterrence = {(Half of the number of eggs laid on both treated and untreated sweet guard - Number of eggs laid on treated sweet guard) × 100}/ Half of the number of eggs laid on both treated and untreated sweet guard. Those experiments were replicated three times and results were subjected to‘t test’.

3. Results and Discussion

In case of repellent activity test, all plants extract offered significant repellency between doses (Tables 1-3), but only *A. indica* shows repellency within time interval (P<0.01; Table 1). This outcome supported the earliest study on repellent activity of Azadirachtin extracted from Neem plant [30, 31]. But the extracts of *P. hydropiper* and *V. negundo* did not show any significant mortality.

Leaf extractives of all three plants provide promising result in making oviposition deterrence to the mature female of *B. cucurbitae*. But the intensity of their activity was different from each other. Out of three plants *P. hydropiper* suppressed overall maximum egg laying of exposed flies which is highly significant. Whereas, *A. indica* showed moderate level of significance and *V. nigundo* less significantly. Highest oviposition deterrence of 61.15% was observed in treated dish with the extract of *P. hydropiper*; lowest oviposition deterrence of 8.36% was found in treated dish with the extract of *V. nigundo*. Among all of the doses applied in this experiment, only 0.2358 mg/cm^2 of *V. negundo* were found non-significant, otherwise all were offered significant result at different level of significance (Table 4).

These findings matched with the result of some previous studies. For instance, it had been clearly shown that extractives of *A. indica* stimulated the reduction of oviposition in insects [32, 33]. Oviposition deterrence by azadirachtin extracts has been reported earlier against oriental fruit fly, *B. dorsalis* and melon fly *B.* cucurbitae [34] and *B.*

zonata [35]. *A. indica* proved best with highest oviposition deterrence of 90.1 and 88.7 per cent against *B. tau* and *B. cucurbitae* respectively [36]; also Singh and Srivastva reported oviposition deterrence of neem extracts against *B. cucurbitae* [37]. It is testified for *A. indica* extracts that it has strong oviposition deterrence effect against oriental fruit fly *Bactrocera dorsalis* and melon fruit fly *B. cucurbitae* [38, 39]. This is in conformity with the work of Singh and Srivastva who reported oviposition deterrence of neem extracts against *B. cucurbitae*.

Oviposition deterrence effect of *P. hydropiper* has also been confirmed against mosquito *Aedes albopictus* which acts as a vector of the dengue fever that supports the egg laying repellent activity of *P. hydropiper* extractives [40]. Another study conveyed that *P. hydropiper* extract offered ovicidal activity against *Oligonychus coffeae* [41]. Oviposition deterrent activity of *Vitex negundo* extractives had been reported against *Aedes aegypti*, *Culex quinquefasciatus* and *Anopheles stephensi* [42].

Table 1: Repellent effect of leaf extract of *Azadirachta indica* (percentage angular transformed)

Dose (mg/cm^2)	Angular transformation of percentage of repulsion of three replications									
	1st day					2nd day				
	1h	2h	3h	4h	5h	6h	7h	8h	9h	10h
0.1729	63.43	63.43	57.86	61.14	62.24	56.79	56.79	64.67	63.43	57.86
0.0865	50.77	56.79	55.73	53.73	51.77	49.78	65.88	58.89	51.77	58.89
0.0432	45.00	57.86	48.85	61.14	52.71	46.89	53.73	47.87	51.77	45.97
0.0216	54.76	45.97	46.89	52.71	41.15	44.03	51.77	54.76	45.00	39.23
0.0108	45.00	51.77	47.87	52.71	43.11	44.03	50.77	52.71	48.85	45.00

Source of Variation	Sum of square	Degrees of freedom	Mean square	Variance ratio (F)	P-value
Between doses	1207.55	4	301.89	18.81	<0.001
Between time interval	385.77	9	42.86	2.67	<0.01

Table 2: Repellent effect of leaf extract of *Persicaria hydropiper* (percentage angular transformed)

Dose (mg/cm^2)	Percentage repulsion of melon fly in different hours after treatment									
	1st day					2nd day				
	1h	2h	3h	4h	5h	6h	7h	8h	9h	10h
0.0786	56.79	60	57.8	61.07	57.8	62.24	55.73	60	54.7	51.71
0.0393	47.87	52.71	56.79	54.7	54.7	49.78	65.88	58.89	50.77	62.24
0.0197	52.71	57.8	48.79	61.07	52.71	51.71	53.73	47.87	45	45.92
0.0098	52.71	45.92	46.89	63.43	41.15	44.03	51.71	54.7	51.71	52.71
0.0049	48.79	49.78	43.05	52.71	43.05	44.03	50.77	52.71	46.89	45.00

Source of Variation	Sum of square	Degrees of freedom	Mean square	Variance ratio (F)	P-value
Between doses	643.05	4	160.76	7.99	<0.001
Between time interval	379.58	9	42.18	2.10	>0.05 ns

Table 3: Repellent effect of leaf extract of *Vitex negundo* (percentage angular transformed)

Dose (mg/cm^2)	Percentage repulsion of melon fly in different hours after treatment									
	1st day					2nd day				
	1h	2h	3h	4h	5h	6h	7h	8h	9h	10h
0.2358	58.89	55.73	65.88	56.79	54.7	57.8	62.24	57.8	55.73	57.8
0.1179	45.92	62.24	49.78	49.78	52.71	56.79	52.71	61.07	60.00	52.71
0.0590	57.8	56.79	46.89	55.73	46.89	60	51.71	49.78	48.79	48.79
0.0295	46.89	45.00	49.78	63.43	40.16	53.73	45.92	54.7	50.77	43.05
0.0147	48.79	49.78	49.78	45.00	43.05	41.15	55.00	45.92	45.00	45.92

Source of Variation	Sum of square	Degrees of freedom	Mean square	Variance ratio (F)	P-value
Between doses	781.99	4	195.50	7.67^{ns}	<0.001
Between time interval	214.60	9	23.84	0.94	>0.05ns

Table 4: Oviposition deterrent effects of leaf extracts of three plants on *B. cucurbitae*

Plant	Dose mg/cm^2	Mean number of eggs laid (Mean ± S.E.)		Egg inhibition %	t value
		Untreated	Treated		
A. indica	0.1729	68.67±6.69	41.33±2.03	24.85	5.86^{**}
	0.0865	73.33±3.71	44.33±4.81	24.65	14.5^{***}
	0.0432	78.67±7.54	50.00±9.87	22.28	12.29^{***}
	0.0216	80.00±2.52	33.67±3.84	40.76	7.32^{**}
	0.0108	80.00±4.16	35.00±5.13	39.13	45.00^{***}
P. hydropiper	0.0786	63.00±2.08	20.33±2.03	51.20	13.42^{***}
	0.0393	83.67±4.37	28.00±4.73	49.85	10.63^{***}
	0.0197	79.33±4.67	24.33±4.09	53.05	95.26^{***}
	0.0098	74.67±2.03	18.00±2.08	61.15	170.00^{***}
	0.0049	83.67±3.84	22.00±2.88	58.36	28.21^{***}
V. negundo	0.2358	54.00±4.62	45.67±10.84	8.36	0.82^{ns}
	0.1179	57.67±1.33	26.67±3.67	36.76	6.20^{**}
	0.0590	52.33±7.53	22.33±4.91	40.18	9.82^{***}
	0.0295	61.67±5.60	35.33±4.63	27.15	21.91^{***}
	0.0147	56.33±7.45	28.33±2.85	33.07	4.65^{*}

$^{***}P<0.1$; $^{**}P<0.01$; $^{*}P<0.05$; ns- not significant.

4. Conclusion
From the present findings we conclude that the extracts from the plant species namely *Azadirachta indica*, *Persicaria hydropiper* and *Vitex negundo* can prove effective alternative in managing fruit flies if applied well in time. Timely sprays of plant extracts will not only avoid egg laying by the fruit flies but will also lead to reduction in insecticidal sprays, thereby minimizing the environmental and other health hazards. Further studies need to be carried out in context with the shelf-life and duration of effectivity of the plant extracts in field conditions so as to fit the management practices in suitable IPM against fruit flies.

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