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Toxicity of three plant leaf extracts against larvae and pupae of melon fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

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Abstract
Methanol extractives of leaves of three selected plants *Azadirachta indica* A. Juss., *Persicaria hydropiper* (Linn.) Spach and *Vitex negundo* Linn. were subjected to larvicidal and pupicidal test against the melon fruit fly, *Bactrocera cucurbitae* (Coq.) (Diptera: Tephritidae). In larvicidal test, offered the highest and lowest mortality has been calculated as 1.161 and 0.758mg cm^{-2} for *A. indica*; 0.853 and 0.275mg cm^{-2} for *P. hydropiper*; 2.213 and 0.732mg cm^{-2} for *V. negundo* after 6 hours and 24 hours of exposure respectively. In case of pupicidal test against melon fly, the highest and lowest activity has been observed for the extract of *A. indica* by LD_{50} 0.26mg cm^{-2} and the extracts of *P. hydropiper* by LD_{50} 8.70mg cm^{-2} respectively. Against *B. cucurbitae*, the pupicidal potentiality of leaf extractives of these three plants can be sequenced in a descending order as *A. indica* > *V. negundo* > *P. hydropiper*.

Keywords: larvicidal, pupicidal, azadirachta indica, persicaria hydropiper, vitex negundo, Bactrocera cucurbitae

Introduction
Tephritid insects, commonly known as "fruit flies" are the utmost substantial agricultural pest among which destroy different fruits and vegetables [1-2]. *Bactrocera cucurbitae* (Coquillett) is one of the most familiar flies of among the tephritids; commonly known as melon fruit fly. It was Bezzi who reported for the first time on melon fruit flies and listed about 39 species in India [3]. The fly mainly attacks cucumber, bitter gourd, watermelon, pumpkin, cantaloupe peach mango, melon, guava, also attack but less frequently some vegetables and other fruits [4-5]. The losses caused due to the infestation of this fly differ from crop to crop as well as season to season. Most of the damage triggered by different larval instars feeding on the fruit and it may reach up to 90% of the total crop revenue [6]. Moreover, together with direct damages, fruit fly causes massive harms in export product overlook for the tight quarantine rules adopted by most of the concerning authorities [7]. In Bangladesh, melon flies delivers 75% of the total number of flies occupying vegetable growing lands; for instances, 10-30% star fruits and mangoes; 30-40% vegetables in Bangladesh annually [8-9].
Hence, to obtain the high crop yield it is highly necessary to control the pest species to keep the pest population beneath economic threshold level. Use of chemical pesticides is frequently thought to be the highly effective approach to control the harmful pests. However, constant use of several pesticides has produced severe problems as these chemical shows direct toxicity to human as well as to other non-target organisms [10]. Moreover, studies have noticeably proved that resistance may also developed in many insects to a wide variety of chemical pesticides [11-12]. Hence, currently distinctive highlight has been given to the conceivable use of plant oriented natural products as promising alternates to chemicals in management of pest population [13-15]. The plants that are found locally now in extensive use in a number of areas throughout the world to save different crops from insect infestation [16-17]. In this present work, the selected three plants *Azadirachta indica* A. Juss., *Persicaria hydropiper* (Linn.) Spach and *Vitex negundo* Linn. was subjected to explore their larvicidal and pupicidal activity and their probable use to eradicate the vicious pest species.

Materials and Methods
Collection and preparation of test materials: The fresh and green leaves of the tested plants were collected from different places of Rajshahi for extraction. 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 were 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.

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 28ºC±5ºC 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.

Dose mortality test on larvae of flies

The experiment for mortality test on *B. cucurbitae* larvae was different from those previous two experiments. To test the leaf extractives in residual film method, firstly the full grown larvae of *B. cucurbitae* were collected from the culture. 5 consequent doses were selected for the final experiments ranged between 0.611 to 1.019mg cm^{-2} for both *A. indica* and *V. nigundo*; 0.1020 to 0.509mg cm^{-2} for *P. hydropiper* were prepared by serial dilution for each extracts. Each dose was prepared by mixing it in 1 ml of distilled water and poured into the petri-dish (50 mm). Group of 10 larva were used for each concentration and put in Petri dishes after extracts treatment (1ml $dish^{-1}$). The control insects were put in Petri dishes treated with water only and they were left for 2 hours at room temperature to dry. Three replicates were carried out for each extracts concentration and for the control. The tested larvae were observed after 6h, 12h, 18h and 24h of exposure. All treatments were inspected for mortality.

Dose mortality test on 1-Day old pupae of *B. Cucurbitae*

The efficacy of these three plants leaf extracts against 1-day old pupae of *B. cucurbitae* was evaluated by sandy soil method. Sand was sieved and put in plastic cups (50g cup^{-1}). Five concentrations of each extracts were selected for the final experiments ranged between 0.04 to 0.64mg cm^{-2} for both *A. indica* and *V. nigundo*; 0.01 to 0.16mg cm^{-2} for *P. hydropiper*, prepared with 7.5ml of water (required amount for saturation) were added in each cup. Following that, the sands were properly stirred with a glass rod to mix the solution homogenously into the sand. Then 10 pupa of 1-day old were confined and buried into sand in each cup. The cups were covered with muslin clothes which tightly secured with rubber bands and left under the above mentioned laboratory conditions till adult emergence. Control experiments using soil saturated with water only also carried out for comparison and correcting mortalities in treatments as previously mentioned in surface contact treatment. After certain days, the numbers of emerging adults from pupa were counted for each cup.

Statistical analysis of the larvicidal and pupicidal tests

The mortality records of the dose mortality experiments done on the larvae and 1-day old pupae of *B. cucurbitae* were corrected by the following formula [18]:

$Pr = \{(Po - Pc)/ (100 - Pc)\} \times 100$; Where, P_r = Corrected mortality (%), P_o = Observed mortality (%), P_c = Control mortality (%).

Then mortality percentages were subjected to statistical analysis according to by using software developed in the Department of Agricultural Environmental Science, University of Newcastle upon Tyne, U.K. The dose-mortality relationship was expressed as a median lethal dose (LD_{50}) [19].

Results

Mortality test on larvae of *B. cucurbitae*

The leaf extractives of *A. indica*, *P. hydropiper* and *V. nigundo* leaves were tested against the larvae of *B. cucurbitae* through larvicidal test and the results found much significant in each cases. As is presented in the Table 01, *A. indica* offered LD_{50} 1.161, 0.999, 0.855, 0.758 and 0.147mg cm^{-2}; *P. hydropiper* offered LD_{50} 0.853, 0.579, 0.347 and 0.275mg cm^{-2}; *V. nigundo* offered LD_{50} 2.213, 1.316, 0.863, 0.732 after 6, 12, 18 and 24h of exposure respectively.

Table 1: LD_{50}, 95% confidence limits and regression equations of tested leaf extracts of three different plants applied on *B. cucurbitae* larvae between 6h and 24h after exposure.

Plant extract	Time of exposure	LD_{50} (mg cm^{-2})	95% confidence limits		Regression equation	χ^2 *value* at 3 df
			Lower (mg cm^{-2})	Upper (mg cm^{-2})		
A. indica	6h	1.161	0.929	1.449	Y = -0.7131977 + 5.366083 X	0.467
	12h	0.999	0.883	1.131	Y = -1.052414 + 6.054165 X	1.383
	18h	0.855	0.796	0.918	Y = -1.701283 + 7.191115 X	3.119
	24h	0.758	0.710	0.809	Y = -1.979523 + 7.934018X	0.749
P. hydropiper	6h	0.853	0.445	1.635	Y = 3.041717 + 2.10308 X	0.701
	12h	0.579	0.378	0.887	Y = 3.485937 + 1.985292 X	1.447
	18h	0.347	0.266	0.453	Y = 3.947133 + 1.948023 X	2.744
	24h	0.275	0.212	0.356	Y = 4.18063 + 1.867183 X	7.105
V. negundo	6h	2.213	0.553	8.859	Y = 1.74286 + 2.421771 X	2.486

	12h	1.316	0.790	2.194	Y = 1.824369 + 2.836901 X	2.253
	18h	0.863	0.761	0.977	Y = 1.213891 + 4.045873 X	1.777
	24h	0.732	0.619	0.866	Y = 2.30568 + 3.115756 X	4.590

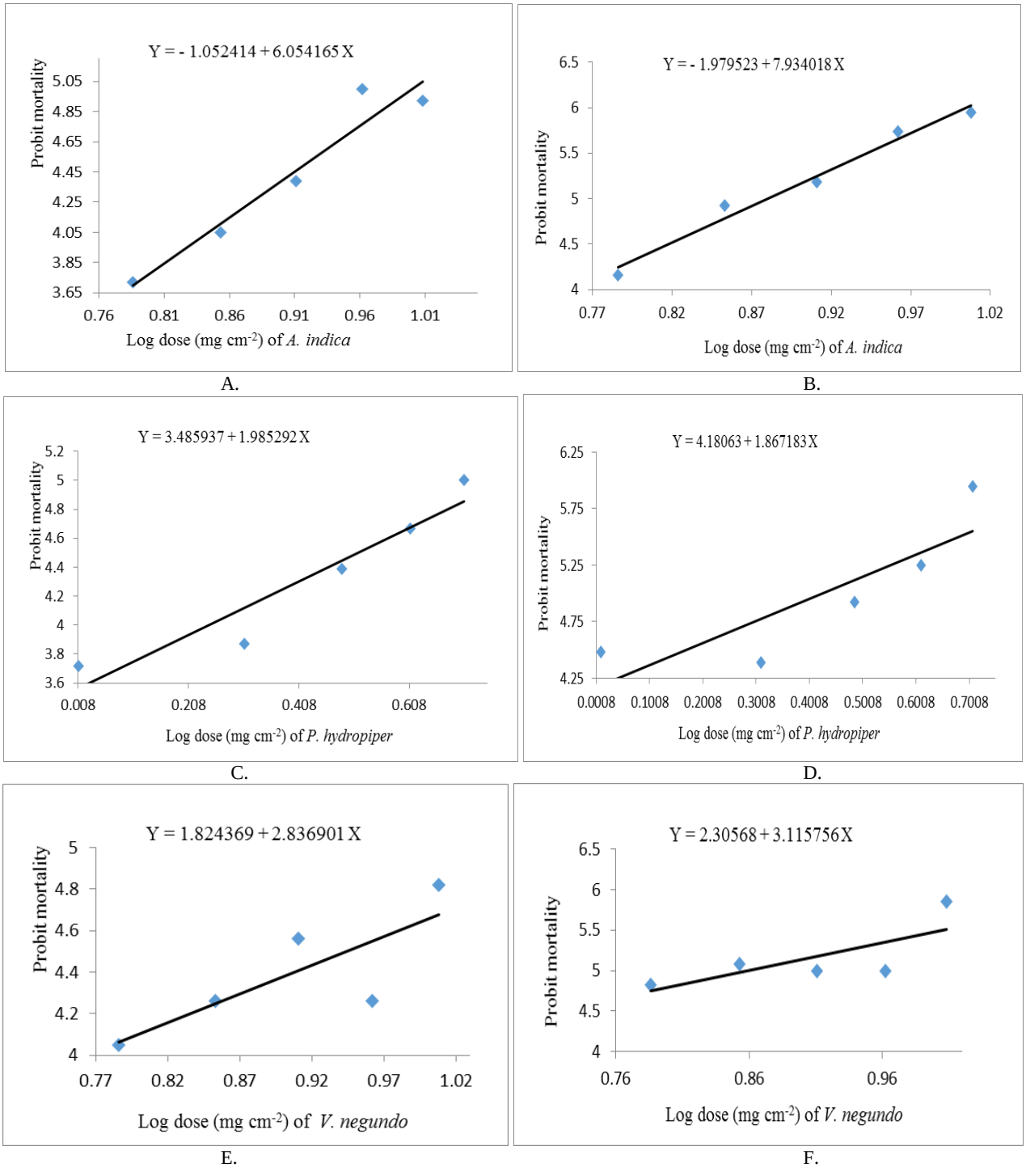

Fig 1: Probit mortality line of log dose (mg cm^{-2}) of leaf extracts of *A. indica* (A and B), *P. hydroppiper* (C and D) and *V. negundo* (E and F) on *B. cucurbitae* larvae after 12h and 24h of exposure respectively

Mortality test on 1 day old pupae of *B. cucurbitae*

It is appeared from the Table 04 that all the extractives have varied degree of mortality on pupae of *B. cucurbitae.* The highest (LD_{50} 0.26mg g^{-1} soil) and lowest (LD_{50} 8.70mg g^{-1} soil) pupicidal activity has been calculated for the extract of *A. indica* and *P. hydropiper* respectively. At the same time, leaf extractives showed moderate pupicidal activity at LD_{50} 0.29mg g^{-1} soil. Hence, the pupicidal activity of leaf extracts of three plants can be ordered in a descending sequence as *A. indica* > *V. negundo* > *P. hydropiper*.

Table 2: LD_{50}, 95% confidence limits and regression equations of tested leaf extracts of three different plants applied on 1 day old pupae of *B. cucurbitae*

Plant extract	LD_{50} mg g^{-1} soil	95% confidence limits		Regression equation	χ^2 at 3 df
		Lower mg/g soil	Upper mg/g soil		
A. indica	0.26	0.08	0.88	Y = 4.386223 + 0.4315063 X	1.59
P. hydropiper	8.70	1.92	0.39	Y = 4.216317 + 0.4040137 X	6.50
V. negundo	0.29	0.16	0.54	Y = 3.694087 + 0.8919702 X	0.29

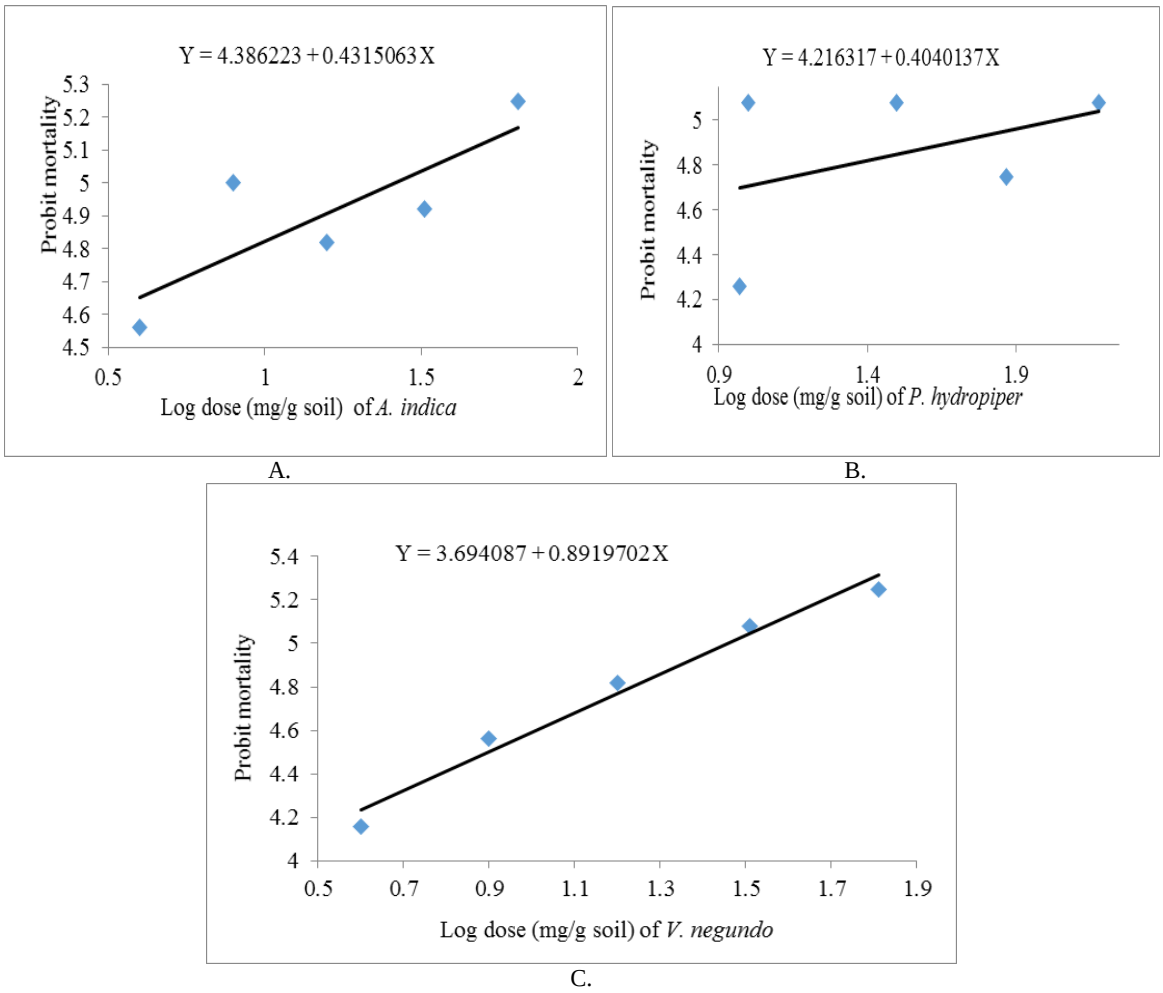

Fig 2: Probit mortality line of log dose (mg g^{-1} soil) of leaf extracts of *A. indica* (A), *P. hydroppiper* (B) and *V. negundo* (C) on 1 day old pupae of *B. cucurbitae*

Discussion

Growers currently depend mostly on the use of chemical insecticides to control different fruit flies. Plant byproducts seem to be a noble source of environment friendly pesticides that can be practiced as successful replacements to the insecticides. These findings get proper support from earlier investigation done on extractives of these three plants. For example, Larvicidal activity of *A. indica* has been confirmed previously against the fleas *Ctenocephalides felts* and *Xenopsylla brasiliensis* [20]. *P. hydropiper* has been tested against the larvae of *Aedes aegypt* mosquitoes which acts as a vector of dengue vector [21]. Leaves extracts of *P. hydropiper* indicated clearly about the repellent and feeding deterrent activity against *T. castaneum* [22]. It is also recorder that extract of *P hydropiper* is very effective against the lepidopteran larvae and termites [23]. In another investigation, the extract of *Vitex negundo* showed promising larval mortality aagainst *Aedes aegypti* at different concentrations to different laval instars [24]. So, all these support the larvicidal activity of these studied three plants leaves extractives. Significant larvicidal and pupicidal activity of *Azadirachta indica* against *Helicoverpa armigera* and *Spodoptera litura* [25]; found in a study that strongly supports the larvicidal and pupicidal efficacy of *A. indica* against fruit flies. A research conducting on leaf extract of *Polygonum hydropiper* has given promising evidence of significant result in cytotoxic antinociceptive and antihyperglycemic activities [26].

Conclusion

After evaluating the results of larvicidal and pupicidal activity of leaves extract of *Azadirachta indica, Persicaria hydropiper* and *Vitex negundo* against melon fruit fly, it can concluded that all these plant's extractives provide promising result that supports the presence of some bioactive compounds that are much effective against larval and pupal stages of *Bactrocera cucurbitae.* Therefore, these all could be used in strategic control of horticultural pests in a ecofriendly way that is highly recommended in the present context of integrated pest management. It can be also suggested that further investigation is needed to detect the specific compound which is responsible for showing this sort of bioefficacy.

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