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Repellent and insecticidal efficacy of some botanical extracts against *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

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Abstract

Botanical insecticides are considered to be the alternative to synthetic chemical pesticides since these compounds are biodegradable and less persistent in the environment. To investigate the repellent and insecticidal efficacy of six selected botanical extracts against *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) at ambient laboratory conditions (27±2°C, RH 65±5%) using acetone solvent was used. The results indicated that all the plant extracts had the repellent and insecticidal effect through ovipositional deterrent against *B. cucurbitae* that inhibited progeny development as well. The order of toxicity of the plant extracts based on repellency and ovipositional deterrent were found as tobacco > water pepper > neem > eucalyptus > bullock's heart > castor. However, progeny recovery, repellency and ovipositional deterrent rate acted as dose dependent manner where repellency and ovipositional deterrent was decreased with the decreased doses of plant extracts. Tobacco and neem plant extracts were showed the complete ovipositional deterrent (100%) whereas the lowest ovipositional deterrent was in castor (52.63%) followed by bullock's heart (58.63%) at 5% concentration of the plant extracts applied. The minimum numbers of progeny were found at 2% concentration in tobacco (0.67), water pepper (1.00) and neem (1.67) whereas the highest at 0.5% concentration in neem (10.00) and tobacco (3.67) meanwhile 4.0% concentration of all extracts totally protects the oviposition. The repellency and ovipositional deterrent activity was increased with the increase of the doses of the plant extract and were found statistically significant ($p < 0.01$). It is evident from the results that at all plant extracts exhibited a remarkable decrease in adult settled, larvae and pupae recovery, and adult emergence through repellent and ovipositional deterrent activities as compared with control.

Keywords: *B. cucurbitae*, repellent, ovipositional deterrent, progeny development

Introduction

In Bangladesh, vegetables are the most important component of our daily food and are rich in vitamins and minerals that are essentials for human health but the production of vegetables is much below than our national requirement. Cucurbits from the largest group of vegetables including bitter gourd, sweet gourd, ridged gourd, sponge gourd, teasel gourd, white gourd, ash gourd, cucumber as the major ones. Among them, bitter gourd is one of the most important vegetables. It is considered one having medicinal properties and with a compound named "Charantin" present which is helpful to reduce blood sugar for diabetic patients [1]. Bitter gourd is also rich in carbohydrates, and iron [2]. Like other south-east Asian countries, fruit fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae) is the most ubiquitous and destructive insect pest of cucurbitaceous vegetables in Bangladesh. It is a frugivorous and multivoltine insect which cause 50.0 to 100.0% damage in different species of cucurbitaceous vegetables [3, 4]. Ramadan and Messing [5] investigated, on an average, 94.4% fruit infestation by *B. cucurbitae* on three cucurbit species in Thailand and Sapkota *et al.* [4] found 40.0% on squash in Nepal. This pest has a major limiting factor which causes intense qualitative and quantitative losses in bitter gourd [6, 7]. The females of the *B. cucurbitae* choose generally soft tender fruit tissues to lay their eggs. It causes enormous economic losses to a wide variety of fruits, vegetables and flowers [8, 9]. The damage starts when the female fruit fly punctures the fruit with its long and sharp ovipositor. The maggots feed inside the fruit [10] and make tunnels in fruits. The infested fruits become rotten, dry up and finally shed up prematurely. If not rotted, become deformed and market value greatly reduced and causes substantial yield losses to growers. However, farmers in Bangladesh solely rely on the use of synthetic insecticides to control the pest in bitter gourd. In some areas, farmers spend about 25% of the cultivation cost

in bitter gourd production only to buy synthetic pesticides [10]. Moreover, repeated and a long time uses of toxic insecticides has some serious drawback such as pesticides resistance, toxic residues, increasing costs of application, environmental pollution and health hazards to human being and domestic animal [11]. Therefore, it is desirable to explore alternative methods to control this pest.

Plants contain thousands of compounds which are virtually an untapped reservoir of pesticides that can be used directly or as templates for synthetic pesticides. It is urgent to find more effective and environmental friendly control strategies that ensure a sustainable production of fruits and vegetables. The environmentally safe method, such as the use of plant extracts, oils, and dusts are growing interest to replace synthetic pesticides [12, 13]. The bagging of fruits in scaffold is an environmentally sustainable method and showed the lowest level of infestation, but the method is very expensive and laborious [14, 15]. Many studies have been carried out on the plant extracts against cucurbit fruit fly [16, 17]. Various botanicals have been found to be effective against different pests; especially water pepper (*Polygonum hydropiper*), neem (*Azadiracta indica*) and tobacco (*Nicotiana tabacum*) are examples of such plants, which can possess medicinal, entomocidal and repellent or antifeedent property [18, 19]. The botanical insecticides are biodegradable and harmless to the environment [20]. As a result, the trend has now been shifted towards an integrated pest management (IPM) for the control of cucurbit fruit flies [21]. Under these circumstances, an investigation was undertaken to find out the entomocidal efficacy of some indigenous plant extracts against *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) and can be used as an alternative to chemical pesticides and can minimize the population.

Materials and Methods

The experiment was conducted in the Laboratory of the Department of Entomology, Faculty of Agriculture, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh during July to December 2016. The entomocidal efficacy of botanical extracts were maintained on *Bactrocera cucurbitae* (Coquillett) in the laboratory in a ambient temperature, humidity and photo period of $27\pm 2^{\circ}\text{C}$, $65\pm 5(\%)$ and L16:D8, respectively.

Collection of leaves and extraction procedure

The fresh healthy leaf of water pepper (*Polygonum hydropiper*), neem (*Azadiracta indica*), tobacco (*Nicotiana tabacum*), bullock's heart (*Anonna reticulata*), castor (*Ricinus communis*) and eucalyptus (*Eucalyptus alba*) were collected from the surrounding area of Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh and were dried in shade but before making powder it was dried in an oven (Model Number: XTDQ-101-4 Drying Oven, Yancheng Hopebond Electric Co. Ltd., China) at $45\text{-}50^{\circ}\text{C}$ for 6 hours. Then the plant materials were grind using electric grinder (Nova Blackberry Blender, model: AD999, Bangladesh). The dust was passed through a 60 mesh sieve to obtain fine powder. Hundred gram of each category of powder were taken in a 600 ml beaker and separately mixed with 300 ml of acetone. The mixture was stirred for 30 minutes in a magnetic stirrer (600 rpm) and left to stand for 72 hours with shaking several intervals. After that the mixture was filtered through a filter paper (whatman no. 1) and was allowed to evaporate with the help of rotary evaporator (Lab Tech EV311H Rotary Evaporator, Manufactured in China).

Then the lyophilized material was stored at refrigerator for further use as an extract.

Mass culture of *B. cucurbitae*

B. cucurbitae (Coquillett) (Diptera: Tephritidae) were reared in partially natural environment in fruit fly rearing cage (60 cm \times 50 cm \times 45 cm) covered with nylon net and newspaper was placed ground of the rearing cage. Infested fruits were collected from the bitter gourd fields and were kept in a plastic jar covered with muslin cloth (Figure 1). After 5-6 days all similar larvae were replaced from bitter gourd to water gourd in the 1st rearing cage. After 2- 3 days later the larvae come out from water gourd and formed pupation under the newspaper. Two rearing cage was needed in where one is for pupae collection (Figure 2) and another for sexually matured flies. All pupae were collected carefully and kept in a petridish and covered with sand or soil (Figure 3). It was placed in 2nd rearing cage (Figure 4). For preparing artificial diet sweet gourd pulp (number 2), egg yolk (number 6), honey (4 table spoon), multi-vitamin syrup (2 table spoon), yeast (1 table spoon) and sugar (8 table spoon) ingredients were used. For making thick syrup solution the ingredients were mixed in a blender and kept in a vacuum protected pot, for further use it was preserved in refrigerator.



Fig 1: Infested fruit kept in the jar



Fig 2: Rearing cage for pupae



Fig 3: Collected pupae



Fig 4: Reared fruit fly

Plant extracts selection and dose preparation

Protocol for primary screening: Six plant extracts viz. bullock's heart, water pepper, castor, eucalyptus, neem and tobacco were taken to find out the effectiveness of repellency and ovipositional deterrent against *B. cucurbitae* (Coquillett) (Diptera: Tephritidae). Before selecting the plant extracts, a pilot experiment was done with 5% concentration of the plant extracts.

Protocol for secondary screening: Three high performance result showing extracts were selected in secondary screening of the botanical extracts to find out the progeny (larvae and pupae recovered, and adult emerged) recover, also in repellent and ovipositional deterrent. The crude extracts were weighed by the help of electronic balance and dissolved with acetone solvent for preparing five concentrations (5.0, 4.0, 2.0, 1.0, and 0.5%) along with control, respectively. Three replications of each concentration were performed in each treatment.

Observation of repellent and ovipositional deterrent

Fresh healthy bitter gourd fruits were spraying with different doses extracts and dried at room temperature for two hours. Treated and untreated bitter gourd fruits were offered to 5 pairs of 10-15 days old gravid flies in plastic cages (measuring 45 cm × 40 cm × 40 cm) for 48 hours in a free choice bioassay for settling and oviposition response [22]. Number of fruit flies settled on treated and untreated bitter gourd fruits were counted after every one hour interval for 10 hours. The experiments were replicated three times. The fruits were removed from the cages and were kept for larval growth. After 12-15 days, number of larvae was counted in each replication separately of the treated and untreated fruits. Percent repellent and percent ovipositional deterrent was calculated as follows:

% Repellent = $\left[\frac{\text{Half of the number of flies settled on both treated and untreated fruits} - \text{number of flies settled on treated fruit}}{\text{Half of the number of flies settled on both treated and untreated fruits}} \right] \times 100$

% Ovipositional deterrent = $\left[\frac{\text{Half of the number of larvae on both treated and untreated fruits} - \text{number of larvae on treated fruit}}{\text{Half of the number of larvae on both treated and untreated fruits}} \right] \times 100$

Observation of progeny (larvae and pupae recovery, and adult emergence)

The counted larvae were kept in petridishes with sand and soil for adult emergence. After 7-8 days later pupae were found in the soil. Finally, after 20- 25 days the emerged adult was counted from the treated and untreated fruits.

Statistical analysis

Data was analyzed by analysis of variance (ANOVA) in accordance with Completely Randomized Design (CRD) through MSTAT - C program. The treatment mean values were separated by Duncan's New Multiple Range Test (DMRT) and the graphical works were done through Microsoft Excel program.

Results

Repellent and ovipositional deterrent effects against *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) in primary screening protocol

Mean number of repellency and ovipositional deterrent effects of six tested plant extract against *B. cucurbitae* on bitter gourd fruits are presented in Table 1. The repellency and ovipositional deterrent of six different plant extracts significantly ($p < 0.01$, $df = 10$) differed among all the treatments applied at 5.0% concentration. The highest repellent rate was observed in tobacco (90.787%) whereas the lowest was found in bullock's heart extract (43.91%) treated bitter gourd fruits. Tobacco and neem plant extracts were showed the complete ovipositional deterrent (100%) whereas the lowest was in castor (52.63%) followed by bullock's heart (58.63%) treated bitter gourd. On the basis of the repellent and ovipositional deterrent, the order of toxicity of the plant extracts were found as tobacco > water pepper > neem > eucalyptus > bullock's heart > castor extracts at 5% concentration.

Table 1: Mean number of repellency and ovipositional deterrent effects of six different plant extracts against *B. cucurbitae* (Coquillett) on bitter gourd fruits at 5% concentration

Plant extracts	Repellency (%)	Ovipositional deterrent (%)
Bullock's heart	62.02 c ± 6.00	58.33 c ± 4.04
Water pepper	88.50 a ± 3.06	100.0 a ± 0.00
Castor	70.00 b ± 4.26	52.63 d ± 4.23
Eucalyptus	71.47 b ± 4.21	59.23 c ± 4.77
Neem	84.43 a ± 3.87	79.52 b ± 3.43
Tobacco	90.78 a ± 2.99	100.0 a ± 0.00
LSD at 5% level	7.157	2.923
CV (%)	9.59	4.07

Each value is a mean of three replications, 5 pairs per replication. Mean values among the columns by the same letter(s) are not significantly different at 5% level by DMRT. ± Values represent standard error (±SE)

Repellent, ovipositional deterrent and progeny (larvae, pupae recovery and adult emergence) development effects in secondary screening protocol

The highest number of adult settled (12.33), number of larvae (8.93) and pupae (8.93) recovery, and adult emergence (8.47) were found in neem treated bitter gourds. The highest repellent was observed in water pepper (60.44%), followed by tobacco (53.37%) whereas the lowest was found in neem (41.88%) extracts. On the other hand, the lowest number of adult settled was found in tobacco (9.73) and water pepper (9.00) which was statistically similar. Same trend of results were found in larvae and pupae recovery as well as adult emergence in all the treatments. The lowest number of larvae and pupae recovery, and adult emergence (5.27) was showed in water pepper, followed by tobacco (5.53). Nevertheless, the highest percent repellency was observed in water pepper (60.44%) whereas lowest in neem (41.88%). Water pepper also showed the highest ovipositional deterrence (89.43%), followed by tobacco (87.88%) but the lowest in neem (68.67%) extracts (Table 2). In all cases, the lowest adult settled and progeny development indicated the highest toxicity of the plant extracts. The order of toxicity of the plant extracts on the basis of the progeny (larvae, pupae recovered and adult emerged) development was tobacco > water pepper > neem against *B. cucurbitae* (Coquillett) (Diptera: Tephritidae).

Table 2: Mean number of adult settled, repellency, ovipositional deterrent and progeny development against *B. cucurbitae* (Coquillett) on bitter gourd fruits

Plant extracts	Mean number \pm SE					
	Adult settled	Repellency (%)	Larvae recovered	Ovipositional deterrent (%)	Pupae recovered	Adult emerged
Water pepper	9.00 b \pm 0.89	60.44 a \pm 4.98	5.27 b \pm 1.02	89.43 a \pm 7.38	5.27 b \pm 1.02	5.27 b \pm 1.02
Neem	12.33 a \pm 0.83	41.88 b \pm 4.40	8.93 a \pm 0.87	68.64 b \pm 4.91	8.93 a \pm 0.87	8.47 a \pm 0.80
Tobacco	9.73 b \pm 0.15	55.37 a \pm 3.94	5.53 b \pm 0.80	87.88 a \pm 5.83	5.53 b \pm 0.80	5.53 b \pm 0.80
LSD at 5% level	1.36	6.42	2.12	11.20	2.12	2.08
CV (%)	17.55	14.43	43.17	16.14	43.17	43.38

Each value is a mean of three replications, 5 pairs per replication. Mean values among the columns by the same letter(s) are not significantly different at 5% level by DMRT.

Effect of different concentrations against tested insect

Figure 5 represents the repellent and ovipositional deterrent effects of different doses of botanical extracts against *B. cucurbitae* on bitter gourd fruits. The highest repellency were observed (70.53%) at 4.0% concentration whereas the lowest were observed (32.86%) at 0.5% concentration. On the other hand, complete oviposition deterrent observed at 4.0% concentration which was statistically similar (92.85%) at 2.0% concentration.

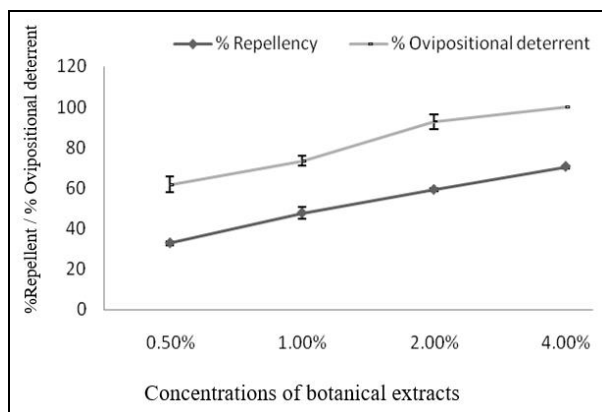


Fig 5: Repellent and ovipositional deterrent effects of different doses plant extracts against *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) on bitter gourd fruits

The results effects of different doses botanical extracts on the adult settled, repellent, ovipositional deterrent and progeny development against *B. cucurbitae* (Coquillett) on bitter gourd fruits were found statistically significant ($p < 0.01$) different in compare to untreated control (Table 3). The highest number of adults settled in 0.5% concentration of neem and tobacco (13.67 and 12.33, respectively) leaf extracts. On the other hand, the lowest were found in 4.0% concentration of tobacco (2.33), followed by water pepper (3.67). On the other hand, no larvae, pupae recovery and adult emergence were observed in 4.0% concentration. The minimum number of larvae, pupae recovery and adult emergence were in 2.0% concentration of tobacco (0.67), followed by water pepper (1.00) whereas maximum number of larvae, pupae recovery in 0.5% concentration of neem (10.00). On the other h and the maximum numbers of adult emergence were in 0.5% concentration of neem (8.67). The highest repellency rate were observed in 4.0% concentration of tobacco (80.23%), followed by water pepper (73.04%) whereas the lowest were observed in 0.5% concentration of neem and tobacco (25.41% and 27.66% respectively). On the other hand, complete ovipositional deterrent showed in 4.0% concentration of tobacco, neem and water pepper. The maximum ovipositional deterrent observed in 2.0% concentration of tobacco and water pepper (95.56% and 93.10% respectively).

Table 3: Effects of different doses plant extracts on the adult settled, repellent, ovipositional deterrent and progeny development against *B. cucurbitae* (Coquillett) on bitter gourd fruits

Plant extracts	Doses (%)	Mean number of					
		Adult settled	Repellency (%)	Larvae recovered	Pupae recovered	Ovipositional deterrent (%)	Adult emerged
Water pepper	4.00	3.67 ef	73.04 ab	0.00 c	0.00 c	100.0 a	0.00 d
	2.00	5.00 def	63.64 bc	1.00 c	1.00 c	93.10 ab	1.00 d
	1.00	5.67 def	59.56 bcd	1.33 c	1.33 c	91.11 ab	1.33 d
	0.50	8.33 cd	45.52 de	3.33 c	3.33 c	73.51 b	3.33 cd
	Control	22.33 a	-	20.67 a	20.67 a	-	20.67 a
Neem	4.00	6.00 de	58.34 cd	0.00 c	0.00 c	100.0 a	0.00 d
	2.00	8.33 cd	46.81 de	1.67 c	1.67 c	89.90 ab	1.67 d
	1.00	10.67 bc	36.94 ef	8.67 b	8.67 b	45.09 c	7.67 bc
	0.50	13.67 b	25.41 f	10.00 b	10.0 b	39.56 c	8.67 b
	Control	23.00 a	-	24.33 a	24.33 a	-	24.33 a
Tobacco	4.00	2.33 f	80.23 a	0.00 c	0.00 c	100.0 a	0.00 d
	2.00	4.33 ef	67.07 abc	0.67 c	0.67 c	95.56 ab	0.67 d
	1.00	8.00 cd	46.50 de	1.67 c	1.67 c	84.13 ab	1.67 d
	0.50	12.33 b	27.66 f	3.67 c	3.67 c	71.83 b	3.67 cd
	Control	21.67 a	-	21.67 a	21.67 a	-	21.67 a
LSD at 5% level		3.04	12.84	12.84	22.41	22.41	22.41
CV (%)		17.55	14.43	43.17	43.17	16.14	43.38

Each value is a mean of three replications, 5 pairs per replication. Mean values among the columns by the same letter(s) are not significantly different at 5% level by DMRT.

Discussion

The plant extracts were assayed for their effect as toxicants against *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) on bitter gourd fruits through repellent, ovipositional deterrent and progeny development. In the present study, the extracts of tobacco showed

the highest repellent (85.34%) and oviposition deterrent (96.46%), whereas the lowest in eucalyptus plant extracts 43.91% and 55.68%, respectively. As plants have a rich source of bioactive compounds, they may give an alternative solution to synthetic insecticides for control plant diseases and pests [23, 24]. Tare *et al.* [25] opined that plant

extracts showed strong entomocidal properties and have additional advantages as these chemicals can be specific for targeted pests, biodegradable to nontoxic products and therefore, considered as appropriate to apply in integrated pest management programs. In an experiment, Solangi *et al.* [26] showed that the botanical extracts, such as neem oil, neem seed powder solution, tobacco leaf solution and eucalyptus leaf solution exhibited strong repellent effects on *B. zonata*. The neem based insecticide has negative effect on pupae formation and adult emergence of Western fruit fly [27]. They also reported an assessing the efficacy of neem leaf extracts by applying *A. calamus* and *A. indica* along with food, an inverse effect on adult survival and on the development of eggs of western fruit fly.

From the above findings, no oviposition was observed at 4.0% concentration of the plant extracts. Siddique *et al.* [28] observed that the highest percent repellency were observed (80.22%) at 4.0% concentration whereas the lowest were observed (27.66%) at 0.5% concentration, and the highest anti oviposition was observed in tobacco (77.48%) at 4.0% concentration which was statistically similar to (63.36%) at 2.0% concentration of plant the extracts. Among the dose effects at 4.0% concentrations extract was the most promising against *B. cucurbitae*. Our results suggested that all the extracts have promising as an effective entomocidal effect against *B. cucurbitae* and this extracts may have some toxic chemicals. Amin *et al.* [29] investigated that the toxicity effect of water pepper (*P. hydropiper*), neem (*A. indica*) and bowstring hemp (*C. gigantea*) leaf extracts on lesser grain borer and reported that at 4.0% concentration water pepper extract exerted strong toxicity effect (80.11%). They also reported that extract toxicity increased with increasing concentration. Abdul *et al.* [30] reported that the complete repellency (100% protectant) was only occurred with *S. oryzae* when treated with the highest concentration (0.8µg/cm²) of the plant extracts. Rouf *et al.* [31] studied the toxicity of leaf powder of water pepper (*P. hydropiper*), neem (*A. indica*) and nishinda (*V. negundo*) against *C. chinensis* on lentil seeds and showed that 4.0 g of *P. hydropiper* leaf powder per 50g of lentil seeds were the most effective in reducing oviposition, and adult emergence. Shahjahan and Amin [32] studied the repellency effect of water peppe (*P. hydropiper*), neem (*A. indica*) and bowstring hemp (*C. gigantea*) plant extract on rice weevil and reported that 4.0% neem extract revealed the highest repellency effect (73.6%) followed by water pepper (68.0%) and bowstring hemp (58.0%). Hussain [33] stated that the highest toxicity of water pepper (*P. hydropiper*) leaf powder extract on the larvae of *T. castaneum* under laboratory condition. The acetone extract of de-oiled kernel powder significantly ovipositional deterred against *B. cucurbitae* at 0.5% and 1.25% [34]. The rate of repellent activity was increased with the increase with the concentration of the botanical extracts [35].

Conclusion

It has been observed that the repellent and ovipositional deterrent increased with the increase of the doses of the plant extract and also found negative effect on progeny development activities. The results on the efficiency of the extracts against *B. cucurbitae* (Coquillett) (Diptera: Tephritidae) as toxicants, repellents, ovipositional deterrent and progeny development can be helpful in developing some competent formulations for commercial use of the botanical extracts against insects.

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