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Ovipositional deterrent and repulsive effect of six botanicals against *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae)

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Abstract
The study was conducted to evaluate the efficacy of six indigenous plant extracts for their anti ovipositional and repellent effects against cucurbit fruit fly, *Bactrocera cucurbitae* (Coqullet) in ambient laboratory conditions (27±2^{0}C, 70% RH) using three different solvents. All the plant extracts had different effect of repellent and anti oviposition as well as effective for controlling *B. cucurbitae*. The order of toxicity was found as tobacco > neem > water pepper > castor > bullock's heart > eucalyptus and the solvents toxicity was acetone > methanol > water at 5.0% concentration. The lowest number of larvae (2.67), pupae and adult emergence (2.00) were found at 4.0% concentration whereas the highest larvae (10.00), pupae and adult emergence (8.00) were found at 0.5% concentration. However, the highest percent repellency were observed (80.22%) at 4.0% concentration whereas the lowest were observed (27.66%) at 0.5% concentration. The highest anti oviposition was observed in tobacco (77.48%) at 4.0% concentration which was statistically similar to (63.36%) 2.0% concentration of plant extracts.

Keywords: *Bactrocera cucurbitae* (Coquillett), Botanical extracts, Repellent, Anti oviposition

1. Introduction
Bitter gourd is one of the most important vegetables grown well in South East Asia including Bangladesh. The immature fruits and the tender leafy shoots or the ripe fruits have both nutritional and medicinal importance like potential health benefits to diabetes, arthritis, rheumatism, asthma, warts, abscesses and ulcers [1]. A compound 'Charantin' present in bitter gourd that is useful to reduce blood sugar in diabetic patients [2]. However, in spite of the immense prospect of bitter gourd in Bangladesh, many biotic, abiotic factors like diseases, insect pests, and adverse environmental conditions hampered bitter gourd productions. The melon fruit fly, *Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae) is the most destructive pest of cucurbitaceous vegetables and causes enormous economic losses of a wide variety of fruits, vegetables and flowers [3, 4]. *Bactrocera cucurbitae* is dominant in all the locations of Bangladesh followed by *Bactrocera tau* and *Dacus ciliatus* [5]. The females of this insect choose generally soft tender fruit tissues to lay their eggs. The damage starts when the female fruit fly punctures the fruit with its long and sharp ovipositor. The maggots feed inside the fruit [6, 7] 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 severely reduced. This pest has been a major limiting factor in obtaining good quality fruits and high yield [8] which cause substantial crop losses to growers and sometimes the extent of losses varies between 30 to 100%, depending on the cucurbit species and the season [6]. About 41-95% fruit infestation by cucurbit fruit fly in bitter gourd crop has also been recorded [9]. However, it is therefore, extremely important to reduce the extent of damage due to fruit flies without affecting the agro ecosystem. Although, a number of management practices, for instance use of pheromone trap, spraying of commercial neem products, bagging of fruits, field sanitation, food baits and spray of chemical insecticides [10, 11, 12, 13] have been used to manage this notorious pest but some of them either fail to control the pest and/or are not financially viable and hazardous to non-target organisms and the environment [14] as well. In fact, the farmers in Bangladesh use of toxic insecticides to control the pest in bitter gourd repeatedly. In some areas, farmers spend about 25% of the cultivation cost in bitter gourd production only to buy toxic pesticides [7].

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 [15]. 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. The botanical insecticides are biodegradable and harmless to the environment [16]. Various botanicals have been found to be effective against different pests; especially water pepper (*Polygonum hydropiper*) and neem (*Azadirachta indica*) are examples of such plants, which can possess medicinal, insecticidal, repellent or antifeedent property [17]. The efficacy of neem extracts on various insect species were noted such as repellent, antifeedent, growth retardant, molt disrupting, progeny development disrupting and oviposition deterrent [18, 19]. A number of attempts have been made to study the insecticidal efficacy of different plant extracts against cucurbit fruit fly [20, 21, 12, 13] but a lot of knowledge is still wanting and it is utmost necessary to the understanding of these pests for successfully managements. Therefore, the present study was conducted to investigate the repellent and anti ovipositional effects of six indigenous plant extracts against *B. cucurbitae* by offering treated and untreated bitter gourd fruits in free choice tests.

2. Materials and Methods

The study was carried out to evaluate the efficacy of six indigenous plant extracts for their anti ovipositional and repellent effects against cucurbit fruit fly, *B. cucurbitae* (Coqullet) in ambient laboratory conditions (27±2^{0}C, 70% RH) during the period of July to December 2016.

2.1 Collection and preparation of plant extract

The fresh healthy leaf of bullock's heart (*Annona reticulata*), water pepper (*Polygonum hydropiper*), castor (*Ricinus communis*), eucalyptus (*Eucalyptus alba*), neem (*Azadirachta indica*) and tobacco (*Nicotiana tabacum*) were collected from the surrounding local area 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 45-50^0 C. The plant materials were ground with the help of electric grinder (Nova Blackberry Blender, 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 methanol, acetone and water solvents. 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). Finally, the extracts were preserved in tightly corked vials in a refrigerator for further experimental use.

2.2 Mass culture of the test insect

Bactrocera cucurbitae infested bitter gourds were collected from the experimental field and fruit was spread in the laboratory conditions (27±2 ºC, 70% RH) on soil covered with fine cloth to prevent escape of larva. Rearing of fruit fly was done according to Rehman *et al*. [20]. In order to get fruit fly pupae daily observation was made. After pupae formation, it was kept in a petridish (150mm) for adult emergence. After getting adult, they were reared in a plastic (PVC pipe) frame cage (60 cm × 50 cm × 45 cm) covered with nylon net. The artificial prepared diet was supplied carefully with water soaked in cotton. Rearing was continued up to experimental needs. For preparation of artificial diet, the ingredient and their quantity were sweet gourd pulp (Number 2), egg yolk (Number 6), honey (4 table spoon), multi-vitamin syrup (2 table spoon), yeast (1 table spoon) and sugars (8 table spoon). The ingredients were mixed in a blender to make a thick syrup solution and the diet was kept in refrigerator with plastic pot for further use.

2.3 Administration of plant extracts

Administration of plant extracts was done to find out the effects of repellent and anti ovipositional effect of six indigenous plant extracts against *B. cucurbitae* following the primary and secondary screening:

2.3.1 Primary screening

For the primary screening, all six plants extract were taken and 5.0% concentration of the crude extracts were used for selecting high performance result. Before selecting the concentration, a pilot experiment was done (data not shown). A total volume of each concentration was made 100 ml with distilled water and 10 mg detergent (commercial product - surf') was added and stirred continuously for 10 minutes with a glass rod to make homologous [22]. Fresh healthy bitter gourd fruits were coated with each solution by dipping for 10 seconds and dried at room temperature for two hours. Treated and untreated bitter gourd fruits were offered to 5 pairs of 18-20 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 [20]. Number of fruit flies settled on treated and untreated bitter gourd fruits were counted after every one hour interval for 10 hours. After that fruits were removed from the cages and were kept for larval growth and about 8-10 days later number of larvae was counted in each replication separately of the treated and untreated fruits. Then it was kept in Petri dishes with sand and soil for adult emergence. Finally, the emerged adult was counted from the treated and untreated fruits.

2.3.2 Secondary screening

From the primary screening tobacco leaf extract with acetone solvent showed the better performed than other plant extracts. Therefore tobacco with acetone extract was tested in lower doses 4.0, 2.0, 1.0 and 0.5%, respectively, along with control. Three replications of each concentration were performed in each treatment, the screening protocol and observations were same as followed in the primary screening.

2.4 Data collection

Number of fruit flies settled on treated and untreated bitter gourd fruits were counted after every one hour interval for 10 hours. After that fruits were removed from the cages and were kept for larval growth. After 8-10 days, number of larvae was counted in each treatment separately of the treated and untreated fruits. Then it was kept in Petri dish with sand and soil for adult emergence. Finally, the emerged adult was counted from the treated and untreated fruits. Percent repellency and anti oviposition was calculated according to the following formulae given by Rehman *et al*. [20].

% Repellency = [{Half of the number of flies settled on both

treated and untreated fruits - number of flies settled on treated fruit} / Half of the number of flies settled on both treated and untreated fruits] × 100

% Anti oviposition = [{Half of the number of larvae on both treated and untreated fruits - number of larvae on treated fruit}/ Half of the number of larvae on both treated and untreated fruits] × 100

2.5 Statistical analysis

The collected data was statistically analyzed in accordance with Completely Randomized Design (CRD) through MSTAT - C program. The treatment mean values were compared by Duncan's New Multiple Range Test (DMRT). The graphical works was done through Microsoft Excel program.

3. Results

3.1 Effects of plant extracts on the adult settled, repellency, anti oviposition and progeny development against *B. cucurbitae* in primary screening

Mean number of adult settled and progeny (larvae, pupae recovery and adult emergence) development from bitter gourd fruits treated with six tested plant extracts are shown in Table 1. The adult settled, larvae and pupae recovery and adult emergence were significantly ($P<0.01$, df = 15) differed among all six tested plants at 5.0% concentration of the extracts. The highest number of adult settled in eucalyptus (17.50), followed by bullock's heart (17.33) whereas the lowest number were found in tobacco (9.83) which is statistically similar to water pepper (10.58). On the other hand, the highest number of larvae and pupae recovery were found in eucalyptus (16.00 and 16.50 respectively), followed by bullock's heart (16.50 and 14.25 respectively) whereas the lowest were found in tobacco (9.08 and 7.42 respectively). The adult emergence was found highest in eucalyptus (16.75) and lowest in tobacco (7.67) which is statistically similar to water pepper (8.17) and neem (8.50). In all cases the lowest adult settled and progeny indicated the highest toxicity of the plant extracts at 5.0% concentration in primary screening. On the basis of the adult settled and progeny development the order of the toxicity of the plant extracts were found in tobacco > neem > water pepper > castor > bullock's heart > eucalyptus.

Table 1: Mean number of adult settled and progeny development on bitter gourd fruits treated with six different plant extracts at 5.0% concentration

Plant Extract	Mean Number of			
	Adult settled	Larvae recovered	Pupae recovered	Adult emerged
Bullock's heart	17.33 a	16.50 a	14.25 b	14.33 b
Water pepper	10.58 cd	10.58 c	7.917 d	8.17 d
Castor	14.17 b	14.08 b	11.33 c	11.58 c
Eucalyptus	17.50 a	16.00 a	16.50 a	16.75 a
Neem	11.75 c	10.33 c	8.25 d	8.50 d
Tobacco	9.83 d	9.08 c	7.42 d	7.67 d
LSD at 5% level	1.81	1.59	1.79	2.28
CV%	16.25	15.17	19.88	24.86

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.

Figure 1 represents the repellency and anti ovipositional effects of six tested plant extracts against *B. cucurbitae* in bitter gourd fruits. The percent repellency and percent anti oviposition of six different plant extracts was significantly ($P < 0.01$, df = 10) differ among all the treatment applied at 5.0% concentration. The highest repellency rate was observed in tobacco (85.34%) whereas the lowest was found in eucalyptus extract (43.91%) treated bitter gourd fruits. Tobacco also showed the highest oviposition deterrent (96.46%) whereas the lowest was in eucalyptus (55.68%) followed by bullock's heart (55.85%) treated bitter gourd fruits.

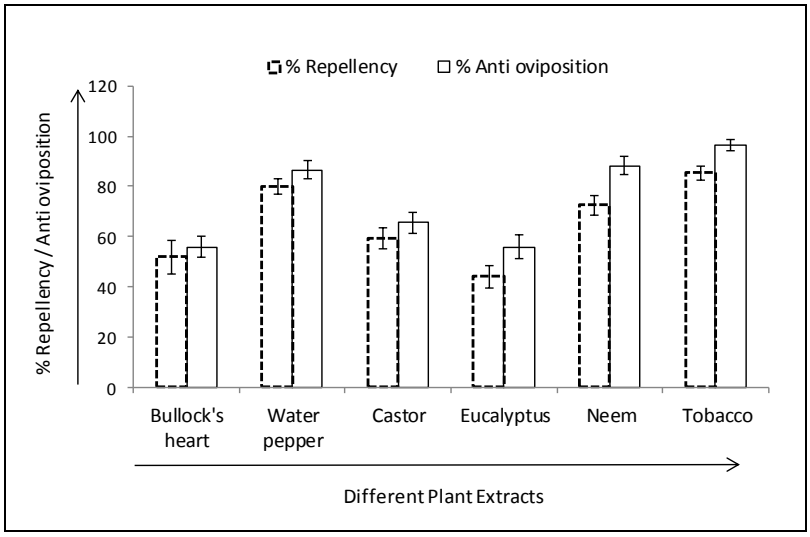

Fig 1: Repellency and anti ovipositional effects of six different tested plant extracts against cucurbit fruit fly, *B. cucurbitae* in bitter gourd fruits

The highest number of adult settled, percent repellency, percent anti oviposition and progeny development were found in control which was significantly ($P < 0.01$) differed from all the solvent extracts used the primary screening at 5.0% concentration of the plant extracts (Table 2). Among the solvent extracts the highest adult settled (10.78), which was significantly similar to larvae (7.94), pupae (6.67) recovery and adult emergence (6.56) were found in water extract whereas the lowest adult settled (4.50), larvae and pupae (3.33) recovery and adult emergence (4.11) were in acetone extract. On the other hand, the percent repellency and anti oviposition deterrent of different solvent extracts was significantly ($P<0.01$, df = 10) differ among all the treatment applied at 5.0% concentration. The highest percent repellency was observed in acetone (76.23%) whereas the lowest was found in water (55.19%). Acetone also showed the highest oviposition deterrent (84.01%). The lowest oviposion deterrent was observed in water (65.74%). On the basis of adult settled, percent repellency and percent anti oviposion, the order of toxicity of the solvent extracts was acetone > methanol > water against *B. cucurbitae* at 5% concentration.

Table 2: Mean number of adult settled, percent, repellency, percent anti oviposition and progeny on bitter gourd fruits treated with different solvent extracts

Solvent Extract	Mean Number of					
	Adult settled	% repellency	Larvae recovered	% anti oviposition	Pupae recovered	Adult emerged
Water	10.78 b	71.66 b	7.94 b	81.86 a	6.67 b	6.56 b
Methanol	7.17 c	78.99 a	5.44 c	64.04 c	5.33 bc	5.33 bc
Acetone	4.50 d	83.03 a	3.33 d	78.96 b	4.11 c	4.11 c
Control	31.67 a	-	34.33 a	-	27.67 a	28.67 a
LSD at 5% level	1.47	5.061	1.29	2.067	1.46	1.86
CV%	16.25	9.59	15.17	4.07	19.88	24.86

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.

The combined effect of six different plants extracts of different solvents on the adult settled, repellency, anti oviposition and progeny development against *B. cucurbitae* on bitter gourd fruits are presented in the Table 3. The highest number of adult settled was found in untreated control which was significantly ($P < 0.01$) differed from all the solvent extracts used in primary screening at 5.0% concentration of the plant extracts. The lowest number of adult settled in tobacco and water pepper with acetone (1.67) extracts indicated the highest toxicity of the plant extracts followed by neem with acetone extract (3.33). The repellency and anti oviposition rate of six plant extracts with different solvents were found statistically significant among all the treatments. The highest percent repellency was observed in tobacco (89.97%) and water pepper (89.87%) with acetone extract whereas the lowest repellency rate was observed in bullock's heart (23.02%) followed by eucalyptus (36.13%) with water extract which is statistically similar to eucalyptus with methanol and acetone extracts (44.72% and 50.88% respectively). On the other hand, no oviposition (100% protectant) observed in tobacco (methanol and acetone extracts), neem and water pepper with acetone extract. The highest number of larvae (34.33) and pupae (27.27) recovery, and adult emergence (28.67) were found in control treatment which was significantly ($P < 0.01$, df = 15) differed of all the plant with solvent extracts used in the primary screening. However, no larvae were found in tobacco (water and methanol extracts), neem and water pepper with acetone solvent extracts. The lowest numbers of larvae were found in neem (1.67) with methanol extract and water pepper (2.67) with water solvent extract. On the other hand, the highest number of larvae were found in bullock's heart (18.33) with water solvent extract, eucalyptus with water, methanol and acetone solvents extract (11.00, 10.00 and 8.67, respectively) which is statistically similar to castor with water extract (8.33), bullock's heart and castor with methanol extract (8.00 and 7.67 respectively). Similarly, no larvae were found in tobacco and neem (methanol and acetone extracts), water pepper (acetone and water solvent extracts). The lowest numbers of pupae were found in tobacco (2.00) and castor (3.67) with water solvent extract. On the other hand the highest numbers of pupae were observed in bullock's heart (16.00) with water solvent extract which is statistically similar to eucalyptus with methanol and acetone solvents extract (14.00 and 15.67 respectively). On the contrary no adult were emerged in tobacco and neem with acetone extracts, and water pepper with water solvent extract. The minimum numbers of adult emerged were in tobacco and castor with water solvent extract (2.00 and 3.67 respectively), whereas the highest number of adult emerged in bullock's heart (15.33) with water solvent extract, followed by eucalyptus with methanol and acetone extracts 14.00 and 15.67, respectively.

On the basis of the adult settled, percent repellency and oviposition deterrent potency showed the highest toxicity in tobacco, followed by neem and water pepper. On the other hand the highest toxicity was found in acetone extract at 5.0% concentration among all the solvents. Hence, tobacco was further assessed at the lower doses 0f 4.0, 2.0, 1.0 and 0.5%, respectively in terms of adult settled, repellency, anti oviposition potency as like primary screening.

Table 3: Combined effects of plants extract with solvents on the adult settled, percent repellency, percent anti oviposition and progeny development against *B. cucurbitae* on bitter gourd fruits at 5.0% concentration

Plant Extract	Solvent	Mean Number of					
		Adult settled	% repellency	Larvae recovered	% anti oviposition	Pupae recovered	Adult emerged
	Water	24.67 b	23.02 h	18.33 b	30.94 e	16.00 b	15.33 b
Bullock's heart	Methanol	8.33 ef	58.58 def	8.00 cd	63.07 cd	8.00 cd	8.00 c
	Acetone	4.67 fgh	74.26 bc	5.33 def	73.54 bc	5.33 cde	5.33 cd
	Water	4.33 fgh	75.89 abc	2.67 efg	86.21 ab	0.00 f	0.00 e

Water pepper	Methanol	4.67 fgh	74.16 bc	5.33 def	73.76 bc	4.00 de	4.00 cde
	Acetone	1.67 h	89.87 a	0.00 g	100.0 a	0.00 f	0.00 e
	Water	10.00 de	52.25 ef	8.33 cd	62.02 cd	8.00 cd	8.00 c
Castor	Methanol	9.67 de	53.82 ef	7.67 cd	63.59 cd	6.00 cde	6.00 cd
	Acetone	5.33 fgh	71.41 bcd	6.00 de	70.67 c	3.67 ef	3.67 cde
	Water	15.00 c	36.13 gh	11.00 c	52.09 d	8.67 c	8.67 c
Eucalyptus	Methanol	13.00 cd	44.72 fg	10.00 c	55.11 d	14.00 b	14.00 b
	Acetone	10.33 de	50.88 ef	8.67 cd	59.85 cd	15.67 b	15.67 b
	Water	7.33 efg	62.56 cde	5.33 def	73.78 bc	5.33 cde	5.33 cd
Neem	Methanol	4.67 fgh	74.26 bc	1.67 g	91.17 a	0.00 f	0.00 e
	Acetone	3.33 gh	80.98 ab	0.00 g	100.0 a	0.00 f	0.00 e
	Water	3.33 gh	81.28 ab	2.00 fg	89.37 a	2.00 ef	2.00 de
Tobacco	Methanol	2.67 h	84.76 ab	0.00 g	100.00 a	0.00 f	0.00 e
	Acetone	1.67 h	89.97 a	0.00 g	100.00 a	0.00 f	0.00 e
Control		31.67 a	-	34.33 a	-	27.67 a	28.67 a
LSD at 5% level		3.61	13.16	3.18	13.08	3.57	4.56
CV%		16.25	12.13	15.17	10.57	19.88	24.86

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.

3.2 Effects of different doses of tobacco plant extracts on the adult settled, repellency, anti oviposition and progeny development against *B. cucurbitae* in secondary screening

The adult settled, percent repellency, percent anti oviposition and progeny development were found in untreated control which was statistically ($P < 0.01$) differed among the different doses tobacco extracts in bitter gourd treated fruits (Table 4). The lowest number of larvae (2.67), pupae and adult emergence (2.00) in 4.0% concentration whereas the highest larvae (10.00), pupae and adult emergence (8.00) were found in 0.5% concentration. On the other hand, the highest percent repellency were observed (80.22%) at 4.0% concentration whereas the lowest were observed (27.66%) at 0.5% concentration. The highest anti oviposition observed in tobacco (77.48%) at 4.0% concentration which is statistically similar to (63.36%) at 2.0% concentration.

Table 4: Mean number of adult settled, percent repellency, percent anti oviposition and progeny development on bitter gourd fruits treated with different doses tobacco plant extracts.

Dose (%)	Mean Number of					
	Adult settled	% repellency	Larvae recovered	% anti oviposition	Pupae recovered	Adult emerged
4.0	2.33 d	80.22 a	2.67 c	77.48 a	2.00 b	2.00 b
2.0	4.33 d	67.07 b	4.67 bc	63.36 ab	3.67 b	3.67 b
1.0	8.00 c	46.50 c	7.33 bc	47.85 bc	6.33 b	6.33 b
0.5	12.33 b	27.66 d	10.00 b	35.99 c	8.00 b	8.00 b
Control	21.67 a	-	21.67 a	-	21.67 a	21.67 a
LSD at 5% level	3.38	13.10	5.42	22.77	5.69	5.69
CV%	19.13	12.56	32.13	21.53	37.57	37.57

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.

4. Discussion

The results of the present study indicated that all the plant extracts had varying degrees of repellent and anti ovipositional effects as well as effective for controlling cucurbit fruit fly. Insecticidal property of any plant material depends on the active constitutes of the plant material. The acetone extract was the most promising against *B. cucurbitae*. The result suggested by Shivendra *et al.* [23] where they opined that the acetone extract of de-oiled kernel powder significantly deterred oviposition against *B. cucurbitae* at 0.5% and 1.25%. Najmeh *et al.* [24] observed that the aqueous extracts of *Nicotiana tabacum* and *Eucalyptus globulus* caused 77.55 and 72.5% mortality of larvae of *Lycoriella auripila* at concentration of 4000 ppm after 72h, respectively. Toxicities of tobacco extract after 24, 48 and 72 h were 1.52, 1.85 and 1.70 times greather than eucalyptus, respectively. Asawalam *et al.* [25] reported that *N. tabacum* and *C. frutescens* were affect adult emergence of *S. zeamais* on maize grains revealing 10.0% and 12.0% emergence respectively. Maximum repellency and anti oviposition effect was recorded in acetone extract of water pepper, neem and tobacco at higher concentration level. Amin *et al*. [26] reported that the toxicity increased with increasing of concentration. Insect repellent activity has been found in many plant species [27]. From the above findings, no oviposition was observed in tobacco with acetone extract at 5.0 and 4.0% concentration of the plant extracts. Our results also suggest that all the extracts have promising potential as an effective insecticide against *B. cucurbitae* and this extracts may have some toxic chemicals. It has been observed that the repellency and oviposition deterrent activity was increased with the increase of the doses of the plant extract. According to Kundu *et al*. [28], the rate of repellency was increased with the increment of concentration.

5. Conclusion

The study revealed the efficacy of six indigenous plant extracts for their repellent and antiovipositional activity against *B. cucurbitae.* Thus, our study clearly indicates that the possible utilization of the chemicals present in the selected leaf extracts could be used as a component of pest management programed and also stability of the compounds for practical application against *B. cucurbitae*. Furthermore to study their chemosterilant, neurotoxic and geno-toxic effects are promising as such compounds may also have effects on hormonal imbalance and reproductive physiology of insects. These findings can be supportive in developing some useful

formulations for commercial use against insects.

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