Screening and Biological Activity of Indigenous Plant Extracts against Pulse Beetle, *Callosobruchus Chinensis* (Bruchidae: Coleoptera)

Md. Abdul Ahad[1], Md Fazlul Haque[2] , Mst. Mottakina Akter[1], Md Najrul Islam[3] and Sheikh Arafatur Rahman[3]

[1]Department of Entomology, [2]Department Medicine Surgery and Obstetrics, [3]Department of Pathology and Parasitology, Hajee Mohammad Danesh Science and Technology University, Dinajpur (5200), Bangladesh

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Correspondence to

E-mail: aahad_hstu@yahoo.com

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Abstract

The experiments were conducted in the laboratory of Department of Entomology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, during June 2009 to June 2010. N-hexane solvent extracts of 13 local plants were tested for screening of their insecticidal activity against pulse beetle, *Callosobruchus chinensis* L. All plants extracts were showed insecticidal activity by affecting through mortality, inhibition of F_1 adult emergence, reduced seed damage and fecundity or acting repellency. *Emblica officinalis* and *Annona reticulata* extracts were showed 100% mortality within 72 hours. Nerium oleander showed 90% and 96.67% mortality in 2%, and 3%, respectively. *Annona reticulata* and *Polygonum hydropiper* extract reduced the seed damage 2% and 9.33% at 0.5% to 3%, respectively. *Annona reticulata* and *Polygonum hydropiper* also reduced the fecundity of pulse beetle. Repellency class varies II to IV; but *Polygonum hydropiper* showed significant results following by *Lantana camara*.

1. Introduction

Pulse is one of the best sources of plant protein and plays an important role in the diet of common people of developing countries like Bangladesh (Darmadi-Blackberry et al., 2004). It has been reported that cultivated area under pulse crops in Bangladesh coverage 3113603.24 ha (769000 acres) with annual production of 259000 tons (BBS, 2007). There are 29 species of pulse such as green gram (*Vigna radiata*); black gram, (*Vigna mungo*), dry peas (*Pisum spp.*) garden pea (*Pisum sativum* var. sativum), pigeon pea (*Cajanus cajan*), lentil (*Lens culinaris*) etc. are cultivated and stored for future consumption (FAO, 1994). Pulses are called "vegetarian's meat" (Darmadi-Blackberry et al., 2004). In Bangladesh 50 species of insect are considered injurious to food grains and their products (Ahad, 2003). But in India there are about 200 species of pest insects which cause damage to stored grains and grain products in storage (Maniruzzaman, 1981). Among these, the pulse beetles *Callosobruchus* spp. are the major pests in stored pulse (Ahad, 2003). Most of the cereals and pulses have to be stored by the producer in their home and by the traders and the Governmental agencies in go-downs for one year or more for future use. Insects pests are the major problem for storing cereals and pulses. It has been reported that pulse beetle, *Callosobruchus chinensis* is a major economically importance pest of all pulses and causes 40-50% in losses of pulses storage (Gosh and Durbey, 2003).

Generally management of stored product pest is done through fumigation (Page and Lubatti, 1963; Lemon, 1967) and also is controlled by synthetic insecticides (Atwal and Dharial, 2005), which have many limitations and undesirable side effects. Insecticides have been used for a long time with serious drawbacks. Indiscriminate use of insecticides to protect pulse beetle in storage may cause serious health hazard as well as destruction of beneficial insect and increasing costs of application (Kavadia et al., 1984; Desmarchelier, 1985; Fishwich, 1988; and Singh et al., 2001). Global warning has cautioned us and the adverse consequences of insecticide use are always alarming and also inducing pest out break because of pest resistance.

In this condition, alternative methods of insect control utilizing botanical products are being used in many countries. Botanical insecticides are biodegradable, relatively specific in the mode of action and easy to use (Das, 1986); and are environmentally safe, less hazardous, less expensive and readily available (Ahmed

et al., 1993). Many workers at home and abroad studied on the insecticidal properties of plant materials (Ahad, 1994; Kestenholzetal et al., 2006; Fokunang et al., 2007; Shukla et al., 2007and Kirubal et al., 2008). It is seen from reviews and literatures that research work on indigenous plants extracts like namely *Vitex negundo* (Nisinda), *Ziziphus jujube* (Barai), *Tgetes erecta*, *Aegel marmelos*, *Polygonum hydropiper*, *Leucas aspara*, *Murraya exotica, Mimosa pudica, Ficus sp, Lantana camara*, *Nerium oleander, Emblica officinalis* and *Annona reticulata* on pulse beetle, C*allosobruchus chinensisa* in Bangladesh condition is limited.

Considering the above problem of synthetic insecticides and benefit of botanical insecticides the present research work was undertaken by thirteen indigenous plants extracts in solvent ethanol. These plants are well distributed in Bangladesh and their leaves and fruits exhibit toxicity, antifeedants, repellent and growth inhibition activity to insects. So, the thirteen indigenous plants extracts were evaluated on pulse beetle, C*allosobruchus chinensis* on green gram, *Vigna radiata* with the following objectives-to study of direct toxicity; to study of total residual toxicity.

2. Materials and Methods

The experiments were conducted in the laboratory of the Department of Entomology and Department of Agricultural chemistry, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur during July '09 to June '10. All insect cultures were maintained in the laboratory at room temperature (30 ± 2° C and with 70 ± 5 % RH) during the experiments. Leaves of *Vitex negundo, Ziziphus jujube Tgetes erecta*, *Aegel marmelos*, *Polygonum hydropiper*, *Leucas aspara*, *Murraya exotica*, *Mimosa pudica*, *Ficus sp.*, *Lantana camara*, *Nerium oleander, Emblica officinalis, and Annona reticulata* were collected from HSTU Campus. These collected materials were washed in running water and then air dried. Finally, the dried plant materials were powdered by the Mortar.

2.1. Preparation of plant dusts and extracts

The leaf powder of 100g of each desired plant were taken in 1.5 liter separated funnel and 130 ml n-hexane were added in separated funnel and were kept for 72 hours with interval of shaking. After 72 hours it was then filtered by Whatman paper No.1 (diameter 40). The filtrates liquid were aqueous extract.

2.2. Isolation of crude extracts

The aqueous extract was collected in a beaker. The solvent was evaporated by using thin film rotary evaporator under reduced pressure. Obtain crude extracts were stored in refrigerator at 0˚C for further investigation.

2.3. Rearing of test insects and maintenance

The pulse beetle, *Callosobruchus chinensis* was used for the present experiments. A small population of *C. chinensis* beetles was obtained from the entomology laboratory stock. They were reared and bred in the laboratory at room temperature (30 ± 2° C and with 70 ± 5 % RH), on diet of the seeds of green gram, *Vigna mungo* in a Jar (28cm × 13cm). Initially, 50 pairs of 1-2 day-old adults were placed in a jar containing green gram seeds. The jars were sealed with net cloth and a maximum of 7 days were allowed for mating and oviposition. Then parent stocks were removed and green gram seeds containing eggs was transferred to fresh green gram seeds in the breeding jars and again were covered with pieces of net cloth fastened with rubber bands to prevent the contamination and escape of insects. The subsequent progenies of the beetles were used for all the experiments.

2.4. Direct toxicity test

Direct toxicity tests of plants extracts on pulse beetle were completed following the method of Talukdar and Howse (1993). Insects were chilled for a period of 10 minutes. The unmobilized insects were individually picked up and one milliliter solutions of different concentrations (0.5, 1.0, 2.0 and 3.0 % w/v) were applied to the dorsal surface of the thorax of each insect by using micro capillary tube. The insects were then transferred into a 9 cm diameter petri-dishes. Ten insects per replication were treated. Insect mortality rates were recorded after 24, 48, 72 and 96 hours after treated. Insects were examined daily and those that did not move or respond to gentle touch were considered dead. All the experiments were conducted completely ramdomized design with five replications and turned to statistical analysis. Finally, the mean values were compared using DMRT (Duncan, 1951).

2.5. Total residual toxicity test

The extracts were mixed with green gram at the rate of 0.5%, 1%, 2% and 3% (w/v). The treated green grams were air dried for 20 minutes and then put into separate plastic pots (6 ×7 cm^2), so that each pot contained 10 g of green gram seeds.

$$\text{Calculation of mortality(\%)} = \frac{\text{Total number of mortality of pulse bettles in each Petridish}}{\text{Total number of pulse bettles released in each petridish}} \times 100$$

2.5.1. Fecundity test

Five pair of newly emerged beetles was released in the plastic pot (6×7 cm^2) containing green gram seeds treated with different concentrations of each plant extracts for recording oviposition and fecundity. Male and female insects were always maintained as 1: 1 ratio. Control treatments were done

side by side. The oviposition and fecundity rate were recorded after 7 days of the release of beetles. The eggs laid on green gram seeds of each treatment in the plastic pot were counted individually by using hand lens.

2.5.2. Adult emergence test

The pulse beetles were started to emerge after 30-40 days of egg laying. The emerge beetles were counted and remove every day from the container. The numbers of adult beetles were counted daily from the date of first emergence to at least 10 days. The adult emergence rate was calculated and the inhibition rates (IR %) was calculated by using the following formula:

Where,

C_n = Number of insects in control plastic pot.

T_n = Number of insects in treated plastic pot.

2.5.3. Seed damage rate test

Each seed was taken out from the pot to determine the hole(s)

$$IR\ (\%) = \frac{Cn\text{-}Tn}{Cn} \times 100$$

on each seed. Seeds containing, hole(s) were considered as damaged seeds. The number of damaged green gram seeds was counted from the random sample of 100 seeds at the end of the experiment and were recorded for each replication.

2.6. Repellency Test

The repellency test was conducted according to the method of Talukder and Howse (1994). For repellency test plants extracts were diluted with respective solvents to prepared (0.5, 1.0, 2.0 and 3.0 % w/v) solutions. Petri-dishes were divided into two parts, treated and fresh grain portion /untreated. With the help of a pipette, 1.0 ml solutions of each plant extract were applied to one half of the petri-dish.

The treated half was then air-dried. Ten insects (5 male and 5 female) were released at the centre of each Petridish and a cover was placed on the Petridish. There were five replications for each plant extract and each dose. Then the insects present on each portion were counted at hourly intervals up to fifth hour. The data were expressed as percentage repulsion (PR) by the following formula:

$PR\ (\%) = (N_C\text{-}50) \times 2$

Where, N_C = the percentage of insects present in the control half.

Positive (+) values expressed repellency and negative (-) values attractions. Data (PR%) were analyzed using analysis of variance (ANOVA). The average values were then categorized according to the following scale (McDonald et al., 1970).

2.7. Chemical Investigation of the best performance plant extract

The plant Species (*Annona reticulata*) leaf extract showed best performance and it was investigate in following way-

2.7.1. Preparation of TLC plate

The slurry was prepared by the slow addition with shaking 30gm of the absorbent silica gel to 100 ml of chloroform in a wide-racked capped bottle. A pair of microscopic slides was held together and dipped into the slurry; slowly withdrawn and allowed to drain momentarily while held over the bottle. The slides were separated carefully and set horizontally in a rack; those were then dried in the electric oven at 30-40°C for 10-15 minutes (Furniss et al., 1988).

2.7.2. Examination of TLC on individual extracts

TLC was checked for the extracts using different solvents to detect the presence of different compounds or components on of extracts. The R_f value of different extracts was calculated using following equation-

The TLC was carried on the glass plates (slides) coated with the silica gel G type 60 (BDH, England).

2.8. Statistical analysis

The experimental data was statistically analyzed by Completely Randomized Design (factorial CRD) using MSTAT statistical software in a microcomputer. Mean values were adjusted by Duncan's Multiple Range test (Duncan, 1951).

$$Rf = \frac{\text{Distance traveled by the component}}{\text{Distance traveled by the solvent front}}$$

3. Results and discussions

3.1. Direct toxicity (through mortality test) effect of different plant extracts on Callosobruchus chinensis

Direct toxicity effect of different plant extracts on *C. chinensis* were calculated by observing mortality at 24 hours, 48 hours, 72 hours, and 96 hours are presented in Table 1 and are described below-

Data were significantly different of each treatment ($p \leq 0.05$). Among the 13 plants extracts, 2 (two) plants extracts were showed statistically best insecticidal activity through performing highest mortality (showed 100 mortality) such as *Emblica officinalis at* 3% in 72 hours; *Annona reticulata* at 2% in 72, hours and at 3% in 48 hours (other than, in the control mortality was 0) and have no significant different in each other. So, these plant extracts showed best performance for the mortality of *C. Chinensis*.But in *Nerium oleander* 90% and 96.67% mortality showed at 2%, and 3%, respectively both in 72 hours; other than, in the control mortality was 0.Nevertheless, 8 plants extracts showed medium performance for the mortality of *C. chinensis* such as *Vitex negundo, Tgetes erecta, Polygonum hydropiper, Leucas aspara, Murraya exotica, Mimosa pudica,* and *Lantana camara* and 60%-70% motality was observed in these

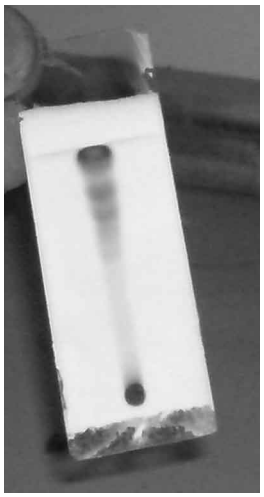

Figure 1: TLC of ethanol crude extract of Ata (*Annona reticulata*) solvent ratio (hexane:ethyleacetate at 3:1)

Solvent mixture	Ratio of solvent	Component	Rf value
Hexane: Ethyl acetate	3:1	S1	0.97
Hexane: Ethyl acetate	3:1	S2	0.91
Hexane: Ethyl acetate	3:1	S3	0.84

extracts of all at 3% in 96 hours; but over 80% mortality was observed in *Nerium oleander, Emblica officinalis* and *Annona reticulata* at 2%, and 3% in 48, 72, and 96 hours.

The above experimental results are quite in harmony with of a lot of previous workers, who found the direct toxicity effect of plant material on stored grain pests and are discuss here- Singh et al., (1989) observed that soybean oil at 0.5% caused 100% adult mortality of *C. chinensis*. Saxena, et al. (1992) pointed out that aerial parts of *Lantana camara* used against *Callosobruchus chinensis* and found that petroleum ether and methanol extracts of the plant caused 10-43% mortality at 1.5% concentrations. Lawati et al. (2002) drew attention that extracts of eight local plants of Oman and found that extracts from the seeds of *A. squamosa* caused 100% mortality of beetles within 24 hours of their exposure in methanol extracts. The other extracts that caused high mortality were *A. nilotica*, *C. juncea*, *M. communis* and *S. aegyptica* in methanol and *B. saca, J. dhofarica, S. aegyptica* and *A. indica* in ethanol. Hossain et al. (2008) pointed out that black pepper (*Piper nigrum*), ceylon cinnamon (*Cinnamonnum zealanicum*), black cardamom (*Amonum subulatum*), nutmeg (*Myristica (fragnans*), black cumin (*Nigella sativa*), turmeric (*Curcuma longa*) and red pepper (*Capsicum frutescens*) (all the plant spices) caused 100% mortality of *C. maculatus* (F.). Okonkwo and Okoye (1996) reported that essential oils of *Dennettia tripetela* and brown pepper (*Piper guineense*) achieved 100% mortality of adults of *C. maculatus* in 24 h.

Though the botanicals tested by above mentioned workers were different from present investigations but the direct toxicity effect of the plants extracts on stored grain pests were almost similar. These results are in general agreement with above findings.

3.2. Total residual toxicity test of different plants extracts on Callosobruchus chinensis

Total residual toxicity effect of different plant extracts on *C. chinensis* were calculated by observing % seed damage, F_1 adult emergence and inhibition of fecundity is presented in Table 2 and are described below-

3.2.1. Seed damage (%) test

Seed damage (%) inhibition by *C. chinensis* is presented in Table 2. Among the 13 plant extracts, in N-hexane solvent two plants extracts such as *Annona reticulata* at 0.5%, 1% 2% and 3% cocentration (seed damage was 2-8.66%) and *Polygonum hydropiper* at 3% (seed damage was 9.33%) were statistically best and have no significantly different between these two plants extracts; whereas in control seed damage was 100%. Consequently, these two plant extrats at these concentration inhibit to less seed damage and showed best performance for the protection of the pulse seed. Next statistically best was *Polygonum hydropiper* at 2% (seed damage was 28%) following the same plant extract at 1% (seed damage was 56%). Moreover, 68-88% seed damage were found in *Vitex negundo* at 2% and 3% (seed damage was 88.67% and 75.33 %, respectively), *Tgetes erecta* at 1%, 2% and 3% (seed damage was 76.67 % and 70% and 68%, respectively), *Polygonum hydropiper* at 0.5% (seed damage was 74.67%), *Nerium oleander* at 3%, (seed damage was 88%), and *Emblica officinalis* at 3% (seed damage was 83.20 %) and these plant extracs showed least performance for the protection of the pulse seed from the infestation of pulse beetle, *C. chinensis*. whereas in control it was 100% seed damage.

3.2.2. Adult (F_1) emergence test

Among the 13 plant extracts, in N-hexane *Polygonum hydropiper* and *Annona reticulata,* at 3% (F_1 adult emergence was 31.96% and 36.67%) was found statistically best, followed by *Polygonum hydropiper* at 1% and 2% (F1 adult emergence was 40.12% and 40.53%, respectively) (Table 2).

Next best were *Vitex negundo* at 3% and *Annona reticulata* at 2% (F_1 adult emergence was 46.11% and 48.96%, respectively) respectively; whereas in control it was 90.74%. Consequently, these 2 (two) plants extracts at these % showed good performance to inhabit for the emergence of pulse beetle *C. chinensis* .Rest 9 (nine) plant plant extracts F_1 adult emergence were above 50% of all % concentration and showed lowest performance for the inhabit for the emergence of pulse beetle *C. chinensis* (Table 2).

3.2.3. Fecudity test

It is observed that among the 13 plant extracts, in N-Hexane solvent 2 (two) plants extracts were statistically best and showed highest insecticidal activity through reducing fecundity such as *Annona reticulata* 1%, 2% and 3% concentration (37.67, 25 and 5.66 eggs, respectively) and *Polygonum hy-*

Table 1: Mortality (%) test of *C. chinensis* in N-Hexane solvent extracts of the different treatments (interaction of extract concentration and time)

Name of the plant (treatment)	Concentration of the plant extracts(%)	% Mortality in hours			
		24	48	72	96
Vitex negundo (Nisinda)	0.5%	0.0000^{j}	0.0000^{l}	0.0000^{q}	23.33^{mq}
	1%	0.0000^{j}	3.333^{l}	3.333^{q}	16.67^{or}
	2%	10.00^{gj}	13.33^{il}	13.33^{oq}	23.33^{nq}
	3%	23.33^{cf}	36.67^{eg}	46.67^{fi}	70.00^{df}
Ziziphus jujube (Barai)	0.5%	0.0000^{j}	0.0000^{l}	0.0000^{q}	3.333^{rs}
	1%	13.33^{fi}	13.33^{il}	13.33^{nq}	20.00^{nq}
	2%	20.00^{dg}	26.67^{i}	30.00^{jn}	36.67^{kn}
	3 %	30.00^{cd}	36.67^{g}	43.33^{gj}	53.33^{gj}
Tgetes erecta (Marigold)	0.5%	0.0000^{j}	0.0000^{l}	3.333^{q}	6.667^{qs}
	1 %	3.333^{ij}	6.667^{kl}	13.33^{oq}	13.33^{ps}
	2%	10.00^{gj}	20.00^{hk}	30.00^{jn}	36.67^{kn}
	3 %	23.33^{cf}	43.33^{ef}	60.00^{ef}	70.00^{d}
Aegel marmelos (Bel)	0.5%	0.0000^{j}	3.333^{kl}	13.33^{oq}	13.33^{ps}
	1 %	6.667^{hj}	13.33^{il}	26.67^{ko}	26.67^{mp}
	2%	16.67^{eh}	26.67^{gi}	40.00^{hk}	43.33^{il}
	3%	30.00^{cd}	46.67^{e}	63.33^{de}	73.33^{ce}
Polygonum hydropiper (Biskatali)	0.5%	0.0000^{j}	3.333^{l}	10.00^{oq}	20.00^{nq}
	1 %	3.333^{ij}	13.33^{il}	23.33^{lp}	33.33^{lo}
	2 %	16.67^{eh}	26.67^{gi}	36.67^{hl}	50.00^{hk}
	3%	33.33^{c}	46.67^{e}	56.67^{eg}	66.67^{eg}
Leucas aspara (Shetodron)	0.5%	0.0000^{j}	0.0000^{l}	16.67^{nq}	26.67^{mp}
	1%	3.333^{ij}	10.00^{jl}	30.00^{jn}	36.67^{kn}
	2 %	3.333^{ij}	10.00^{jl}	36.67^{hl}	53.33^{gj}
	3%	10.00^{gj}	26.67^{gi}	56.67^{eg}	73.33^{ce}
Murraya exotica (Kamini)	0.5%	0.0000^{j}	0.0000^{l}	13.33^{oq}	20.00^{nq}
	1 %	0.0000^{j}	3.333^{kl}	23.33^{lp}	36.67^{kn}
	2 %	3.333^{ij}	13.33^{il}	36.67^{hl}	50.00^{hk}
	3%	6.667^{hj}	23.33^{gj}	50.00^{eh}	63.33^{eh}
Mimosa pudica (Lazzabati)	0.5%	0.0000^{j}	0.0000^{l}	13.33^{oq}	23.33^{mq}
	1%	0.0000^{j}	0.0000^{l}	16.67^{nq}	23.33^{mq}
	2%	0.0000^{j}	3.333^{kl}	26.67^{ko}	43.33^{il}
	3%	6.667^{hj}	16.67^{hl}	40.00^{hk}	60.00^{eh}
Ficus sps (Dumur)	0.5%	0.0000^{j}	3.333^{k-l}	10.00^{pq}	20.00^{nq}
	1%	3.333^{ij}	3.333^{l}	13.33^{oq}	23.33^{nq}
	2%	0.0000^{j}	6.667^{kl}	20.00^{mp}	33.33^{lo}
	3%	6.667^{hj}	16.67^{hl}	33.33^{im}	53.33^{gj}
Lantana camara (Lantena)	0.5%	0.0000^{j}	0.0000^{l}	3.333^{q}	13.33^{ps}
	1%	0.0000^{j}	0.0000^{l}	10.00^{q}	20.00^{nq}
	2%	3.333^{ij}	10.00^{jl}	23.33^{lp}	30.00^{lp}
	3%	10.00^{gj}	16.67^{hl}	43.33^{gj}	63.33^{eh}

	0.5%	3.333^{ij}	30.00^{f-h}	40.00^{hk}	40.00^{jm}
Nerium oleander (Korobi)	1%	6.667^{hj}	46.67^{e}	60.00^{ef}	63.33^{eh}
	2%	23.33^{cf}	66.67^{d}	90.00^{ac}	90.00^{ab}
	3%	33.33^{c}	76.67^{cd}	96.67^{ab}	96.67^{ab}
	0.5%	3.333^{ij}	36.67^{eg}	46.67^{fi}	56.67^{fi}
Emblica officinalis	1%	10.00^{gj}	46.67^{e}	60.00^{ef}	60.00^{eh}
	2 %	26.67^{ce}	76.67^{cd}	83.33^{bc}	83.33^{bd}
	3 %	33.33^{c}	83.33^{bc}	100.0^{a}	100.0^{a}
	0.5%	3.3.33^{ij}	70.00^{cd}	76.67^{cd}	86.67^{ac}
Annona	1%	13.33^{fi}	83.33^{bc}	93.33^{ab}	96.67^{ab}
reticulata (Ata)	(Ata)	46.67^{b}	96.67^{ab}	100.0^{a}	100.0^{a}
	3%	70.00^{a}	100.0^{a}	100.0 ^{a}	100.0^{a}
Control	0.00	0.0000^{j}	0.0000^{l}	0.0000^{q}	0.0000^{s}

Mean within a column followed by no common letter (s) are significantly different (DMRT, $p \leq 0.05$)

dropiper at 3%, (21 eggs); whereas in control it was 391 eggs. Consequently, these plant extracs at these % inhabit to lay less egg and showed statistically best performance for the reducing of fecundity of pulse beetle *C. chinensis*.

Over 300 egg was found in *Ziziphus jujube* at 0.5% and 1% (334.3 and 301.3 eggs, respectively); *Leucas aspara* at 0.50 % (362.7 eggs); *Murraya exotica* at 0.50 % (343.3 eggs); *Mimosa pudica* at 0.5% and 1% (348.3 and 311.7 eggs, respectively); *Ficus* sp. at 0.50 % (321 316 eggs), and *Lantana camara* at 0.5% 1% and (389.3, 372.3 and 322.7 eggs, respectively); whereas in control it was 391 eggs and there were no statistically differences between the control; as well as showed no performance for the reducing of fecundity of pulse beetle, *C. chinensis* (Table 2).

The above experimental results about total residual toxicity are fairly synchronization with those of the earlier of a lot of workers such as-

Ahad et al. (1994) showed that Coconut oil, Mustard oil, Soybean oil protect pulse from the attacked of pulse beetle *C. chinensis* L. up to 9 months. Elhag (2000) tested extracts from nine plant materials as oviposition deterrents against *C. maculate.* Mulatu and Gebremedhin (2000) showed that the oils of *A. indica*, *Milletiaie ferruginea* and *Chrysanthemum cinerariaefolium* were the most effective in partially or completely preventing egg laying, and pulse beetles emergence from the laid eggs. Tapondjou et al. (2000) found that the dry ground leaf of *Chenopodium ambrosioides* inhibited F_1 progeny and adult emergence of the *C. chinensis* and *C. maculatus*. Kestenholzetal et al. (2007) observed that extracts (1% and 5% w/w) of *Cassia sophera* were effective at reducing *C. maculatus* infestation and adult emergence. Shukla et al. (2007) cited that the leaf powders of *Murraya koenigii* and *Eupatorium cannabinum* were most effective in reducing oviposition and causing the mortality of bruchids at dose of 2% (w/w). The F_1 emergence from the infested chick pea was significantly reduced.

The above literatures more or less confirm or common agreement about the results of total residual toxicity of the present research. The above literatures more or less confirm or common agreement about the results of total residual toxicity of the present research.

3.3. Repellency test

Percent mean (of 1hour 2 hour, 3 hour, 4 hour, and 5 hour) repellency rate against C. *chinensis* in n-hexane solvent extracts are presented in Table 3. Data were statistically significantly different of each treatment ($p \leq 0.05$). % Mean repellency rate was varies repellency class II to IV. Among the 13 plants extracts, in n-hexane solvent 1 (one) plants extracts, *Polygonum hydropiper* at 3% showed statistically best insecticidal activity through showing highest repellency rate (repellency rate was 78.89 % and class was IV) following by *Lantana camara* at 3% (repellency rate was 77.22% and class was IV)); whereas in control it was 29.45% and class was II.

Least repellency or third category repellency rate (that is 20-40% repellency rate, class II) was observed was only in *Tgetes erecta* at 0.5% (mean repellency rate was 32.78%) and in control (mean repellency rate was 29.45% and class II).

The above experimental results are quite in harmony with of a lot of previous workers, who found the repellent effect of plant material on stored grain pests and are discuss here-

Shah et al. (2008) evaluated the repellent properties of leaves of *Typhonium trilobatum*, *Cleome viscosa*, *Cassia occidentalis*, *Pongamia pinnata*, *Mesua ferrea*, and *Trewia nudiflora* against *Oryzaephilus surinamensis* at 2.5, 5.0, 7.5, and 10% concentrations and observed that mean repellency rate of extracts

Table 2: Total residual toxicity test of different leaf extract on pulse beetle, *C. chinensis* using n-hexane solvent

Treatments	Concentration of the plant extracts (%)	No. of Eggs	% F1 adult emergence	% Seed damage
Vitex negundo (Nisinda)	0.5%	314.3^{c-h}	61.80^{g-o}	100.0^{a}
	1%	200.0^{n-s}	79.87^{b-d}	98.67^{a}
	2%	148.7^{s-v}	69.79^{d-m}	88.67^{b}
	3%	91.67^{w-y}	48.96^{o-t}	75.33^{de}
Ziziphus jujube (Barai)	0.5%	334.3^{b-f}	73.65^{d-h}	100.0^{a}
	1%	301.3^{d-i}	72.81^{d-i}	100.0^{a}
	2%	239.7^{j-o}	72.02^{d-i}	100.0^{a}
	3 %	191.7^{o-s}	63.42^{e-o}	100.0^{a}
Tgetes erecta (Marigold)	0.5%	206.7^{m-r}	65.91^{d-n}	96.67^{a}
	1 %	122.0^{u-x}	73.67^{d-h}	76.67^{cd}
	2%	99.33^{v-y}	55.22^{k-r}	70.00^{de}
	3 %	66.67^{y-z}	42.92^{q-u}	68.00^{e}
Aegel marmelos (Bel)	0.5%	268.3^{g-l}	80.10^{b-d}	100.0^{a}
	1 %	253.3^{i-m}	80.12^{b-d}	100.0^{a}
	2%	218.3^{l-q}	90.75^{a-b}	100.0^{a}
	3%	216.3^{l-r}	78.75^{d-e}	100.0^{a}
Polygonum hydropiper (Biskatali)	0.5%	131.0^{t-w}	48.20^{o-t}	74.67^{de}
	1 %	75.00^{x-z}	40.53^{r-u}	56.00^{f}
	2 %	45.67^{y-z}	40.12^{s-u}	28.00^{g}
	3%	21.00^{z}	31.96^{t-u}	9.333^{h}
Leucas aspara (Shetodron)	0.5%	362.7^{a-c}	61.48^{g-o}	100.0^{a}
	1%	294.3^{d-j}	68.44^{d-m}	100.0^{a}
	2 %	201.7^{n-s}	89.06^{a-c}	100.0^{a}
	3%	181.0^{p-t}	56.42^{j-q}	100.0^{a}
Murraya exotica (Kamini)	0.5%	343.3^{a-e}	66.30^{d-n}	100.0^{a}
	1 %	296.0^{d-j}	59.39^{h-p}	100.0^{a}
	2 %	288.7^{e-j}	73.83^{d-h}	100.0^{a}
	3%	198.0^{n-s}	57.94^{i-p}	100.0^{a}
Mimosa pudica (Lazzabati)	0.5%	348.3^{a-d}	58.57^{h-p}	100.0^{a}
	1%	311.7^{c-h}	68.41^{d-m}	100.0^{a}
	2%	217.7^{l-q}	71.02^{d-j}	100.0^{a}
	3%	174.3^{p-u}	62.65^{f-o}	100.0^{a}
Ficus sp (Dumur)	0.5%	321.0^{b-g}	72.37^{d-i}	100.0^{a}
	1%	290.7^{e-j}	66.07^{d-n}	100.0^{a}
	2%	246.0^{i-o}	70.31^{d-l}	100.0^{a}
	3%	227.7^{k-p}	46.11^{p-u}	100.0^{a}
Lantana camara, (Lantana)	0.5%	389.3^{a}	74.03^{d-h}	100.0^{a}
	1%	372.3^{a-b}	65.62^{d-n}	100.0^{a}
	2%	322.7^{b-g}	65.53^{d-n}	100.0^{a}
	3%	281.0^{f-k}	58.02^{i-p}	100.0^{a}

Nerium oleander (Korobi)	0.5%	168.3^{q-u}	94.82^{a}	96.00^{a}
	1%	170.0^{q-u}	76.33^{c-g}	97.73^{a}
	2%	160.0^{r-u}	61.37^{g-o}	98.67^{a}
	3%	100.0^{v-y}	77.96^{b-f}	88.00^{b}
Emblica officinalis (Amloki)	0.5%	263.3^{h-m}	70.48^{d-k}	100.0^{a}
	1%	260.0^{h-m}	56.57^{j-q}	100.0^{a}
	2 %	240.0^{j-o}	67.37^{d-m}	99.33^{a}
	3 %	121.7^{u-x}	54.98^{l-r}	83.20^{bc}
Annona reticulata (Ata)	0.5%	58.33^{y-z}	45.44^{p-u}	8.667^{h}
	1%	37.67^{z}	51.10^{n-t}	4.400^{h}
	2%	25.00^{z}	48.89^{o-t}	4.400^{h}
	3%	5.667^{z}	36.67^{t-u}	2.000^{h}
Control	0.00	391^{a}	90.74^{a-b}	100.0^{a}

Table 3: Repellency test of different plant extracts on *Callosobruchus chinensis* using treated filter paper in n-hexane solvent

A	B	C	D
Nerium oleander (Korobi)	0.5%	43.89^{f-j}	III
	1%	47.78^{e-j}	III
	2%	53.33^{c-i}	III
	3%	54.45$^{c- i}$	III
Emblica officinalis (Amloki)	0.5%	47.78^{e-j}	III
	1%	53.33^{c-i}	III
	2.0%	53.33^{c-i}	III
	3 %	57.22^{a-I}	III
Annona reticulata (Ata)	0.5%	34.44^{ij}	II
	1 %	42.78^{g-j}	III
	2%	49.44^{e-j}	III
	3%	62.22^{a-h}	IV
Vitex negundo (Nisinda)	0.5%	61.11^{a-h}	IV
	1%	62.78^{a-h}	IV
	2%	66.67^{a-f}	IV
	3%	67.22^{a-e}	IV
Ziziphus jujube (Barai)	0.5%	43.89^{f-j}	III
	1%	47.22^{e-j}	III
	2%	57.22^{a-i}	III
	3%	59.45^{a-h}	III
Tgetes erecta (Marigold)	0.50%	29.45^{j}	II
	1%	40.00^{h-j}	III
	2 %	52.78^{c-i}	III
	3 %	53.33^{c-i}	III
Polygonum hydropiper (Bishkatali)	0.5%	63.33^{a-g}	IV
	1 %	73.33^{a-d}	IV
	2 %	75.56^{a-c}	IV
	3%	78.89^{a}	IV
Aegel marmelos (Bel)	0.5%	40.00^{h-j}	III
	1%	42.22^{g-j}	III
	2. %	48.89^{e-j}	III
	3. %	54.44^{c-i}	III
Lantana camara (Lantena)	0.5%	56.11^{b-i}	III
	1%	65.00^{a-g}	IV
	2 %	70.00^{a-e}	IV
	3 %	77.22^{ab}	IV
Ficus sp (Dumur)	0.5%	58.89^{a-h}	III
	1 %	61.11^{a-h}	IV
	2%	63.89^{a-g}	IV
	3 %	69.44^{a-e}	IV
Mimosa pudica (Lazzabati)	0.5%	53.89^{c-i}	III
	1 %	55.56^{b-i}	III
	2%	56.67^{b-i}	III
	3%	57.78^{a-h}	III
Murraya exotica (Kamini)	0.5%	47.22^{e-j}	III
	1 %	51.11^{d-j}	III
	2 %	57.78^{a-h}	III
	3 %	58.34^{a-h}	III
Leucas aspara (Shetrodron)	0.5%	43.33^{g-j}	III
	1 %	51.11^{d-j}	III
	2 %	51.67^{d-j}	III
	3 %	65.00^{a-g}	IV
Control (Pure n-hexane)		29.45^{j}	II

A: Name of the plant; B: Concentration of the plant extracts (%); C: % mean repellency rate in n-hexen solvent extracts (1hour 2 hour, 3 hour, 4 hour, and 5 hour); D: Repellency class; Mean within a column followed by no common letter(s) are significantly different (DMRT, $p \leq 0.05$)

of acetone and ethanol solvents of all six plants were in the same repellency class i.e. class III and the rate of repellency increased with the increase of dose level. Shahjahan and Amin (2000) reported that water extracts and powdered material of akanda, *Asclepias calotropis*, biskatali, *Polygonum hydropiper* and neem, *Azadirachta indica* were evaluated for their repellency and found that 2, 3 and 4% water extracts of all three plants repellency class varies II to IV (37.6 % to77.6%) against *Rhizopertha dominica* and repellency class varies II to IV (35.2% to73.65) against *Sitophillus oryzae.* Qureshi et al. (1988) tested 19 plant extracts for their repellent activity against *T. castaneum* and reported that an acetone extract of *Ageratum houstonianum* flowers and alcohol extract of *Valeriana wallichi* rhizomes had the strongest repellent activity.

These literatures more or less support/general agreement about the results of repellency effect against *C. chinensis* of the present investigation.

4. Chemical Investigation of the best performance plant extract

The ata (*Annona reticulata*) leaf extract showed best performance only. The result in this experiment indicated that the aqueous extract of ata *Annona reticulata* leaf showed highest insecticidal activities on *Callosobrushus chinensis* L.. The above interesting results encouraged taking why and how this type of plant is responsible for insecticidal activities. So, the crude compounds were extracted from the powder of that plant *Annona reticulata* with non-polar solvent ethanol and N-hexane. The crude compound was then preceded for TLC examination. The TLC (Thin Layer Chromatography) of ethanol extract of common ata *Annona reticulata* leaf showed four compounds distinctly at Hexane: Ethyleacetate, this result suggested that it contained three distinct compounds, designated as S_1, S_2 and S_3 respectively.

The R_f value of (Figure 1) were also summarized in tabular form. From the R_f value it is clear that ethanol extracts of ata *Annona reticulata* contain three distinct compounds. Any of these three compounds may toxic nature or all of them may contain toxic nature, which may responsible for controlling the insects.

4. Conclusion

All extracts tested were effective to causing mortality, reduced the ovipositional preferences and adult emergence of F_1 progeny of pulse beetle and reduced seed damage. *Annona reticulata, Nerium oleander, Emblica officinalis* plant extracts may be useful as a seed protectant materials against the pulse beetle, *Callosobruchus chinensis.* So, the above plant extracts can be highly effective against the stored product insects like pulse beetle.

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