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Biological Activities of Five Weed Extracts against *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

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식물추출물의 팥바구미에 대한 생물활성

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ABSTRACT: This study was conducted with n-hexane extracts of sensitive plant *Mimosa pudica*, mexican poppy *Argemone mexicana*, panimarich *Leucus aspara*, water pepper *Polygonum hydropiper* and shialmutra *Blumea lacera* weeds against pulse beetle *Callosobruchus chinensis* (Coleoptera: Bruchidae) for protection of mung bean *Vigna radiata* grains. The LC_{50} values of the weed extracts ranged from 4.5 to 6.4, 4.1 to 5.6 and 3.6 to 5.5 g/100 mL at 24, 48 and 72 hours of post treatment, respectively. The extracts showed 35 to 69% fecundity and 33 to 63% adult emergence inhibitory effect on the pest, and revealed 13 to 49% grain protection of mung beans. Insect mortality, fecundity and adult emergence inhibitory effects, and grain protection activity increased with increased concentration of the extracts. The shialmutra followed by water pepper extracts revealed better performances in fecundity and adult beetle emergence inhibitory effect compared to the other weeds. The findings proved that the n-hexane extracts of the five weeds are sources of botanical insecticides which may be used in the integrated management of *C. chinensis*.

Key words: Bruchids, mortality, plant extract, *Vigna radiata*

초 록: *Mimosa pudica* (미모사), *Argemone mexicana* (멕시코 가시양귀비) *Leucus aspara* (꿀풀과 일종), *Polygonum hydropiper* (여뀌), *Blumea lacera* (국화과 일종) 등 5종의 식물 헥산추출물들의 팥바구미 성충에 대한 살충, 성충우화억제력 및 녹두 종실피해 방제력이 검증되었다. 그 결과, 팥바구미 성충 살충력은 35-69%이었으며, 녹두에 추출물을 처리한 후 성충우화 방제율은 33-63%, 종실 피해 방제율은 13-49%이었다. 추출물의 농도가 증가할수록 살충력, 성충우화 및 종실피해 방제율 등이 증가하는 것으로 나타났다. 이와 같이 5종의 식물 헥산추출물들은 팥바구미 친환경 방제제로 이용가능성이 있을 것으로 판단된다.

검색어: 팥바구미, 살충력, 식물추출물, 녹두

The pulse beetle *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is one of the most destructive pests of mung bean *Vigna radiata* L. (Leguminosae) in Bangladesh. They are reported to be a serious damaging insect of cowpeas, lentils, black gram and other stored legumes in Bangladesh, India and other tropical and subtropical countries (Park et al., 2003; Mummigatti and Krishnaiah, 2007; Roy et al., 2012a). The beetles breed rapidly in the storage of the tropical and subtropical environment. Their larvae can easily penetrate into the grains and feed the endosperms (Roy et al., 2014). The infested grains become unsuitable for human consumption, deteriorate nutritional value and loss

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germination potential (Deeba et al., 2006).

Ratnasekera and Nayanathara (2010) reported that the moisture content in pulse grains to less than 10% could significantly reduce the pulse beetle infestation. But this precautionary measure is not practiced in Bangladesh as the pulses are sold by weight. Fumigation with synthetic chemicals like methyl bromide and phosphine is an effective method being used only in the warehouses. This tactics is expensive and unbearable to rural farmers, and impractical in the primitive nature of storage in many of the villages. On the contrary, injudicious application of the synthetic fumigants creates serious health hazards and environment pollution (Kim et al., 2003). These problems directed the need for biodegradable pesticides in the management of stored grain pests (Daglish, 2008).

Now-a-days, the pesticides of botanical origin, pressurized carbon dioxide and temperature management techniques are becoming popular to pest management experts in the worldwide (Yuya et al., 2009). The plant-derived materials are selective in action and their compounds are readily biodegradable, and less likely to contaminate the environment. A number of authors have reported the toxicity, repellency, antifeedant, growth and progeny inhibition activity of plant materials against field and stored grain insects (Roy et al., 2005; Cosimi et al., 2009; Saroukolai et al., 2010). Moreover, the farmers and small-scale industrialists can easily produce and store the crude or partially purified extracts from leaves, stems, fruits or roots of the plants.

In Bangladesh, little attention has given on pulse beetle *C. chinensis* management with the rationalize use of botanical pesticides, especially with the extracts of indigenous weeds. The purpose of this study was to evaluate the n-hexane extracts of sensitive plant *Mimosa pudica,* mexican prickly poppy *Argemone mexicana,* panimarich *Leucus aspara,* water pepper *Polygonum hydropiper* and shialmutra *Blumea lacera* weeds on the mortality, fecundity and adult emergence of *C. chinensis* reared on mung bean grains. Effect of the extracts on grain damage inhibition was also examined.

Materials and Methods

Mass culture of the test insect

Fifty pairs of unsexed *C. chinensis* beetles were obtained from the Entomology Laboratory stock of Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur, Bangladesh. The beetles were introduced into 1 L glass jar containing 500 g of mung bean grains. The jar was covered with a piece of cloth fastened with rubber band and kept in the same laboratory at 27 ± 2°C, 80 ± 5% RH and 12:12 h (light: dark). The insects were allowed to copulate and lay eggs for 7 days. Then the parent stocks were removed from the jar, and the eggs laid in the pulses were allowed to hatch. Mass culture was repeated and subsequent progenies were used for the experiments.

Sample preparation of test weeds

Whole plants of lajjaboty, mexican poppy, panimarich, water pepper and shialmutra weeds were collected from the rural areas of Bangladesh, conditioned in clearly labeled plastic bags and transported to the Entomology Laboratory. The weeds were washed with tap water and dried under shade at ambient temperatures (25-28˚C) for 7 days and further oven dried at 50-60˚C for 24 h to obtain constant weight. Dry samples were ground using an electric blender (Braun Multiquick Immersion Hand Blender, B White Mixer MR 5550 CA, Germany) and passed through a 25 mesh sieve to obtain fine dusts. The dusts were then stored in air tight glass jars in the laboratory under cool and shade conditions. Each weed sample (10 g) was mixed with 1 L n-hexane (96% analytical pure) in 2.5 L capacity bottles and stirred for 30 minutes using a magnetic stirrer and then left to stand for 24 h. The mixtures were then filtered and the filtrates were considered as 1 g/100 ml concentration. Concentrations of 2 g/100 mL and 4 g/100 mL extracts of the weed species were made by dissolving 20 g dusts in 2 L and 40 g dusts in 2 L n-hexane, respectively.

Mortality test

Ten adult beetles (2-3 days old) were placed at the center of a piece of filter paper and the paper was twisted to enclose them. The papers along with the insects were then dipped separately in 1, 2 and 2% extract concentrations or n-hexane for 30 seconds. The insects were removed, air-dried and released to Petri dishes containing 5 g of mung bean grains. Insect mortality was observed at 24, 48 and 72 h of post treatment and % mortality was calculated. Data were corrected for control mortality according to Schneider-Orelli's (1947) formula as mortalities in the control ranged between 5 and 20%.

$$\text{Corrected mortality (\%)} = \frac{\text{\%Mortality in control} - \text{\%Mortality in treatments}}{100 - \text{\%Mortality in conrtol}} \times 100$$

Toxicity ratios (TR) were calculated using the following formula: TR = LC_{50} and /or LC_{95} of the extract with less toxicity /LC_{50} and /or LC95 of the other extract, individually (Gusmão et al., 2013). All tests were replicated five times.

Fecundity test

In each 250 mL glass jar, 100 g mung bean grains were put and the extract doses were distinctly added to the grains with pipette and subjected to manual agitation for 2 minutes. Five pairs of 0-72 h old *C. chinensis* beetles were packed in each glass jar with perforated lid, coated with thin fabric (voile) to allow gas exchange. After 7 days, the number of eggs laid (fecundity per five females) by the females in the jars was counted by using hand lens. A control treatment was made with n-hexane treated grains, and the treatments were replicated four times. The fecundity inhibition (%FI) of each extract concentration was calculated using the following formula.

$$\text{\%FI} = \frac{\text{Eggs laid in control grains} - \text{Eggs laid in treated grains}}{\text{Eggs laid in control grains}} \times 100$$

Adult insect emergence and grain damage

Mung bean grains treated with different doses of the extracts were air dried and put on different Petri dishes (100 grains/Petri dish). Five pairs of newly emerged beetles were released in each of the Petri dish and the treatments were replicated four times. The beetles were removed after one week and the Petri dishes along with pulse grains were kept in the laboratory. After one month (bruchids start to emerge 30 days after eggs are laid) daily observation was started in each Petri dish and continued up to 15 days to record and remove the newly emerged beetles. The adult emergence inhibitory rate (%IR) was calculated by using the formula-Total number of damaged grains in each Petri dish was counted at the end of the experiment and percent grain damage inhibition (%DI) was calculated by the following formula-

$$\text{\%IR} = \frac{\text{No. of insects in control grains} - \text{No. of insects in treated grains}}{\text{No. of insects in control grains}} \times 100$$

Total number of damaged grains in each Petri dish was counted at the end of the experiment and percent grain damage inhibition (%DI) was calculated by the following formula-

$$\text{\%DI} = \frac{\text{No. of damaged grains in control} - \text{No. of damaged grains in treatments}}{\text{No. of damaged grains in control}} \times 100$$

Statistical analysis

Probit analysis was employed in analyzing the dose-mortality response. LC_{50} and LC_{95} values and their fiducial limits were estimated. Data of fecundity, adult emergence and grain damage inhibition were expressed as mean ± SE. Significant differences among the treatments were statistically compared using GLM. The individual pair wise comparisons were made using DMRT at 5% probability level with SPSS (IBM SPSS statistics 21).

Results and Discussion

The contact toxicity effects of the weed extracts on adult *C. chinensis* at 24 h post treatment showed LC_{50} and LC_{95} values from 4.5(3.8-6.1) to 6.4(4.7-13.9) and 9.0(7.0-13.4) to 12.4 (8.4-30.5) g/100 mL, respectively (Table 1). The $\chi 2$ values of the data differed significantly ($p < 0.05$) and the shialmutra weed extract was found most effective. Its concentration response curves showed the steepest slopes indicating that small variations in the concentrations induced greater responses in mortality. The mortality activity of the weed extracts at 24 h post treatment showed shialmutra > water pepper > panimarich > mexican poppy > sensitive plant. The toxicity results of the tested weed extracts against *C. chinensis* at 48 h post treatment showed LC_{50} and LC_{95} values from 4.1(3.4-5.8) to 5.6(4.7-7.6) and

9.0(6.9-14.3) to 11.8(9.3-17.0) g /100 mL, respectively. The results exerted that the χ^2 values of the data were significant different ($p < 0.05$). The shialmutra extract revealed the lowest LC_{50} and LC_{95} values and the concentration response curves of this weed also showed the steepest slopes. The toxicity responses of the weed extracts at 72 h post treatment were significantly different ($p < 0.05$). The LC_{50} and LC_{95} values of the extracts ranged from 3.6(3.2-4.4) to 5.5(4.3-8.7) and 7.9(6.4-10.8) to 11.8(8.7-20.9) g/100 mL, respectively. Among the treatments, shialmutra revealed the most toxic effect as it showed the lowest LC_{50} and LC_{95} values as well as steepest slopes of the concentration curves.

The tested weed extracts showed significant effects on *C. chinensis* and their toxicity were dose and exposure duration dependent. Among the treatments, 4% shialmutra revealed most toxicity effect at 72 h of post treatment. The findings of this study indicated that the weed species acted as valuable sources of insecticides, which proved for the rationalize use of botanical pesticides against stored grain pests. The ingredients of these weeds may have the ability to inject into the body of the beetles and dysfunction their nutritional valance, and eventually to death. Fouad et al. (2014) reported that the toxic substances in the plants interrupted insect normal physiology and resulted mortality.

The fecundity inhibition effects of the weed extracts on *C. chinensis* are presented in table 2. The weed species ($F_{4,45}$ = 15.3, $p < 0.001$), extract concentrations ($F_{2,45}$ = 205.9, $p < 0.001$) and interaction of weed species and extract concentrations ($F_{8,45}$ = 2.4, $p< 0.05$) had significant effects on the fecundity inhibition. The treatments showed 35 to 69% fecundity inhibition and the most promising results were obtained by 4% shialmutra.

Plants are great sources of broad spectrum insecticides. Their compounds can affect the life stages of the insects and inhibit their feeding, mating and oviposition. Our study showed that the weed extracts inhibited 35-62% fecundity of the *C. chinensis,* and the highest inhibition was obtained by 4% shialmutra. Ambrósio et al. (2008) reported that *Tithonia diversifolia* plant possessed sesquiterpene lactones which had broad spectrum action and inhibited the oviposition of herbivore insects. The fecundity inhibition effects of *Capparis decidua* plant materials on *C. chinensis* have been demonstrated by Upadhyay et al. (2006).

Table 1. Toxicity effect of five weed extracts on adult *Callosobruchus chinensis*

Weed plant	24 h post treatment						48 h post treatment						72 h post treatment					
	Slope (±SE)	LC^a50 (95%fl)	TR50	LC95a (95%fl)	TR95	χ^2 (df)	Slope (±SE)	LC^a50 (95%fl)	TR50	LC95a (95%fl)	TR95	χ^2(df)	Slope (±SE)	LC^a50 (95%fl)	TR50	LC95a (95%fl)	TR95	χ^2(df)
Sensitive plant	0.31±0.05	6.4 (4.7-13.9)	-	12.4 (8.4-30.5)	-	30.0	0.26±0.05	5.6 (4.7-7.6)	-	11.8 (9.3-17.0)	-	16.6	0.27±0.05	5.5 (4.3-8.7)	-	11.8 (8.7-20.9)	-	19.7
Mexican poppy	0.32±0.05	5.8 (4.4-11.6)	1.10	11.5 (7.9- 6.1)	1.07	34.1	0.27±0.04	4.9 (3.9-7.5)	1.14	11.2 (8.2-19.8)	1.05	21.7	0.31±0.04	4.4 (3.7-6.2)	1.25	9.9 (7.6-15.9)	1.19	22.5
Panimorich	0.35±0.05	5.7 (4.4-10.3)	1.12	10.9 (7.7-22.1)	1.14	31.8	0.28±0.05	5.1 (4.0-8.7)	1.09	10.8 (7.7-21.2)	1.09	31.3	0.36±0.05	4.6 (3.8-6.2)	1.20	9.4 (7.3-13.9)	1.26	22.1
Water pepper	0.36±0.05	5.3 (4.1-9.6)	1.20	10.1 (7.2-21.2)	1.23	41.4	0.32±0.04	4.1 (3.4-6.1)	1.37	9.4 (7.0-16.3)	1.26	33.9	0.37±0.04	3.9 (3.3-5.0)	1.41	8.4 (6.7-12.3)	1.40	26.2
Shialmutra	0.39±0.05	4.5 (3.8-6.1)	1.42	9.0 (7.0-13.4)	1.38	25.7	0.36±0.05	4.1 (3.4-5.8)	1.37	9.0 (6.9-14.3)	1.31	28.7	0.40±0.04	3.6 (3.2-4.4)	1.53	7.9 (6.4-10.8)	1.49	23.7

Each datum represents the mean of five replicates, each set up with 10 adults (n = 50). Concentrations are expressed as g/ 100 mL. fl stands for fiducial limits.
[a]Different concentrations (1%, 2% and 4%).

Table 2. Effect of five weed extracts on the fecundity inhibition (%mean ± SE) of *Callosobruchus chinensis*

Weed plant	% Fecundity inhibition		
	1% extract	2% extract	4% extract
Sensitive plant	34.9 ± 3.9 bB	51.5 ± 4.3 bA	56.4 ± 3.3 bA
Mexican poppy	35.2 ± 2.4 bB	50.3 ± 3.6 bA	55.6 ± 4.1 bA
Panimorich	38.9 ± 1.9 abB	52.2 ± 4.5 abA	54.7 ± 4.4 bA
Water pepper	38.7 ± 1.7 abC	53.8 ± 3.6 abB	66.5 ± 4.2 aA
Shialmutra	42.2 ± 2.4 aC	57.9 ± 2.7 aB	69.2 ± 4.1 aA

Values followed by the same lowercase letter (s) on the same column or by the same uppercase letter (s) in the same row are not significantly different, as assed by DMRT ($p \leq 0.05$).

Table 3. Effect of five weed extracts on the adult emergence inhibition (%mean ± SE) of *Callosobruchus chinensis*

Weed plant	% Adult beetle emergence inhibition		
	1% extract	2% extract	4% extract
Sensitive plant	34.2 ± 1.7 cB	48.3 ± 2.9 bA	49.9 ± 3.1 bA
Mexican poppy	34.2 ± 2.5 cC	45.1 ± 2.2 bB	52.0 ± 3.1 bA
Panimorich	33.3 ± 3.7 cC	45.9 ± 3.0 bB	51.7 ± 3.2 bA
Water pepper	42.0 ± 3.1 bC	52.6 ± 1.8 aB	61.4 ± 2.6 aA
Shialmutra	48.5 ± 2.6 aC	53.1 ± 3.1 aB	62.8 ± 2.0 aA

Values followed by the same lowercase letter (s) on the same column or by the same uppercase letter (s) in the same row are not significantly different, as assed by DMRT ($p \leq 0.05$).

The weed species ($F_{4,45} = 44.0$, $p < 0.001$), extract concentrations ($F_{2,45} = 192.1$, $p < 0.001$) and interaction of weed species and extract concentrations ($F_{8,45} = 2.3$, $p < 0.05$) indicated significant effects on the adult emergence inhibitory of *C. chinensis* (Table 3). The beetle emergence inhibitory in different treatments varied from 33 to 63%. Among the treatments, 4% shialmutra and 1% panimarich revealed the highest and lowest percentages of inhibition, respectively.

The tested weed extracts depicted potential effects to protect mung bean grains from the attack of *C. chinensis*. The compounds in the weeds could partly be involved in modification of the physical properties of the mung bean grains that reduced inter-granular air spaces, amount of oxygen in the Petri dishes, there by discouraged insect penetration and feeding. Roy et al. (2014) isolated the aromatic ester (n-hexyle salycilate or O-hydroxy-n-hexyl-benzoate) and a long-chain ketone from the leaf of the *Xanthium strumarium* weed and reported that the aquous extract of the weed caused mortality, inhibited fecundity and adult emergence of *C. chinensis,* as well as protected black gram grains.

Our study proved that the weed extracts prevented fecundity and adult emergence of *C. chinensis* and the activity of the extracts increased with increased doses. The bioactive compounds in the weed caused death of the beetles and seemed to be inhibited egg hatching, larval development to the pupal and adults. Nennah (2011) observed deformed larvae and pupae of *Tribolium castaneum* and *Rhizopertha dominica* when the insects were reared on wheat grains mixed with methanolic extracts of *Peganum harmala* seed. Roy et al. (2012b) observed dose dependent inhibitory effect of *X. strumarium* fruit extract on the fecundity and adult emergence of *C. chinensis*.

Table 4 showed that the weed extracts possessed inhibitory ability against the attack of *C. chinensis* on mung bean grains. The effects of weed species ($F_{4,45} = 24.9$, $p < 0.001$), extract concentrations ($F_{2,45} = 123.9$, $p < 0.001$) and interaction of weed species and extract concentrations ($F_{8,45} = 3.6$, $p < 0.05$) differed significantly. Grain damage inhibition among the treatments varied from 13 to 49%. The highest and lowest percentages of inhibition were found by 4% shialmutra and 1% sensitive plant, respectively.

Many authors reported the toxicity, repellent and antifeedant properties of plant materials on stored grain insects (Amin et

Table 4. Effect of five weed extract on the grain damage inhibition (%mean ± SE) of mung bean from the attack of *Callosobruchus chinensis*

Weed plant	% Grain damage inhibition at		
	1% extract	2% extract	4% extract
Sensitive plant	13.0 ± 4.4 bC	23.5 ± 2.1 cB	31.8 ± 6.3 bA
Mexican poppy	17.2 ± 5.3 abC	33.4 ± 4.5 bB	43.0 ± 6.7 aA
Panimorich	17.2 ± 5.1 abB	22.1 ± 4.1 cB	41.1 ± 5.7 aA
Water pepper	19.1 ± 4.1 abB	44.3 ± 6.1 aA	48.6 ± 6.3 aA
Shialmutra	23.8 ± 2.1 aB	47.9 ± 6.9 aA	49.4 ± 2.8 aA

Values followed by the same lowercase letter (s) on the same column or by the same uppercase letter (s) in the same row are not significantly different, as assed by DMRT ($p \leq 0.05$).

al., 2000; Shahjahan and Amin, 2000; Roy et al., 2010). Koona et al. (2007) stored legume grains in jute bags impregnated with the aqueous extracts of the *Lantana camera* plant and observed 80% reduction in damage by *C. maculatus* over a six months period. The bitter gourd, small bitter gourd, bottle gourd and ridge gourd oils were found to be effective against *C. chinensis* up to 60 days for protection of legume-pulse grains (Mishra et al., 2006). Rahman and Talukder (2006) reported that 3% acetone extract of *Vitex negundo* revealed good protection for black gram seeds against *C. chinensis* infestation.

It is evident from our findings that the tested weeds are potential source of bioactive pesticides. The mixtures of these weed materials with rapid and slow action insecticide might be useful in the protection of mung beans from the attack of *C. chinensis*. The plant materials are generally recognized as safe and ecosocio-friendly insecticide in contrast to synthetic ones. Moreover, the broad spectrum bioactivity of the weeds coupled with their abundance in the rural areas of Bangladesh, and processing facility makes them more acceptable and cost effective alternatives to synthetic pesticides for farmers and small scale storage holders.

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