

INSECTICIDAL EFFECTS OF TWO MEDICINAL PLANTS *Polygonum hydropiper* L. AND *Abrus precatorius* L. LEAVES AGAINST THE RICE WEEVIL *Sitophilus oryzae* L. (COLEOPTERA: CURCULIONIDAE)

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Abstract

During the study, the leaves of two medicinal plants, *Polygonum hydropiper* and *Abrus precatorius* were extracted with water, ethanol, methanol and petroleum ether solvents and tested against the rice weevil *S. oryzae* L. for insecticidal properties. Response varied with plant species. The mortality of adults increased with increasing dose concentrations from 1000 ppm to 5000 ppm each solution, with an exposure time of 72 hours. LC₅₀ values calculated were found to be 15091.436 ppm with water, 5051.534 ppm with ethanol, 5111.063 ppm with methanol and 4305.348 ppm with petroleum ether extracts of *P. hydropiper* and 9687.292 ppm with water, 6263.849 ppm with ethanol, 4682.683 ppm with methanol and 3222.984 ppm with petroleum ether extracts of *Abrus precatorius* leaves corresponding their concentrations of the leaf extract solutions at 1000ppm, 2000 ppm, 3000 ppm, 4000 ppm and 5000 ppm respectively. The results of the study showed that methanol and petroleum ether extracts of *P. hydropiper* and ethanol, methanol and petroleum ether extracts of *A. precatorius* showed good toxicity. It appeared that the leaf extracts had some insecticidal activities against *S. oryzae* adult.

Key words: *Polygonum hydropiper*; *Abrus precatorius*; Leaf; Mortality; LC₅₀; Relative potency.

INTRODUCTION

Stored grain losses of major cereal crops can be attributed primarily to attack by insect pests. There is a need to protect the stored grains against deterioration both qualitatively and quantitatively during storage due to insect attack. Agricultural stored and animal origin products are attacked by more than 600 species of beetles, 70 species of moths and about 355 species of mites, which cause quantitative and qualitative losses (Rajendra and Sriranjini 2008). This damage may cause loss to about 5 to 10% in temperate zone and 20 to 30% in tropical zone (Hague *et al.* 2000). These stored commodities are vulnerable to insect attack and the quality is deteriorated. Loss in weight of grains is a great economic problem. One of the major cosmopolitan pests of stored commodities in tropical countries is 'rice weevil', *Sitophilus oryzae* L. (Coleoptera: Curculionidae). One pair of *S. oryzae* can reproduce about one million of its species within a period of three months under favorable conditions (Thomas *et al.* 2002) and the adults are internal feeders and cause serious quantitative and qualitative losses to the grains.

Control of weevil population worldwide has been provided principally by the use of synthetic chemical insecticides. However, these insecticides are expensive and mostly out of reach of most small holder farmers and also led to insect resurgence, resistance and negative effect on non-target organisms (Duke *et al.* 2003). Therefore, there is need to look for an alternative way of control measure that are readily available, less poisonous and less detrimental for pest control (Talukder and Howse 1995). Little research has been performed on the development of affordable organic pesticides which offer the same control levels as synthetics to weevils (Cosmas *et al.* 2012). Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. Different types of plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for

their insecticidal activity including their action as fumigants, repellents, antifeedants, anti-ovipositions and insect growths regulators (Isman 2000, Weaver and Subramaniam 2000).

Plants are rich source of bioactive organic chemicals and offer an advantage over synthetic pesticides as these are less toxic, less prone to development of resistance and easily biodegradable. However, some works have been done to determine the efficacy of medicinal plant materials against stored grain insect pests. Investigations on such botanicals have been carried out to determine their insecticidal activity, toxicity, repellency, mortality, residual effects, larval growth and progeny production by Amin *et al.* (2000), Roy *et al.* (2005), Jbilou *et al.* (2006), Rahman *et al.* (2007), Kundu *et al.* (2007), Mondal *et al.* (2012), Ahalya and Mikunthan (2012), Gogoi (2012), Padin *et al.* (2013), Sagheer *et al.* (2014), Khan *et al.* (2014) and Karunakaran *et al.* (2016) on different stored grain insects.

The present study has been focused to investigate the comparative effectiveness of different concentrations of four extracts of two locally available medicinal plants *Polygonum hydropiper* L. and *Abrus precatorius* L. leaves for insecticidal activity against the rice weevil *Sitophilus oryzae* L.

MATERIAL AND METHODS

Experiments were conducted in the BCSIR laboratory, Chittagong and Entomological Research Laboratory of Department of Zoology, Chittagong University, for a period of six months from May to October 2017. *Sitophilus oryzae* was the test insect which was exposed to the various test doses of leaf extracts of *Polygonum hydropiper* and *Abrus precatorius* separately. The adult *S. oryzae* were collected from infested rice grain samples from the stored godowns of markets and were reared in plastic jars with fresh rice grains as culture media in the Entomological Research laboratory of the Department of Zoology, Chittagong University. The lids of the jars were perforated for proper aeration of the test insects and the jars were kept in a dark place in the laboratory. After some days, the test insects increased in number. The test insects were collected and used for the experiments when required. *P. hydropiper* (Bishkatali) and *A. precatorius* (Kuch) leaves were collected from Rangamati Hill Tracts. Fresh leaves were washed with tap water. All leaves were examined for signs of diseases, variegation and presence of external materials such as insects, their eggs and larvae. Only the fresh, disease less and uninfected leaves were taken for the experiments. The bigger leaves were cut into small pieces, air dried for some days and then dried in an oven at 40°C for five to six hours before grinding in an electronic grinding machine. The leaf dust thus obtained was passed through a 60 mesh sieve to obtain fine powder and was stored in glass jars under normal laboratory conditions.

Hundred grams of each powdered leaves were immersed into 500ml of the distilled water, ethanol, methanol and petroleum ether individually in 1L spirator bottles and left to stand for 72 hours and shaken several times at intervals. After 72 hours the mixture was filtered through Whatman filter paper no. 1 and the residue was again immersed in the same way for another two times and filtered. All the filtrate was evaporated at 40-60°C using a rotary vacuum evaporator under reduced pressure to separate the solvent from the extract. After evaporation, the crude extract was isolated and stored in tightly corked bottles and kept in the refrigerator for experimental use.

Stock Solution

Stock solution was prepared with 1g of each crude extract dissolving in 1000 ml of the test solvents, i.e. distilled water, ethanol, methanol or petroleum ether. Experimental doses in terms of ppm were prepared according to APHA (2005) from the stock solution.

Mortality Test

To test the mortality rate or dose response of *S. oryzae* adults to Bishkatali, *P. hydropiper* and Kuch, *A. precatorius* leaf extracts, Surface/Residual Film Method (Busvine 1971) was applied. The

experiments were conducted in Entomological Research Laboratory of the Department using medium sized petridishes (12 cm diameter). One ml extract of each dose was poured in each of the upper and lower parts of each petridish pair with the help of pipette and spread evenly throughout the petridish. Then the petridishes were air dried. The doses used during the experiments were of five different concentrations (1000, 2000, 3000, 4000 and 5000 ppm) of different extracts of *P. hydropiper* and *A. precatorius* leaves. The test insects were shifted from culture media to the petridishes with the help of fine brush. The number of test insects released in each set was always ten. Three replications were maintained for each concentration. A control set was maintained for each experiment with the same number of insects and similar replications with the solvent only. The petridishes were then kept in an incubator without food at $25 \pm 5^{\circ}\text{C}$ for 72 hours. Mortality of *S. oryzae* was recorded after 72 hours exposure to the toxicants.

Statistical Analysis

Dose-mortality relationships were calculated by Probit analysis. The regression equations were calculated from empirical probit and weighting probit, the values of which were taken from the tables of Finney (1971). Chi-square with P-values was calculated using observed and expected mortality data with the tables of the statistics at 0.01 and 0.05 level significance. The LC_{50} values with confidence limits were analyzed in a computer based Probit analysis program. Relative potency values were calculated from LC_{50} values of experimental doses taking the highest LC_{50} value as unit.

RESULTS AND DISCUSSION

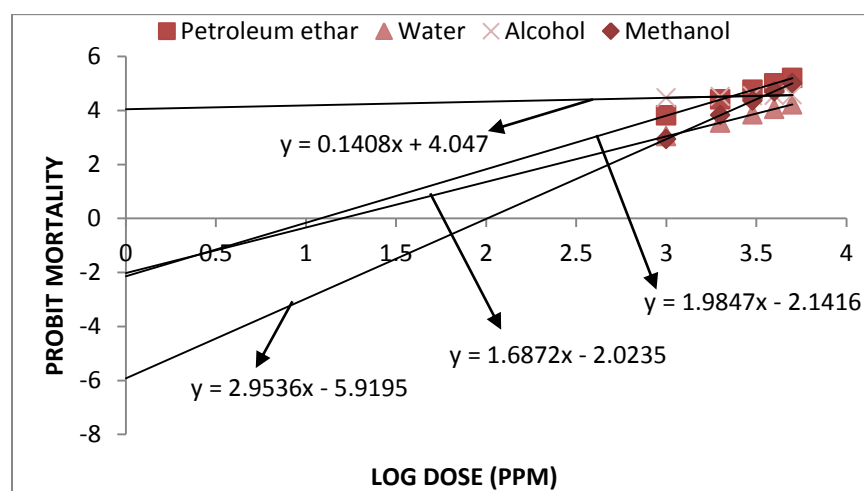
The result of the percentage mortality of *Sitophilus oryzae* were recorded at 72 hours interval exposure to different concentrations of the two experimental plant leaf extracts. It was seen that a particular extract of different concentrations caused different percentage of mortality in the experimental stored grain insect, *S. oryzae*.

Effect of *Polygonum hydropiper* leaf extracts on mortality of *Sitophilus oryzae*

With the water, ethanol, methanol and petroleum ether extracts of *Polygonum hydropiper* leaf, the lowest mortality (3.33%, 3.33%, 3.33% and 6.67%, respectively) was recorded with 1000 ppm and the highest mortality (23.33%, 46.67%, 53.33% and 66.67%, respectively) with 5000 ppm doses at 72 hours exposure (Table 1). The percentage mortalities recorded were 3.33%, 6.67%, 10.00%, 16.67% and 23.33% for water extract; 3.33%, 23.33%, 33.33%, 40.00% and 46.67% for ethanol extract; 3.33%, 10.00%, 26.67%, 33.33% and 53.33% for methanol extract; and 6.67%, 13.33%, 30.00%, 40.00% and 66.67% for petroleum ether extract corresponding their concentrations of the leaf extract solutions 1000, 2000, 3000, 4000 and 5000 ppm, respectively. For water extract the regression equation was estimated to be $1.687x-2.023$, the chi-square value was 1.42 ($P > 0.01 > 0.05$) and the LC_{50} value was 15091.436 ppm with confidence limits ranging from 7149.395 to 10683569.246 ppm; for ethanol extract the regression equation was estimated to be $0.140x+4.047$, the chi-square value was 44.23 ($P < 0.01 < 0.05$) and the LC_{50} value was 5051.534 ppm with confidence limits ranging from 3889.852 to 8910.509 ppm; for methanol extract the regression equation was estimated to be $2.953x-5.919$, the chi-square value was 3.09 ($P > 0.01 > 0.05$) and the LC_{50} value was 5111.063 ppm with confidence limits ranging from 4118.602 to 7929.172 ppm; and for petroleum ether extract the regression equation was estimated to be $1.984x-2.141$, the chi-square value was 25.47 ($P < 0.01 < 0.05$) and the LC_{50} value was 4305.348 with confidence limits ranging from 3557.034 to 5912.285 ppm (Table 1). Analysis of probit mortality relationship of the four extracts of the experimental plant leaf is plotted in Fig. 1.

Table 1. Toxicity parameters of *Polygonum hydropiper* leaf extracts on *Sitophilus oryzae* exposed for 72 hours.

Extracts	Water	Ethanol	Methanol	Petroleum Ether
Toxicity Parameters				
Dose Range (ppm)	1000-5000	1000-5000	1000-5000	1000-5000
Mortality Range (%)	3.33-23.33	3.33-46.67	3.33-53.33	6.67-66.67
Regression Equation	$1.687x-2.023$	$0.140x+4.047$	$2.953x-5.919$	$1.984x-2.141$
Chi-Square (χ^2)	1.42	44.23	3.09	25.47
Degree of Freedom (χ^2)	4	4	4	4
P-value (χ^2)	$P>0.01>0.05$	$P<0.01<0.05$	$P>0.01>0.01$	$P<0.01<0.05$
LC ₅₀	15091.436	5051.534	5111.063	4305.348
Confidence limit (lower)	7149.395	3889.852	4118.602	3557.034
Confidence limit (upper)	10683569.246	8910.509	7929.172	5912.285

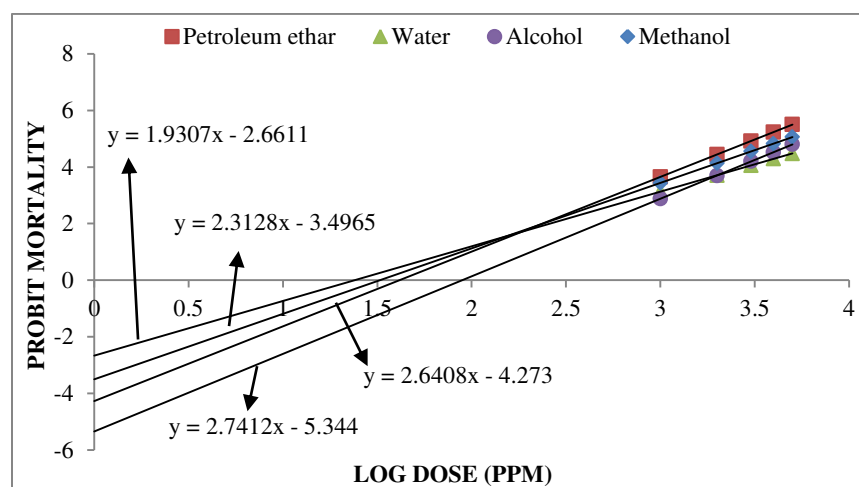
Fig. 1. Regression lines for determining the LC₅₀ values of water, ethanol, methanol and petroleum ether extracts of *Polygonum hydropiper* leaves on *Sitophilus oryzae* after 72hr of exposure.

Effect of *Abrus precatorius* leaf extracts on mortality of *Sitophilus oryzae*

With the water, alcohol, methanol and petroleum ether extracts of *Abrus precatorius* leaf, the lowest mortality (3.33%, 3.33%, 6.67%, and 10.00%, respectively) was recorded with 1000 ppm and the highest mortality (30.00%, 50.00%, 60.00% and 73.33%, respectively) with 5000 ppm doses at 72 hours exposure (Table 2). The percentage mortalities recorded were 3.33%, 10.00%, 16.67%, 23.33% and 30.00% for water extract; 3.33%, 10.00%, 16.67%, 26.67% and 50.00% for ethanol extract; 6.67%, 20.00%, 30.00%, 36.67% and 60.00% for methanol extract; and 10.00%, 30.00%, 40.00%, 60.00% and 73.337% for petroleum ether extract corresponding their concentrations of plant extract solutions 1000, 2000, 3000, 4000 and 5000 ppm, respectively. For water extract the regression equation was calculated to be $1.930x-2.661$, the chi-square value was 0.42 ($P>0.01>0.05$) and the LC₅₀ value was 9687.292 ppm with confidence limits ranging from 5846.594 to 88375.416 ppm; for alcohol extract the regression equation was calculated to be $2.741x-5.344$, the chi-square value was 5.54 ($P>0.01>0.05$) and the LC₅₀ value was 6263.849 ppm with confidence limits ranging from 4717.580 to 13069.702 ppm; for methanol extract the regression equation was calculated to be $2.312x-3.496$, the chi-square value was 3.86 ($P>0.01>0.05$), and the LC₅₀ value was 4682.683 ppm with confidence limits ranging from 3702.092 to 7410.714 ppm; and for petroleum ether extract the regression equation was calculated to be $2.640x-4.273$, the chi-square value was 3.06 ($P>0.01>0.05$) and the LC₅₀ value was 3222.984 with confidence limits ranging from 2666.017 to 4040.298 ppm (Table 2). Analysis of probit mortality relationship of the four extracts of the experimental plant leaf is plotted in Fig. 2.

Table 2. Toxicity parameters of *Abrus precatorius* leaf extracts on *Sitophilus oryzae* exposed for 72 hours.

Extracts	Water	Ethanol	Methanol	Petroleum Ether
Toxicity Parameters				
Dose Range (ppm)	1000-5000	1000-5000	1000-5000	1000-5000
Mortality Range (%)	3.33-30.00	3.33-50.00	6.67-60.00	10.00-73.33
Regression Equation	1.930x-2.661	2.741x-5.344	2.312x-3.496	2.640x-4.273
Chi-Square (χ^2)	0.42	5.54	3.86	3.06
Degree of freedom (χ^2)	4	4	4	4
P-value (χ^2)	P>0.01>0.05	P>0.01>0.05	P>0.01>0.05	P>0.01>0.05
LC ₅₀	9687.292	6263.849	4682.683	3222.984
Confidence limit (lower)	5846.594	4717.580	3702.092	2666.017
Confidence limit (upper)	88375.416	13069.702	7410.714	4040.298

Fig. 2. Regression lines for determining the LC₅₀ values of water, ethanol, methanol and petroleum ether extracts of *Abrus precatorius* leaves on *Sitophilus oryzae* after 72hr of exposure.

Relative potency values of the experimental plant leaf extracts

Relative potency values of an extract are reciprocal of an equitoxic extract. The relative potency values of different extracts of the two experimental plant leaves of *Polygonum hydropiper* and *Abrus precatorius* were calculated and are given in Table 3. From the LC₅₀ and relative potency values, it is seen that the most toxic extract was the petroleum ether extract of *A. precatorius* with LC₅₀ of 3222.984 ppm and the least toxic was the water extract of *P. hydropiper* with LC₅₀ of 15091.436 ppm.

Table 3. The LC₅₀ (ppm) and Relative Potency values of different extracts of *Polygonum hydropiper* and *Abrus precatorius* leaves on *Sitophilus oryzae*.

Plant	Extract	LC ₅₀ (ppm)	Relative Potency	Rank
<i>Polygonum hydropiper</i>	Water	15091.436	1.000	8
	Ethanol	5051.534	2.987	4
	Methanol	5111.063	2.953	5
	Petroleum Ether	4305.348	3.505	2
<i>Abrus precatorius</i>	Water	9687.292	1.558	7
	Ethanol	6263.849	2.409	6
	Methanol	4682.683	3.223	3
	Petroleum Ether	3222.984	4.682	1

The order of toxicity of the extracts for *P. hydropiper* was Petroleum Ether > Ethanol > Methanol > Water and that for *A. precatorius* was Petroleum Ether > Methanol > Ethanol > Water. The sequence of

toxicity of the four extracts of the two experimental plant leaves was: Petroleum Ether extract of *A. precatorius* > Petroleum Ether extract of *P. hydropiper* > Methanol extract of *A. precatorius* > Ethanol extract of *P. hydropiper* > Methanol extract of *P. hydropiper* > Ethanol extract of *A. precatorius* > Water extract of *A. precatorius* > Water extract of *P. hydropiper* leaves. Of the two plants, extracts of *A. precatorius* leaves were more toxic than that of *P. hydropiper* leaves.

The result obtained from the bioassay revealed that all the extracts of the two experimental plant leaves showed varying levels of insecticidal property in terms of mortality against *S. oryzae*. Mortality of *S. oryzae* adults increased with the increase of concentrations. Mortality percentage showed parallel response to the different levels of concentrations with both the plant leaf extracts. For tested extracts the percentage of mortality depended on efficacy of concentrations. The mortality increased from 3.33% to 66.67% with increase of dose concentration of 1000 ppm to 5000 ppm at 72 hours exposure in case of *P. hydropiper* leaves. The mortality value increased from 3.33% to 73.33% mortality with increase of dose concentration from 1000 ppm to 5000 ppm at 72 hours exposure in case of *A. precatorius* leaves. Very low mortality had been observed for water extracts of both medicinal plant leaves which were 3.33% to 23.33% for *P. hydropiper* and 3.33% to 30.00% for *A. precatorius* leaves. Ethanol extracts of both plant leaves gave almost same mortality which was 3.33% to 46.67% for *P. hydropiper* and 3.33% to 50.00% for *A. precatorius* leaves. Methanol extracts of both the plant leaves showed greater mortality and it was 3.33% to 53.33% for *P. hydropiper* and 6.67% to 60.00% for *A. precatorius* leaves. In both cases, the highest mortality was observed for petroleum ether extracts and it was 6.67% to 60.00% for *P. hydropiper* and 10.00% to 73.33% for *A. precatorius* leaves. In every leaf extract, the highest mortality dose was 5000 ppm but a longer interval or higher concentration would be required in some of the extracts to bring about 100% mortality. High mortality rate observed at higher dose agreed with the findings of Kundu *et al.* (2007) who examined the effects of chloroform and ethyl alcohol extracts of *Polygonum hydropiper* plant on *Tribolium castaneum* and Khan *et al.* (2014) who experimented the chloroform soluble fraction of ethanol extract of rhizome of *Drynaria quercifolia* against *T. castaneum* using Surface/Residual film method. Effects of different plant extracts having insecticidal property as in causing mortality due to direct toxicity is also supported by the findings of Talukder and Howse (1993), Roy *et al.* (2005), Rahman *et al.* (2007), Kundu *et al.* (2007), Ahalya and Mikunthan (2012) and Khan *et al.* (2014).

Chi-square tests done for all the extracts of the two experimental plant leaves showed insignificant values in most of the cases indicating that there was no difference between the observed mortality and expected mortality. From the LC₅₀ values, it was observed that petroleum ether extract of *A. precatorius* leaves was the most toxic and water extract of *P. hydropiper* leaves was the least toxic. However, in all the leaf extracts the dose-response slopes were more or less identical, suggesting a common mechanism of death. Hence, LC₅₀ is of great significance as biological constant in biological data analysis (Zbinden and Flury-Roversi 1981, Wallance-Hayes 1982).

The study was conducted to evaluate the mortality and relative potency value of extracts of *P. hydropiper* L. and *A. precatorius* leaves on rice weevil *S. oryzae* adults. The results obtained from the assay revealed that all extracts of *P. hydropiper* and *A. precatorius* leaves showed various levels of insecticidal property against *S. oryzae* and that the potential of the leaf extracts to cause mortality increased with concentration. The findings of this study agree with earlier reports that both the plant leaf extracts have insecticidal property and can control the experimental insect pest through causing direct mortality. The results demonstrated that leaf extracts of *P. hydropiper* and *A. precatorius* could be applied against rice weevil *S. oryzae* to protect stored grains.

Laboratory based toxicity experiments of plant products can give near optimal information about the efficacy of the plant toxins. It is evident from the present study that botanical extracts might have

promising stored grain insect control efficacy. Findings of the study reveal in implementing environmentally friendly and ecologically sustainable rice weevil management practices. This information could also be useful in formulating rice weevil management tactics.

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