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Chemical Methods of Leaf Extraction of Bankalmi, *Polygonum hydropiper* for Controlling Rice Hispa Beetles, *Dicladispa armiger* (Olivier) (Coleoptera: Chrysomelidae) in Bangladesh

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Abstract: Chemical analysis of Bankalmi, *Polygonum hydropiper* was evaluated against rice hispa beetle. Hot water extracts of Bankalmi (*Ipomoea sepiaria*) and Bishkatali (*Polygonum hydropiper*) (1:10; W:V) can efficiently controlled the hispa beetle. Bankalmi leaf extract with 25-95% ethyl alcohol gave 70-100% mortality of rice hispa. However, 95% alcohol itself killed 100% of the test insect population indicating unsuitable for Bankalmi leaf extraction. But it can be extracted with 25-50% ethanol. Hexane itself killed all the insects and was not suitable as solvent to extract Bankalmi active ingredient. Comparing the three methods of leaf extraction (Soxhlet and two crude methods), Soxhlet methods gave higher mortalities of rice hispa beetles than the others.

Key words: Botanicals, rice hispa, *Dicladispa armigera*, Bankalmi leaf extracts

Introduction

Rice hispa, *Dicladispa armigera* (olivier) is a menacing rice pest in Bangladesh (Chowdhury *et al*., 1989; Hedayetullah, 1941; Rabbi and Karim, 1989). Outbreaks of hispa in recent years were unprecedented, causing huge losses to rice crops. Tactics of hispa control are limited. Presently, insecticides are a major and popular component for its control (Chowdhury *et al*., 1989; Rabbi and Karim, 1989; Anonymous, 1995). Continuous or heavy usage of some pesticides has created serious problems arising from factors such as direct toxicity to parasites, predators, pollinators, fish and man (Pimental, 1981). It has therefore, become necessary to complement our reliance on synthetic pesticides with less hazardous, safe and biodegradable substitutes (Islam, 1994). This awareness has created a world-wide interest in the re-evaluation, use of age-old and traditional botanical pest control agents. In recent years, the use of local botanical has gained much importance mainly among the researchers because of their high bio-efficacy against many crop pests and relative safety to the environment as compared to the synthetic pesticides (Karim and Haque, 1999). Leaf extracts of *Datura* and *Ipomoea*, when sprayed on hispa infested plants, were toxic to the insect, causing 82.5-91.2% mortality 48 h after spary in the greenhouse test (Rabbi and Karim, 1989). Plant semio-chemicals, which act as attractants, repellents, arrestants or deterrents by manipulating the insect behaviour are now being investigated as an alternatives to synthetic organic insecticides to control insect pest (Khan and Shahjahan, 1998). Karim and Haque (1999) mentioned that bankalmi (*Ipomoea sepiaria*), bishkatali (*Polygonum hydropiper*), castard apple (*Annona squamosa*), jute (*Corchorus capsularis*), neem (*Azadirachta indica*), nishinda (*Vitex negundo*), pithraj (*Amoora ruhitaka*) and rayna (*Aphanomixis polystachya*) were effective against rice insect pests. Therefore, the present studies were taken to determine efficacy of Bankalmi leaf extract and also to find out their suitable extraction procedures.

Materials and Methods

Preparation of extracts: Bankalmi leaves were collected from middle portion of mature plants. The leaves were air-dried in a shaded place ensuring sufficient air flow to avoid damping. After drying at the moisture level below 20%, the leaves were chopped into very fine pieces. With these leaf pieces extracts were made with different ratio of alcohol, hexane and plain water in the following methods:

1) Crude method-I
2) Crude method-II
3) Soxhlet method

Crude method-I: In this method, the leaf samples were put in the solvent e.g. alcohol, hexane, cold water and hot water at the ratio of 1:10 (w/v) in a beaker for five minutes at room temperatures and stirred intermittently to get a better extraction. After 5 minutes the leaves were separated from the solvents through cheesecloth and the extracts were stored in a refrigerator (5°C) and sprayed on the following morning. The extracts were sprayed directly without diluting in any other solvent.

Crude method-II: In this method, extracts were prepared in two steps. The first step was exactly similar to the procedure described in the first method in case of alcohol and hexane. The extracts obtained in this method were then heated in boiling water bath (60°C) and the solvents were allowed to evaporate to dryness. The powder remaining in the surface of the beaker was dissolved in cold water at the ratio of 1:10 (original leaf material weight/volume) and stirred well with a glass rod. In this process the oily component of the powder remained insoluble in water and stuck on the glass rod surface. For cold-water extraction, the leaf material was dissolved in water for five minutes at the stipulated ratio and then the leaf residues were filtered out. For hot water extraction, the leaf materials were dissolved in boiling water and kept boiling for 5 min. Then the leaf residues were separated out through filtration. In all the cases, the extracts were stored overnight in a refrigerator (5°C).

Soxhlet method: In this method, the leaf samples (10 gm/500ml solvent) were extracted with water, ethanol 25% and ethanol 75% for 4h in soxhlet apparatus. The extracts were then evaporated to 100 ml, to make the solution 1:10 (w/v) ratio of solute:solvent.

Application of extracts: The extracts were applied on rice plants before or after the release of rice hispa to determine the effects of the materials as antifeedent or contact toxicity, respectively. Rice hispa beetles were collected from the stock culture maintained in the net house of Bangladesh Rice Research Institute. The beetles were released on BR3 rice plants raised in earthen pots of 14 cm diameter and 13 cm deep. The plants were used when they were 45-50 days old. The insects were caged on the plant by 60 cm tall and 10 cm diameter mylar cages. A one-litre capacity hand sprayer was used to spray the extracts with an average estimated spray volume of 700 L ha^{-1}. To see the contact toxicity of the extracts 10 adult beetles were confined on the plant and extracts were sprayed directly on them. Mortality of the beetles were recorded at 24 and 48 h after spray. The mortality was adjusted by Abbott's (1925) formula as follow:

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$$\text{Corrected \% Mortality} = \frac{\text{\% Mortality in treatment} - \text{\% Mortality in control}}{100 - \text{\% Mortality in control}} \times 100$$

To test the efficacy of the materials as feeding and egg laying deterrent, the spray was done on the plant before releasing insects. After 10 minutes of spray, 10 beetles were confined on each plant. The beetles were kept starved for three days before releasing. Leaf area fed by the beetles and number of eggs laid by them was recorded after 3 days of treatments.

In both the cases the experiments were laid in CRD. The experiments were repeated 4-5 times considering each pot as replication.

Results and Discussion

Contact toxicity of the extracts on adult hispa: In the T. aman (summer rice) season 1993, Bankalmi leaf extraction with 95% ethanol by Crude method-I gave a high mortality of more than 95% hispa beetles at 24 and 48 hrs of application (Table 1). Ethanol (95%) itself was not so toxic to the insect and caused less than 15% mortality indicating that toxic alkaloid of Bankalmi was extracted out with this solvent (Table 1). Bankalmi leaf extraction with 25% ethanol had moderate killing effect on the pest. The mortality in this case was about 55%. Hexane extract of Bankalmi leaf also gave a high mortality of more than 80% hispa adults. However, hexane itself was a highly toxic material having the same mortality effect on the beetles. So it could not be determined whether the alkaloid responsible for hispa mortality in Bankalmi leaf was extracted by hexane (Table 1).

Both cold water and hot water extracts of Bankalmi offered very low mortality of rice hispa (10-20% mortality) (Table 1). Previous studies showed that Bankalmi leaf was effective on hispa when extracted by boiling for 3 min (Rabbi *et al.*, 1989). In the present study the leaf was not continuously boiled for 5 min rather than the water containing leaf pieces were removed from the heating agent just at the boiling point and stirred away from the heat source. Thus the water gradually lost heat causing less extraction or no extraction of the active ingredient.

Same materials were tested in two trials in Aus, 1994 season. The test materials gave more or less similar results as obtained in the previous trial. However, in these trials 95% ethanol itself caused high mortality of the beetles (100% mortality) (Table 2), which contradict with the results of the T. aman, 1993 experiment. The alcohol used in 1994 experiment contained a very little amount of lead, which probably made it toxic to the insect.

A grade of 50% ethanol extracts of Bankalmi was introduced in the second trial of Aus, 1994. This grade was also found to be a good solvent for Bankalmi leaf extraction as it killed more than 90% hispa at 48 h of spray (Table 2). The solvent itself had very low toxic effect on the insect causing not more than 20% mortality (Table 2).

Although the chemical extraction of Bankalmi leaf was done following Crude method-I, the hot water extraction method was a bit different in the Aus, 1993 trial from the experiment conducted in T. aman, 1993. In this case the Bankalmi leaf was continuously boiled in water for 5 min. This caused a better extraction of the alkaloid causing 74-78% mortality of the insect (Table 2).

Extracts of Bankalmi with the same solvents prepared following crude method-II was tested in a trial conducted in T. aman, 1994 season. None of the extracts were found effective against rice hispa (Table 2). In this method the extracts obtained from the crude method-I was dried to powder. When these powders were mixed with water and stirred the oily portion of the powder was separated out and stuck on the glass rod used for stirring. As a result, the water was permanently devoid of the active ingredient, which probably was oily in nature. This was the reason of failure of the extracts prepared by Crude method-II in killing hispa beetles. However, hot water extract gave a moderate mortality of 60% at 48 h of spray (Table 2).

Table 1: Efficacy of different extracts of Bankalmi leaves on rice hispa adults, BRRI nuthouse, T. aman, 1993 (Crude method –I)

Treatments	Mortality percent (corrected) 24 HAT	48 HAT
95% ethanol + Bankalmi	96.69a	96.67a
95% ethanol	13.33c	36.91cd
25% ethanol + Bankalmi	56.67b	56.91bc
25% ethanol	00.00d	01.67f
Hexane + Bankalmi	83.34ab	84.88ab
Hexane	83.34ab	83.33ab
Cold water + Bankalmi	11.67c	10.12ef
Hot water + Bankalmi	16.66c	21.67de
Cold water	00.00d	00.00f

Data are averages of 4 replications. In a column, means followed by a common letter are not significantly different at 5% level.

Table 2: Determination of chemical nature of Bankalmi extract responsible for hispa mortality. Aus and T. Aman 1994, BRRI, Gazipur

	Crude method-I				Crude method-II	
	Aus 1		Aus 11		T. aman	
	% mortality		% mortality		% mortality	
Treatments	24 h	48 h	24 h	48 h	24 h	48 h
Ethyl Alcohol 95% + Bankalmi	100c	100c	100.0c	100.0d	13.3ab	13.3ab
Ethyl Alcohol 95% only	100c	100c	100.0c	100.0d	--	--
Ethyl Alcohol 50% + Bankalmi	--	--	82.6bc	92.5d	--	--
Ethyl Alcohol 50% only	--	--	15.0a	20.0b	--	--
Ethyl Alcohol 25% + Bankalmi	84bc	88bc	15.0a	17.5b	16.7ab	16.7bc
Ethyl Alcohol 25% only	5a	10a	5.0a	5.0ab	--	--
Hexane + Bankalmi	84bc	100c	82.5bc	92.5d	0.0a	0.0a
Hexane only	96c	100c	97.5c	100.0d	--	--
Hot water extract of Bankalmi	72b	74b	62b	77.5c	46.7c	60.0d
Cold water exract of Bankalmi	10a	20a	5.0a	7.5ab	30.0bc	33.0c
Plain water (Control)	6a	8a	0.0a	0.0a	0.0a	0.0a

Data are average of 3-4 replications. In a column means followed by a common letter are not significantly different at 5% level (DMRT). In Aus the complete solutions (solvent + solute) were sprayed on potted plants caged with 10 adult hispa. But in T. aman the dissolved portion of Bankalmi leaf was separated from the solvent by volatilization and sprayed on the insect.

Soxhlet method of extraction of Bankalmi with 25, 50 and 75% ethanol was compared with the extracts prepared by crude method-II in T. Aus season, 1995. It was found that 25 and 75% ethanol extracts of Bankalmi killed 73 and 83% of the test insects at 48 h of spray (Table 3). However, in control ethanol 75%, the mortality was also high (86% mortality) probably due to some toxic elements in the solvent (Table 3). An inconsistent result was obtained with the Bankalmi extracts with 50% ethanol. The reason of such inconsistency could not be explained. Results indicate that the higher concentration of ethyl alcohol and hexane themselves killed the insects and were not suitable as solvent to determine the chemical nature of Bankalmi active ingredient. They were not suitable even when the solvents were evaporated and the ingredient was diluted with water (Table 2). Kabir *et al.* (1998) followed similar procedure of leaf extraction and they found that acetone extract of *Datura metal* L. leaves was highly toxic against *Periplaneta americana* L. again. Rahman *et al.* (1999) also reported that the acetone and alcohol extracts of urmoi, neem and turmeric showed better repellent action than the water extract. The efficiency of these chemical extracts was higher, perhaps due to the cumulative effects of both solvent and plant extracts.

Effect of extracts on egg laying by rice hispa beetle: An experiment in T. Aus, 1995 season indicated that Soxhlet method of Bankalmi leaf extraction with 25, 50 and 75% ethanol worked as an egg laying deterrent of rice hispa. Egg laying capacity of the female decreased with increased grade of alcohol. Thus 75% ethanol extract allowed the females to lay only 42 eggs/female in

Table 3: Effect of different ethanol extracts of Bankalmi leaves on Hispa beetle greenhouse T. Aus, 1995.

	Mortality at 24 HAS (%)			Mortality at 48 HAS (%)			Mortality at 72 HAS (%)		
Concentrations	Soxhlet method of extraction	Crude method-II extraction	Control	Soxhlet method of extraction	Crude method-II extraction	Control	Soxhlet method of extraction	Crude method-II extraction	Control
Water	6.67bA	3.33aA	6.67bA	10.00bA	3.33aA	10.0aA	16.67aA	3.33aA	16.67cA
25% Ethanol	70.00aA	6.67aB	13.33bB	73,33aA	16.67aB	16,67bA	76.67abA	23.33aB	16.67cB
50% Ethanol	40.00abA	6.67aA	36.67aA	43.33abAB	10.00aB	50.00bA	46.67abAB	13.33aB	60.00bA
75% Ethanol	73.33aA	16.67aB	86.67aA	83.33aA	16.67aB	90.00aA	90.00bA	23.33aB	96.67aA

In columns and rows means followed by a common letter are not statistically different at 5% level by DMRT. Small and capital letters indicate comparison within columns and rows respectively. Data are average of three replications. HAS = Hours after spraying

Table 4: Effect of different ethanol extracts of Bankalmi leaves on feeding and egg laying of hispa beetle in treated leaves, Greenhouse, T. Aus, 1995.

	Eggs laid by ten hispa beetle (Average no.)			Feeding area by ten hispa beetle (mm^2)		
Concentrations	Soxhlet method of extraction	Crude method-II extraction	Control	Soxhlet method of extraction	Crude method-II extraction	Crude method-II extraction
Hot water	203.3aA	157.3aA	161aA	1378.3aB	2436aA	1439.3aA
25% Ethanol	107.7abA	163.3aA	118aA	1026.3abA	1466aA	1334.3aA
50% Ethanol	82.3abB	177.7aA	*	670.7abA	1473aA	*
75% Ethanol	42.7bB	166.7aA	*	435.7bB	2135aA	*

In a column and row means followed by a common letter are not statistically different at 5% level by DMRT. Small and capital letters indicate comparison within columns and rows respectively. Data are average of three replications. * Data could not be taken due to phytotoxicity.

comparison to 203 eggs/female in hot water extraction by three days. However, extraction by crude method-II had no significant effect on egg laying (Table 4).

Effect on feeding by rice hispa beetle-: Similar to the effect on egg laying, the Soxhlet method of extraction with ethanol had an negative effect on hispa feeding. While feeding by a beetle was 1378 mm^2 on the plants sprayed with hot water extraction, it was only 436 mm^2 on the plants treated with 75% ethanol extract three days after treatment application (Table 4).

In soxhlet method, the feeding area (mm) and eggs laid in treated leaves were comparatively lower in ethyl alcohol extracts than the water extracts of Bankalmi. Islam (1983) also observed that oil of neem, *A. indica* as well as extracts of leaves and seeds of *Azadirachta indica, Melia azadirach, Annoa reticulata, Amoora ruhituka* with hexane, diethyl ethar, 95% ethyl alcohol and acetone showed potential as antifeedant or feeding deterrents for the control brown plant hopper (BPH), green leafhopper (GLH), rice hispa (RH) and lesser rice weevil. However, there were no differences in feeding area and eggs laid in treated leaves in dried methods of leaf extraction (Table 4).

In this case crude method-II also failed to reduce hispa feeding. The results obtained from the above experiments indicate that Bankalmi extracts with different chemicals have varying effect on hispa mortality, feeding and egg laying. Hot water and ethanol extract have a high mortality effect on the pest. More over they can reduce hispa feeding and egg laying on rice plants. This indicates that the active ingredient of Bankalmi leaf is soluble in hot water and ethanol.

Method of extraction is also a major factor in determing the alkaloid dissolve in the solvent. The Soxhlet method and Crude method-I was found superior to Crude method-II in extraction the active ingredient from the leaves. In Soxhlet methods of leaf extraction, the mortalities of hispa beetles were higher in all the concentration of ethyl alcohol (25, 50 and 75%) compared to water extracts of Bankalmi (Table 3). Similarly, Khalequzzaman and Islam (1992) extracted a liquid from the leaves of *Datura metal* with petroleum spirit, ethyl acetate, acetone and methanol using a Soxhlet apparatus serially. They found that the leaf extract of dhutura with methanol caused the highest mortality to the adults *Tribolium castaneum* (Herbst) beetles followed by extraction in petroleum sprit and acetone.

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