

Investigation of *Sapindus Mukorossi* Extracts for Repellency, Insecticidal Activity and Plant Growth Regulatory Effect

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Abstract: Ethanolic extract of *Sapindus mukorossi* was investigated for repellency and insecticidal activity against *Sitophilus oryzae* and *Pediculus humanus*. Average mortality percentage indicated that the extracts caused significant mortality and repellency on the target insects and bioassays indicated that the toxic and repellent effect was proportional to the concentration and higher concentration has stronger effect. Observed mortality percentage increased with increase in time intervals after treatment. Mortality percentage showed parallel response to the level of concentration at different time intervals after treatment. The water extract of *Sapindus mukorossi* was used to investigate the allelopathic effects of different concentration on germination. The effects of the different concentration were compared to that of distilled water (control). The result revealed that different concentration of the extracts caused significant inhibitory effect on germination, root and shoot elongation. Bioassays indicated that the inhibitory effect was proportional to the concentration and higher concentration has stronger inhibitory effect of the extract.

Key words: *Sapindus mukorossi*, *Sitophilus oryzae*, *Pediculus humanus*, Allelopathy, Toxicity, Repellency.

INTRODUCTION

Insect pests have mainly been controlled with synthetic insecticides in the last fifty years. The protection of stored grains from insect damage is currently dependent on synthetic pesticides. Most insecticidal compounds fall within four main classes, the organochlorines, organophosphates, carbamates and pyrethroids. There are problems of pesticide resistance and negative effects on non-target organisms including man and the environment. Botanicals are promising source of pest control compounds. These have generated extraordinary interest in recent years as potential sources of natural insect control agents. In the middle of the 17th century, pyrethrum, nicotine, and rotenone were recognized as effective insect-control agents^[1]. Leaf extracts of *Blumea lacera* showed botanical insecticidal activity against lesser grain borer and rice weevil^[2]. The insecticidal and antifeedant activity of extracts derived from different parts of the Mangrove tree *Rhizophora mucronata* also reported^[3]. Neem extract in benzene was most effective repellent of red pumpkin beetle, followed by Bakain extract in benzene^[4]. Hermal extract in ethanol was found significantly least effective, followed by Hermal extract

in benzene, Bakain and Neem extracts in ethanol. Hot water extracts of *Ipomoea sepiaria* and *Polygonum hydropiper* (1:10; w/v) can efficiently controlled the hispa beetle^[5]. Nadi *et al* showed the toxicity of aqueous, methanolic and acetonetic extracts of three plants *Rhazya stricta*, *Azadirachta indica*, and *Heliotropium bacciferum* to *Trogoderma granarium everts* larvae^[6].

The direct or indirect effect of one plant to adjoining plants through release of chemical substances has come to be known as allelopathy^[7]. Different types of naturally occurring organic, bioorganic compounds have been isolated from different types of plants, weeds, and animal sources, having effective medicinal, insecticidal or pesticidal activity. Different types of plants beside crop field may through their leaves and fruits on field and due to rainfall make aqueous extracts of toxic compounds which may damage germination and the growth of the crops. Allelopathic interactions are widely known in different groups of plants such as algae, lichens, crops as well as annual and perennial weeds^[7-9]. The plant-plant chemical compound interactions are of vital importance in the field of agriculture^[10]. Several investigations have been reported on the effect of primary and secondary

metabolites from different plants and weeds on germination, growth behavior and development of various crops^[11,12]. Patterson *et al.*^[13] reported the effect of secondary extract metabolites from *Sorghum halepense* on germination, growth and development of various crops. The allelopathic effects of *Eucalyptus camaldulensis dehn.* on some agricultural crops in Bangladesh was reported in the literature^[14]. Allelopathic of *Eupatorium odoratum* on germination and growth behavior of six agricultural crops also reported^[15]. Chemicals that inhibit the growth of some species at certain concentrations can stimulate the growth of the same or different at lower concentrations^[7]. Hence, it should be expected that due to perceived ambiguous nature of allelopathy, the phenomenon is hesitantly accepted, or even refuted, as an important factor in crop production. Present work has been carried out to investigate *Sapindus mukorossi* extracts for repellency, insecticidal activity. We have also monitored the allelopathic effect of the plant extract, whether it shows any regulations on plant growth.

MATERIALS AND METHODS

Plant Materials: Matured fruits of Rittha, (*Sapindus mukorossi*) were collected and cut into small pieces, powdered and dried. The air-dried materials were then further dried in an oven at 40°C. The dried fruits were macerated. Dried powdered fruits (100g) extracted with 80% ethanol (300 ml), and another 100g with distilled water (500ml) for three consecutive terms. The ethanolic extracts were concentrated using a rotary evaporator at 45°C. The dried crude extract was 33.7% in 80% ethanol as a solvent. The crude extract was then dissolved in distilled water to prepare solutions of different concentrations (0.80%, 1.60% and 3.20%). The bioassays of the extracts were done for repellency and direct toxicity (mortality) test.

Method for Toxicity Test: Direct toxicity test with rice weevil was done following the standard method^[16]. Insects were chilled for a period of 10 minute. The immobilized insects were individually picked up and 1 ml solutions of different concentrations (0.0%, 0.8%, 1.6% and 3.2%) were applied to the dorsal surface of the thorax of each insect by using a micro capillary tube. Ten insects per replication were treated. The insects were then transferred into a 9 cm diameter petridish containing food. Insect mortality rate was recorded after 0.25, 0.50, 0.75, 1.0 and 1.5 hour after treatment (HAT). All the experiments were conducted in completely randomized design with four replications and turned to statistical analysis. Finally the mean values were compared using Duncan's Multiple Range Test (DMRT)^[17].

Repellency Test: Repellency test was conducted following the standard method^[16]. The dried extracts were dissolved in distilled water to make solution of

different concentrations. Solutions of three different concentrations as 0.8%, 1.6% and 3.2% (w/v) were prepared. Nine-centimeter diameter filter papers (Whatman No. 40) were marked into two portions. One-milliliter solution of each extract was applied to one half of the filter paper (treated half) and on the other half 1 ml of distilled water was applied (controlled half). The treated disks were then air dried and placed in a petridish. 20 Insects were placed there, 10 on the controlled half and 10 on the treated half. Number of insects on each side was counted at 30 min intervals up to the second hour after treatment. Percent repellency was calculated by using the following Abbots formula^[18]:

$$\text{Percent Repellency} = \{(A - B) / A\} \times 100$$

Here,

A = Average number of insects present on untreated portion.

B = Average number of insects present on treated portion.

The percentages of repellency were then categorized according to the following scale:

Class	Repellency	Class	Repellency
Rate (%)		Rate (%)	
0	>0.01-0.10	III	40.10 to 60.00
I	0.10 to 20.00	IV	60.10 to 80.00
II	20.10 to 40.00	V	80.10 to 100.00

Test for Allelopathic Effect (Plant Growth Regulatory Effect):

Fresh fruits of Rittha (*Sapindus mukorossi*) (100g) were air dried, powdered and soaked in 500ml of distilled water for 72 hours for three consecutive terms. The aqueous extracts were filtered through 9 centimeter diameter filter papers (Whatman No. 40). This extract was taken as 100% stock solution and further diluted to prepare 20%, 40%, 60%, 80% solution and stored for seed treatment. The following treatments were used in the experiments:

- T₀ = Seeds of receptor plants grown in distilled water only (Control).
- T₁ = Seeds of receptor plants grown in fruit extracts of 20% concentration.
- T₂ = Seeds of receptor plants grown in fruit extracts of 40% concentration.
- T₃ = Seeds of receptor plants grown in fruit extracts of 60% concentration.
- T₄ = Seeds of receptor plants grown in fruit extracts of 80% concentration.
- T₅ = Seeds of receptor plants grown in fruit extracts of 100% concentration.

Germination and Growth Records: The germination test was carried out in sterile petridishes of 12 cm in size placing filter paper (Whatman No. 40) on

petridishes. The extract of each concentration was added to each petridish of respective treatment daily in such an amount just to allow the seeds to get favorable moisture for germination and growth. The control was treated with distilled water only. 30 seeds of each agricultural crop were placed in the petridish replicating five times. The experiment extended over a period of ten days to allow the last seed germination and measurement of the root and shoot length. The results were determined by counting the number of germinated seeds, number of lateral roots and measuring the length of primary root and main shoot on the 10th day of the experiment. The data were subjected to analysis of DMRT^[17].

The Ratio of germination and elongation were calculated as suggested by Hoque *et al.*^[15].

- Relative Germination Ratio (RGR) = (Mean Germination of Tested Plant /Germination rate of Control) × 100
- Relative Elongation Ratio (RER) of Shoot = (Mean Shoot length of Tested Plant/Mean Shoot length of Control) × 100
- Relative Elongation Ratio (RER) of Root = (Mean Root length of Tested Plant/Mean Root length of Control) × 100

RESULTS AND DISCUSSIONS

Direct Toxicity Test: With the ethanolic extract of Rittha (*Sapindus mukorossi*) average mortality percentage indicated that 3.20% concentration resulted the higher toxicity of 88.1% for *Sitophilus oryzae* and 70.1% for *Pediculus humanus*. It was also notable that, 0.80% concentration showed average toxicity of 79.5% for *Sitophilus oryzae* (Table 1) and 23.2% for *Pediculus humanus* (Table 2), whereas 1.6% concentration showed the average toxicity of 82.5% and 45.54% for *Sitophilus oryzae* and *Pediculus humanus* respectively. The order of toxicity of three different concentrations were 3.20 > 1.60 > 0.80%. The highest toxicity was observed in *Sitophilus oryzae* compared to *Pediculus humanus*. Observed mortality percentage increased with increase in time intervals after treatment. Mortality percentage at 0.25, 0.50, 0.75, 1.00, and 1.50 HAT indicated that 3.2% solution showed the highest mortality (88.1%) in *Sitophilus oryzae* at 1.50 HAT compared to *Pediculus humanus*. Mortality percentage showed parallel response to the level of concentration at different time intervals after treatment. Mortality after 1.00 to 1.50 HAT showed a little variance signifies that the effect of the extracts decrease after a long interval of time.

Table 1: Corrected mortality with ethanol extract of Rittha (*Sapindus mukorossi*) for *sitophilus oryzae*:

Concentration	Corrected Mortality					Mean Mortality
	Hour After Treatment (HAT)					
	0.25	0.50	0.75	1.00	1.5	
0.00%(Control)	0.00	0.00	5.00	5.00	5.00	3
0.80%	65.00 a*	67.50 a	82.50 a	90.00 a	92.50 a	79.5
1.60%	62.50 a	77.50 b	87.50 ab	92.50 a	92.50 a	82.5
3.20%	77.50 b	87.50 c	90.00 b	90.00 a	95.50 a	88.1

*Within column values followed by same letter(s) did not differ significantly at 5% level by DMRT

Table 2: Corrected mortality with ethanol extracts of Rittha (*Sapindus mukorossi*) for *Pediculus humanus*:

Concentration	Corrected Mortality					Mean Mortality
	Hour After Treatment (HAT)					
	0.25	0.25	0.25	0.25	0.25	
0.00%(Control)	0.00	0.00	2.50	7.50	10.00	4
0.80%	5.00 a*	17.50 a	27.50 a	33.50 a	32.50 a	23.2
1.60%	10.00 a	37.50 b	60.00 b	82.50 b	82.50 b	54.5
3.20%	42.50 b	60.00 c	77.50 c	85.50 b	85.00 b	70.1

* Within column values followed by same letter(s) did not differ significantly at 5% level by DMRT

Table 3: Repellency of different Concentration of dried Ethanol extracts *Sapindus Mukorossi* on rice weevil.

Extract Concentration (%)	Average Repellency Rate (%)				Mean Repellency Rate	Repellency Class
	Minute After treatment					
	30	60	90	120		
0.80	37.84 a*	42.10 a	57.14 a	53.66 a	47.69	III
1.60	48.72 b	50.00 b	57.14 a	57.14 a	53.25	III
3.20	53.66 b	53.66 b	60.47 a	53.66 a	55.36	III

*Within column values followed by same letter(s) did not differ significantly at 5% level by DMRT.

Table 4: Repellency of different Concentration of dried Ethanol extracts *Sapindus Mukorossi* on *Pediculus humanus*.

Extract Concentration (%)	Average Repellency Rate (%)				Mean	Repellency Rate Class
	Minute After Treatment					
	30	30	30	30		
0.80	12.5 a*	33.33a	42.11 a	33.33 a	30.32	III
1.60	23.53 b	42.11 b	42.11 a	37.84 ab	36.40	II
3.20	37.84 c	46.15 b	50.00 b	42.11 b	44.03	III

*Within column values followed by same letter(s) did not differ significantly at 5% level by DMRT

Table 5: Effect of Water Extract of Rittha (*Sapindus mukorossi*) fruit on Germination of the receptor crops.

Treatment	Receptor Plants.									
	Red Amaranth (<i>A. tricolor</i>)		Mustard (<i>B. campestris</i>)		Sword bean (<i>C. esculanta</i>)		Lady's finger (<i>A. esculantus</i>)		Chick pea (<i>C. arietum</i>)	
	RGR	PIE	RGR	PIE	RGR	PIE	RGR	PIE	RGR	PIE
T ₀	80.00a*	0.00	90.00a	0.00	86.67a	0.00	83.33a	0.00	93.33a	0.00
T ₁	36.67b	-54.17	23.33b	-74.07	10.00b	-88.46	33.33b	-60.00	26.67b	-71.43
T ₂	33.33b	-58.33	23.33b	-74.07	13.33b	-84.62	26.67c	-68.00	20.00c	-78.57
T ₃	13.33c	-83.33	20.00c	-77.78	13.33b	-84.62	10.00d	-88.00	16.67c	-82.14
T ₄	6.67d	-91.67	16.67c	-81.48	10.00b	-88.46	10.00d	-88.00	20.00c	-78.57
T ₅	20.00e	-75.00	6.67d	-92.59	13.33b	-84.62	13.33d	-84.00	20.00c	-78.57

(Within column, values followed by same letter(s) did not differ significantly at 5% level by DMRT. Here, RGR=Relative Germination Ratio. And PIE= Percent of Inhibitory Effect.)

Repellency Test: Among the different concentration of extracts the 3.20% extract solution of *Sapindus mukorossi* showed maximum repellency 55.36% in case of rice weevil and 44.03% in case of *Pediculus humanus*. The repellent action increased with the increase in concentrations of the extract applied (Table 3 and Table 4).

Allelopathic Effect Test:

Effect on Germination: Relative germination percentages of the receptor agricultural crops are shown in (Table 5). Highest germination percentage was observed at control. Germination percentage of most of the crops was reduced significantly with increase in concentration of the extract but to a minimum value

and again showed an upper trend on highest concentrations except Mustard (*Brassica campestris*). Irregular inhibitory effect was observed in Lady's finger (*Abelmoschus esculantus*). Highest inhibition (-92.59%) was found in case of Mustard (*Brassica campestris*) at T₄ treatment and lowest (-54.17%) on Red Amaranth (*A. tricolor*) at T₁.

Effect on Shoot Length: Table 6 represents average shoot length (mm) of the germinated seedlings of all the receptor agricultural crops. No stimulatory effect was found. Maximum reduction of shoot length was caused by T₃ treatment followed by T₄ treatment in most of the receptors. The inhibitory effect decreased on highest concentration. Among the survivors highest inhibitory

Table 6: Effect on Shoot Elongation of Water Extract of Rittha (*Sapindus mukorossi*) fruit

Treatment	Receptor Plants									
	Red Amaranth (<i>A. tricolor</i>)		Mustard (<i>B. campestris</i>)		Sword bean (<i>C. esculanta</i>)		Lady's finger (<i>A. esculantus</i>)		Chickpea (<i>C. arientum</i>)	
	A	B	A	B	A	B	A	B	A	B
T ₁	64.89a*	-35.11	58.95a	-41.05	67.77ac	-32.23	73.74a	-26.26	70.13a	-29.87
T ₂	68.00ac	-32.00	54.59a	-45.41	58.29b	-41.71	76.26a	-23.74	68.83a	-31.17
T ₃	49.78b	-50.22	71.18b	-28.82	64.45c	-35.55	70.20a	-29.80	63.64b	-36.36
T ₄	72.89c	-27.11	53.71a	-46.29	69.67a	-30.33	64.65b	-35.35	63.20b	-36.80
T ₅	72.44c	-27.56	75.11b	-24.89	71.09a	-28.91	66.16b	-33.84	60.17b	-39.83

Within column values followed by same letter(s) did not differ significantly at 5% level by DMRT [Here, A = Relative Elongation Ratio (RER) B= % Inhibition. (-ve sign indicates inhibition.)]

Table 7: Effect on Root Elongation of Water Extract of Rittha (*Sapindus mukorossi*) fruit

Treatment	Receptor Plants									
	Red Amaranth (<i>A. tricolor</i>)		Mustard (<i>B. campestris</i>)		Sword bean (<i>C. esculanta</i>)		Lady's finger (<i>A. esculantus</i>)		Chick pea (<i>C. arientum</i>)	
	A	B	A	B	A	B	A	B	A	B
T ₁	18.33ac*	-81.67	19.90ac	-80.10	21.88a	- 8.13	35.19a	-64.81	31.68a	-68.32
T ₂	17.78ac	-82.22	15.92ab	-84.08	25.52a	-74.48	38.27a	-61.73	16.83b	-83.17
T ₃	10.56b	-89.44	11.44b	-88.56	16.67b	-83.33	19.75b	-80.25	16.34b	-83.66
T ₄	16.67c	-83.33	22.39c	-77.61	20.31ab	-79.69	17.28b	-82.72	22.28c	-77.72
T ₅	22.22a	-77.78	19.90ac	-80.10	18.75ab	-81.25	25.31c	-74.69	15.84b	-84.16

* Within column values followed by same letter(s) did not differ significantly at 5% level by DMRT [Here, A = Relative Elongation Ratio (RER) B= % Inhibition. (-ve sign indicates inhibitory effect)]

Table 8: Effect on Number of Lateral Roots developed in Receptor agricultural crops

Treatment	Receptor Plants									
	Red Amaranth (<i>A. tricolor</i>)		Mustard (<i>B. campestris</i>)		Sword bean (<i>C. esculanta</i>)		Lady's finger (<i>A. esculantus</i>)		Chick pea (<i>C. arientum</i>)	
	A	B	A	B	A	B	A	B	A	B
T ₁	59.35a*	-40.65	102.72a	2.72	73.02a	-26.98	65.03a	-34.97	94.30a	-5.70
T ₂	35.77b	-64.23	72.83b	-27.17	66.67b	-33.33	43.36b	-56.64	83.54b	-16.46
T ₃	17.07c	-82.93	92.93c	-7.07	53.97c	-46.03	54.55c	-45.45	69.62c	-30.38
T ₄	15.45c	-84.55	91.85c	-8.15	43.92d	-56.08	90.21d	-9.79	60.76d	-39.24
T ₅	56.91a	-43.09	92.39c	-7.61	37.57e	-62.43	102.80e	2.80	60.13d	-39.87

Within column values followed by same letter(s) did not differ significantly at 5% level by DMRT [Here, A = Relative Number of Lateral Roots developed B= % Inhibition. (-ve sign indicates inhibitory effect)]

effect (-50.22%) was found on Red Amaranth (*A. tricolor*) at T₃ treatment and lowest (-24.89%) on Mustard (*B. campestris*) at T₅ treatment. Lady's finger (*A. esculantus*), and Chick Pea (*Cicer arientum*) showed decreasing RER (shoot) with concentration and for Red Amaranth (*A. tricolor*), Mustard (*B. campestris*), and Sword Bean (*Canvalia esculanta*) showed a decrease of RER followed by an increase.

Effect on Root Elongation: Table 7 represents average

shoot root length (mm) of the germinated seedlings of all the receptor agricultural crops. No stimulatory effect was found. Maximum reduction of root length was caused by T₃ treatment followed by T₄ and T₅ treatment in most of the receptors. The inhibitory effect decreased on highest concentration. Among the survivors highest inhibitory effect (-89.44%) was found on Red Amaranth (*A. tricolor*) at T₃ treatment and lowest (-61.73%) on Lady's finger (*A. esculantus*) at T₂ Treatment.

Effect on Number of Lateral Roots developed: The Study revealed that the number of lateral root development was significantly inhibited from the treatment T₁ and onwards. Except some exception T₅ and T₄ treatment inhibited the lateral root development largely. T₄ showed maximum inhibitory effect on Red Amaranth (*A. tricolor*) and T₁ showed minimum inhibitory effect on Chick Pea (*C. arietum*). T₅ showed stimulatory effect on Lady's finger (*A. esculantus*) (Table 8).

The study clearly demonstrated the suppressive effect of *Sapindus mukorossi* fruit extracts on the germination and seedlings growth of selected bioassay species. The suppressive effect was significantly reduced the germination and overall seedling growth of the receptor plants. This findings were correlated with the findings of Haque *et al.*^[15] who found the inhibitory effect of leaf extracts of some plants often grows or cultivated beside crop fields on certain food crops. Suppressive effect was increased with an increase of extract concentration indicating that the effect of plant extracts dependent very much on their concentrations. Similar observations was found by others^[14,15,19-21].

The suppressive effect of *Sapindus mukorossi* may be caused by allelopathy. This results were correlated with the findings of others^[11-12]. Patterson reported the effect of secondary extract metabolites from Jhonson grass (*Sorghum halepense*) on germination, growth and development of various crops^[13]. Ahmed *et al.* reported the allelopathic effects of *Eucalyptus camaldulensis dehn.* on some agricultural crops in Bangladesh^[14]. Hoque *et al.* also reported allelopathic effects of *Eupatorium odoratum* on germination and growth behavior of six agricultural crops^[15]. The present study also revealed that development of roots and lateral roots were severally impeded compared to germination and shoot growth. These results correlated with findings of Haque *et al.*^[15]. Those studies were found that growth effects were more sensitive and respond more strongly to small variation in toxin concentrations. The biological activity of plant extracts is due to the various phytotoxic compounds present in the extracts. These compounds may independently or jointly contribute to cause plant growth regulatory effect and inhibit germination. Further investigation is needed to identify the active compound(s) of the extract responsible for its activity and to examine the effect of *Sapindus mukorossi* extract against a wider range of receptor plants. Though laboratory bioassays are of great importance to single out the allelopathic effect extensive field study may recommended. However, it is difficult to draw a definite conclusion from this study about the particular compound(s) responsible for the biological activity of Rittha. Nevertheless, from this result it is better to suggest

the farmer to remove the *Sapindus mukorossi* rapidly during germination of Red amaranth (*Amaranthus tricolor*), Mustard (*Brassica campestris*), Sword bean (*Canavalia esculanta*), Lady's finger (*Abelmoschus esculantus*), Chick pea (*Cicer arietum*) seeds.

In our view, biopesticides from plant origin may contribute to an effective vector control tools. These new agents should preferentially to be applied in integrated control strategies to gain maximum and safer impact on insect growth.

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