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Toxicity and repellant activity of organic extracts of bioactive Indian medicinal plant, *Alstonia scholaris* against *Tribolium castaneum* (Herbst) adults

Nilesh S.Pawar*[1], Snehal P.Salunke[2], Vijay V.Bhadane[2]

[1]Synthetic Research Laboratory, Department of Chemistry, Pratap College, Amalner-425 401, M.S., (INDIA)

[2]Department of Botany, Pratap College, Amalner- 425 401, M.S., (INDIA)

E-mail: nileshpawar1329@rediffmail.com; bhadanevv2009@rediffmail.com

ABSTRACT

The effect of polar and non-polar extract of leaves and stem barks of *Alstonia scholaris* (L.) R.Br. (Apocynaceae) were evaluated for its repellent activity. The study of repellency was revealed that as the concentration increases 25, 50 and 100 mg/ml with increased in repellent effect. Such study will be helpful to increase the pest management potency and efficacy of plant derived products.

KEYWORDS

Alstonia scholaris;
Isolation;
Tribolium castaneum;
Repellent;
Extraction;
Polar and non-polar.

INTRODUCTION

Stored food grains face severe damage due to infestation by insects. The insect damages are ranging from 5-30% of the world's total agricultural production. The reasons for their widespread presence range from evolutionary adaptations to the actions of humans who transport them throughout the world and offer a protected habitat within stored food stuffs[3]. The red flour beetle, *Tribolium castaneum* (Herbst) is a major pest in human stored food and has been found in association with a wide range of commodities including grain, flour, peas, nuts, dried fruits and spices. It also infesting groundnut kernels and pods. Milled grain products remain its preferred food. This insect causes substantial loss in storage because of its high reproductive potential and can breed throughout the year in warm areas[7]. Usage of synthetic pesticides to control these pests is highly discouraged because of their adverse effect on human beings and environment. Plants are known as a good source of grain protectant[4,8,9]. Although a large number of plant products in various forms have been screened against major pests of stored grain, thousands are still untouched. Plants, which have rich source of secondary metabolites, can acts as insecticides, ovicidal, ovipositional deterrents, feeding deterrents and growth retardants. Most of the plant products are non pollutant, less toxic and are easily biodegradable in nature. Hence in the present investigation, studied repellent activity of plant on the basis of plant bioassay.

The plant *Alstonia scholaris known* as "Devil's Tree" (Family Apocynaceae) is an important medicinal plant which is used all over the world. A tall tree with bitter milky juice, evergreen, branches arranged in whorls. Leaves 3-7 at a node, in whorl, coriaceous, oblong-lanceolate, or obovate, lower surface with whitish bloom, intrastaminal nerve present. Flowers (Sep-Nov) greenish-white, in terminal corymbose or umbellate cymes, hairy. Calyx- lobes 5, oblong, obtuse, ciliate. Corolla salver shaped, hairy inside and near the mouth, lobes 5, cuneate oblong, rounded, hairy. Stamens 5, included, carpels 2, free. Follicles long, pendulous, cylin-

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drical, seeds many, comose on both the ends[1,3]. The tree is found throughout the tropical and subtropical region of India, especially in the west coast forests, but is nowhere very abundant. It also occurs in Burma, Ceylon, Malaya, East Indies and in the Philippines[1,2].

Different parts i.e. stem bark, leaf, milky juice of plant were used in various indigenous medicine[5]. The chemical constituents present in the plant are Diamine, Echitamine, Alstonine and Echitamidine[1]. The survey of literature revealed that stem bark are astringent, febrifuge, alterative, bitter tonic, antimalerial. They are used in malaria fever, diarrhoea and dysentery[1,5]. Milky juice applied on ulcers. In the present study we evaluated the potential repellent activity of crude ethanolic and Benzene extract from the leaves and stem barks of *Alstonia scholaris* on *Tribolium castaneum*.

The aim of the present work is to control stored grains pest using plant compound.

MATERIAL AND METHODS

Collection and preparation of the test materials

The fresh leaves and stem bark of *A. scholaris* were collected from the campus of the North Maharashtra University, Jalgaon. The plant material was taxonomically identified (voucher specimen: PCA-C 21/2011 and preserved in the Department of Botany, Pratap College, Amalner) by the taxonomist of Botany department, Pratap College, Amalner, M.S. India. Collected leaves and bark were washed with distilled water and dried in shade. Subsequently, it was coarsely powdered and used for obtaining the extracts.

Collection and culture of the test insect

T. castaneum were collected from private store houses of Amalner tehsil of Jalgaon district, Maharashtra, were used throughout this study. *T. castaneum* used in the present experiment was reared in whole-wheat flour in the Laboratory of P.G. and Research Department of Zoology, Pratap College, Amalner-425 401 [M.S.] India. The cultures were maintained in the laboratory at 29-32°C and 70-80% RH.

Plant extract preparation[6]

- Preparation of polar (Alcohol) extract
- Preparation of Non-polar (Benzene) extract.

The leaves and stem bark of the selected plant species of locally available Indian Medicinal plant *Alstonia scholaris.* The leaves were washed with distilled water and dried in shade and crushed in mixer, to form a powder type material.

As same, the stem bark of *A. Scholaris* washed with distilled water and dried in shade and crushed in mixture to form a powder type material and then (100 g each plant sample) extracted with Ethanol (polar) and Benzene (Non-polar) using Soxhlet's extraction apparatus. The solvents were removed in the beaker and evaporated it on water bath greenish-black semi-solid mass was obtained. The output extracts were removed to glass vials and preserved in a refrigerator at 4°C with proper labeling.

Repellent activity bioassay

In present study, the stored grain pests, viz. red flour beetle (*Tribolium castaneum*) as selected for the repellent activity bioassay. The repellency test used was adopted from the method (No. 3) of McDonald *et al.* (1970)[10] with some modifications by Talukder and Howse (1995)[11]. The filter paper disc (Whatman No. 40, diameter 8 cm) were prepared and cut into two equal halves. Separately on each half, 25, 50 and 100 mg/ml of the solutions of compounds and acetone (control) were added. These halves were air dried for 10 min. Each treated half-disc was then attached lengthwise, edge-to-edge, to control half-disc with adhesive tape and placed in a petridish (diameter 8 cm), the inner surface of which was smeared with glue-stick to prevent insect escaping. The orientation of the seam was changed in the replicates to avoid the effect of any external directional stimulus affecting the distribution of the test insects. Twenty adult insects of either species were released in the middle of each filter-paper circle and a plastic cover with some small holes was placed on the petridish. Each compound was tested twice times. Insect that settled on each half of the filter paper disc were counted after 1h and then at hourly intervals for 5 h. No significant difference was noted between the repellency of acetone impregnated and untreated filter papers in tests designed to check for and possible influence of acetone. The average of the counts was converted to percentage repellency (PR) using the Talukder and Howse formula.

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(a) Percent of insects on untreated half of the disc

Percent of insects on untreated half of the disc = 100 [No of insects on untreated half of disc/Total no of insects taken for experiment]

(b) Percent repellency

Percent repellency = 2 (C -50)

Where, C = The percent of insects on the untreated half of the disc.

Positive values express repellency and negative values attractancy. The results of repellent activity against red flour beetle are summarized respectively in TABLE 1, 2, 3 and 4.

TABLE 1 : Repellent activity of *Alstonia scholaris*, for polar (Alcohol) extracts of leaves.

Conc. /ml	Hours	No. of insects on untreated half of disc			No. of Insects taken for the Experiment	% of Insects on untreated half of disc	Percent Repellency	Mean percent Repellency
		1st disc	IInd disc	Mean				
25 mg	1	10	08	09	20	45	-10	-27
	2	09	05	07		35	-30	
	3	14	03	8.5		42.5	-15	
	4	07	04	5.5		27.5	-45	
	5	09	04	6.5		32.5	-35	
50 mg	1	14	08	11	20	55	10	07
	2	10	06	08		40	-20	
	3	12	08	10		50	00	
	4	13	11	12		60	20	
	5	13	12	12.5		62.5	25	
100 mg	1	11	12	11.5	20	57.5	15	18
	2	14	16	15		75	-50	
	3	12	17	14.5		72.5	45	
	4	14	18	16		80	60	
	5	11	13	12		60	20	

RESULTS AND DISCUSSION

The data presented in above TABLE 1, 2, 3 and 4 exhibits the differences due to different period of exposures of insect to the extracts. Considering the average of all state of leaves and stem barks, extraction media and concentrations of extract into consideration, the highest mean repellency of 18% was observed at 100 mg/ml concentration while, the least mean activity of -27% was observed at 25 mg/ml concentration. It indicates that repellency increased with increasing concentration of the extracts.

TABLE 2 : Repellent activity of *Alstonia scholaris*, for non-polar (Benzene) extracts of leaves.

Conc. /ml	Hours	No. of insects on untreated half of disc			No. of Insects Taken for the Experiment	% of insects on untreated half of disc	Percent Repellency	Mean percent Repellency
		1st disc	IInd disc	Mean				
25 mg	1	11	12	11.5	20	57.5	15	+12
	2	12	10	11		55	10	
	3	14	09	11.5		57.5	15	
	4	15	07	11		55	10	
	5	18	04	11		55	10	
50 mg	1	08	11	9.5	20	47.5	-05	-31
	2	06	07	6.5		32.5	-35	
	3	09	03	06		30	-40	
	4	09	01	05		25	-50	
	5	08	07	7.5		37.5	-25	
100 mg	1	12	09	10.5	20	52.5	05	+19
	2	12	10	11		55	10	
	3	15	11	13		65	30	
	4	14	12	13		65	30	
	5	11	13	12		60	20	

Similarly, the data presented in TABLE 2, shows the highest mean repellency of 19% was observed at 100 mg/ml concentration while, the least mean activity of -31% was observed at 50 mg/ml concentration. The repellency increased at 25 mg/ml and 100 mg/ml concentration. But it decreases at 50 mg/ml concentration. It indicates that repellency increased with increasing concentration of the extracts.

In case of TABLE 3, the highest mean repellency of 26% was observed at 100 mg/ml concentration while, the least mean activity of 16% was observed at 25 mg/ml concentration. It indicates that repellency increased with increasing concentration of the extracts.

Also the data obtained from TABLE 4, it indicates that the highest mean repellency of 58% was observed at 50 mg/ml concentration while, the least mean activity of 20% was observed at 25 mg/ml and 100 mg/ml concentration. The repellency increased from 25 mg/ml to 50 mg/ml concentration, but it decreases for 100 mg/ml concentration with the same repellency. It indicates that repellency increased with increasing concentration of the extracts.

In overall mean repellent effect was increased with increasing time over the 5 hours experimental period. It

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indicates that active ingredients in extracts could be non-volatile.

TABLE 3: Repellent activity of *Alstonia scholaris*, for polar (alcohol) extracts of Stem bark.

Conc. /ml	Hours	No. of insects on untreated half of disc			No. of insects taken for the Experiment	% of insects on untreated half of disc	Percent Repellency	Mean percent Repellency
		1st disc	IInd disc	Mean				
25 mg	1	14	12	13	20	65	30	+16
	2	12	10	11		55	10	
	3	10	12	11		55	10	
	4	14	04	09		45	-10	
	5	10	18	14		70	40	
50 mg	1	12	08	10	20	50	00	+24
	2	10	14	12		60	20	
	3	14	10	12		60	20	
	4	16	12	14		70	40	
	5	18	10	14		70	40	
100 mg	1	06	06	06	20	30	-40	+26
	2	12	18	15		75	50	
	3	10	18	14		70	40	
	4	18	10	14		70	40	
	5	18	10	14		70	40	

TABLE 4 : Repellent activity of *Alstonia Scholaris*, for non-polar (benzene) extracts of Stem bark.

Conc. /ml	Hours	No. of insects on untreated half of disc			No. of insects taken for the Experiment	% of insects on untreated half of disc	Percent Repellency	Mean percent Repellency
		1st disc	IInd disc	Mean				
25 mg	1	16	10	13	20	65	30	+20
	2	10	10	10		50	00	
	3	14	18	16		80	60	
	4	08	14	11		55	10	
	5	08	12	10		50	00	
50 mg	1	18	16	17	20	85	70	+58
	2	18	16	17		85	70	
	3	18	16	17		85	70	
	4	16	16	16		80	60	
	5	14	10	12		60	20	
100 mg	1	12	14	13	20	65	30	+20
	2	12	10	11		55	10	
	3	14	10	12		60	20	
	4	12	14	13		65	30	
	5	10	12	11		55	10	

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