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The Herbal Insecticides from Root Stock of *Alocasia indica* as Potential Sources for Control of Stored-Product Insect Pests

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Abstract

In the present study the insecticidal activity of organic extracts from root stock of *Alocasia indica* (Linn.) against three major important stored grain insect pest viz; rice weevil, *Sitophilus oryzae*; the lesser grain borer, *Rhyzopertha dominica* and the rust-red flour beetle, *Tribolium castaneum* is reported. The fumigant toxicity of hexane extract shows LC50 value 108.4 µg/l for adult *T. castaneum*, 96.2 µg/l for *S. oryzae* and 84.6 µg/l for R. dominica, respectively and toxicity was species specific. A concentration of 300 µg/l for a period of 72 h exposure resulted in 100% mortality of mixed age culture of these three species. An extended exposure period of 72 h has increased the rate of mortality in all three species. Further the results confirm that there are no significant differences between seed germination of control and treated grains. The study proved that efficacy of *Alocasia indica* when extracted with hexane is more effective when compared with other type of solvent extractions. The probable use of this extract as a bio fumigant is here with discussed.

Keywords: *Alocasia indica*; Fumigant toxicity; Root stock extracts; Stored-product insect pests; Seed viability; Grain protectant

Introduction

Stored-product insect pests cause extensive damage during the postharvest storage in developing countries [1,2]. As a control measure many synthetic chemical insecticides and fumigants are being widely used against various insect pests. However, due to their slow biodegradable nature which results in environmental pollution, and also with concern to human health hazards, many insecticides have been replaced by modern insecticides [3,4]. Further, due to the problem of resistance to insecticides, there is a growing demand to develop newer eco-friendly safer and effective biofumigant [5]. Natural products are an important source of novel active compounds that can be used to control stored grain insect pests [2,6,7].

Natural compounds have the advantage of providing novel modes of action against insects that can reduce the risk of cross-resistance as well as offering new leads for design of target-specific molecules [7,8]. Natural compounds can be useful for stored grain protection and also for the management of the stored product insect pests. Plant extracts and its derivatives have important role to play in the preservation of food grains against insect infestation and plants are in rich source of new biopesticides [1,9,10]. At present, there are no biopesticides to replace phosphine and methyl bromide which are used for protection of stored grain from insect infestation. Biopesticides of plant origin are considered to be the best source of newer compounds for development of new, ecofriendly, and safer insect control agents since they show selectivity to insect species, easily biodegradable, and nontoxic to mammalian system [11-13].

Alocasia indica Linn. (Araceace), commonly called as giant taro, is a mainly found in . The different parts of this plant are used for treating inflammation and disease of abdomen and spleen [14]. *Alocasia indica* has showed antifungal properties and juice prepared from the leaves of this is used as digestive, laxative, astringent and also for the treatment of rheumatic arthritis [15,16]. However, there are no literatures on use of *Alocasia indica* as a grain protectant. In the present paper we have evaluated the fumigant activity of different organic extracts from root stock of *A. indica* against the storage pests viz. rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and lesser grain borer, *Rhyzopertha dominica* (F.) and rust red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae). The results of the present study would be useful in promoting research aiming at the development of promising grain protectants from natural origin.

Material and Methods

Cultures of *S. oryzae* and *R. dominica* were maintained on whole wheat (*Triticium aestivum*) whereas, *T. castaneum* were reared on the whole wheat flour with 2% yeast powder. Insect cultures were maintained at 25 ± 1°C and 70 ± 2% r.h. [17]. From these cultures, adults (1-2 week old) were taken for preparation of mixed-age cultures. About 300 adults of *S. oryzae* and *R. dominica* were released into 1 kg of whole wheat grains kept in glass jars. Similarly 300 adults of *T. castaneum* were allowed to breed separately in glass jars containing 1 kg of wheat flour.

Healthy mature root stocks (vertical rhizome) of *A. indica* (10 kg) were collected in the month of June from Imphal valley region of Manipur, North East India. The root stock was cut into small pieces, dried at 40°C and powdered. One hundred gram of root stock powder was sequentially extracted with a series of solvents of increasing polarity *viz.*, hexane, ethyl acetate, acetone and methanol in a Soxhlet apparatus. The solvent was evaporated *in vacuo* and the residue was dissolved in a known volume of methanol/acetone. This solution was screened for insecticidal activity through fumigant toxicity (Figure 1).

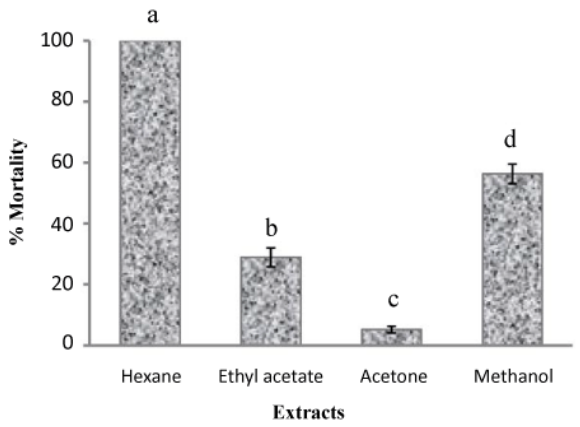

Figure 1: Insecticidal activity of the extracts of *Alocasia indica* to *Tribolium castaneum*. The extracts were applied for fumigant toxicity at 200 µg/l (n= 4, error bars, standard deviation).

The insecticidal activity of extracts of *A. indica* against adults of *S. oryzae*, *R. dominica and T. castaneum* was studied by fumigation. 50 numbers and known age of (adults) insects were released into cloth bags (20 cm x 14 cm size) and bags were placed individually in 0.85-l desiccators that served as the fumigation chambers. In each desiccator, a Whatman No. 1 filter circle (9cm size) was placed to serve an evaporating surface for injecting active extract. For each species, there were four replicates for each dose of the active extract, with equal number of untreated control replicates.

Another experiment was designed for the mixed age culture of different stored grain insect species were exposed to active extract (hexane extract) of *A. indica* for 24 h and 72 h exposure at 25 ± 2°C. The active extract doses were chosen based on the preliminary experiments and their mixtures to the insects could be determined. Rearing media containing mixed age cultures of the insects were weighed in 50 g aliquots into cloth bags (20 cm x 14 cm size) and bags were placed individually in 0.85-l desiccators that served as the fumigation chambers. In each desiccator, a Whatman No. 1 filter circle (9 cm size) was placed to serve an evaporating surface for injecting active extract and along with an equal number of untreated control desiccators were maintained. After 24 h and 72 h, the fumigation was terminated and the test insect bags were taken out. The contents of the bag were transferred to individual bottles and kept in the rearing temperature (25 ± 1°C) and humidity (70 ± 2% r.h.) conditions for weeks. At weekly intervals, the F1 progeny produced were recorded for 8 consecutive weeks. Percentage reduction in adult emergence of F1 progeny or inhibition rate (%IR) was calculated as

$$IR\ (\%) = (C_n\text{-}T_n)\ 100/C_n$$

Where C_n is the number of newly emerged insects in the untreated jar and T_n is the number of insects in the treated jar [18].

The hexanel extract of *A. indica* was analyzed on a gas chromatograph (HP6890; Agilent Technologies USA Ltd) directly linked to a HP5973 mass selective detector (Agilent Technologies) operated in electron impact mode (source temperature 230°C; transfer line 250°C). The HP-5 MS phenyl methyl siloxane non polar capillary column (0.25 mm x 30 m x0. 25 µ.) max 350°C (Agilent part No 190915 – 433) was used. The mobile phase was Helium 99.999% purity (Praxair India Ltd) passed through the universal trap for removing the contaminants. The split inlet was used with a split ratio of 50:1 and inlet temperature of 280°C. The oven temperature program was set at 70°C min^{-2} to 2 minute hold and a ramp of 6°C min^{-1} till 260°C and held for 5 mins with column flow of 1 ml/mi. The mass spectral detector was maintained at a temperature of 280°C with the interface temperature of 230°C. The mass spectra created using the MS was compared with the Wiley mass spectral library (Wiley W9N11.L and NIST 2.0 version).

Wheat grains were treated with hexane extract of *A. indica* at 200 and 400 µg/l and germination tests were done at 24 h and 72 h of exposure. Fifty grains from each treatment were randomly selected from each group and soaked in distilled water for about 30 min, and kept on filter paper (Whatman No. 1) in a petri dish, moistened daily with distilled water and allowed to germinate at room temperature (25 ± 2°C). After 5 d, germinated seeds were counted and percentage of germination was calculated [2].

LC_{50} and LC_{90} were determined by Probit analysis [19]. The Mortality data were analyzed using one way Analysis Of Variance (ANOVA) ($p < 0.05$) by a Newman-Keul's multiple range test using Statplus 2007 software and computer program SAS (version 6.12, SAS Institute Inc. Cory, NC, USA).

Results and Discussion

Various plant species belonging to *Acoraceae, Annonaceae, Apiaceae, Asclepidaceae, Asteraceae, Areceae, Clusiaceae, Fabaceae, Lamiaceae, Leguminosae, Meliaceae, Myrtaceae, Verbenaceae* and *Zingiberaceae* families were known to contain many promising compounds which can be used as insecticides [1]. Many of the plants from these families provide a potential alternative or substitute for synthetic insecticides currently used for stored-product pest control. Azadirachtin from t neem (*Azadirachta indica*) tree is used as antifeedant and an insect growth regulator. However due to lack of fumigant toxicity commercialization of the product was failed but managed to find a place in integrated pest management of field crop pests [20]. Another compound, Rotenone one of the earliest plant-derived insecticide from the Derris root, was found effective however, this compound found to be toxic to the mammalian systems and its use as a protectant for stored grain pests was not accepted [11,21]. At present widely used and most successful synthetic pyrethroids were originally derived from the flowers of *Chrysanthemum cinerariaefolium* [22,23]. The organic extracts and its bioactive molecules from root powder of *Decalepis hamiltonii* (Wight and Arn) showed potential to be used as grain protectant against grain insect pests [7,18].

Plant derived products composed of many bioactive molecules, which exhibit fumigant activity. Zapata and Smagghe [24] reported that the four essential oils extracted from the leaves and bark of *Laurelia sempervirens* and *Drimys winteri* was found to be toxic to *T. castaneum*. Simialarly, our preliminary screening showed that, the hexane extract root stalk of *A. indica* was most effective against *T. castaneum* when used as fumigant followed by ethyl acetate, hexane and acetone (Figure 1). Based on LC50 values (Table 1) and their respective confidence intervals, the hexane extract was found to be significantly more effective in *S. oryzae* compared to *T. castaneum* adults and this extract showed potent fumigant toxicity. The LC50 value for all the three species was variable and species-specific. The

results clearly demonstrated that the extract showed potent fumigant activity against stored grain insect pest and results indicated that the insecticidal mode of action of the active extract may be largely attributed to fumigant action.

Insect species	LC50[a,b]	LC_{90}	Slope ± SE	Degrees of freedom	Chi square ($©^2$)
T. castaneum	108.4 (97.6- 117.6)	190.2	0.671 ± 0.020	7	12.09
S. oryzae	96.2 (87.2 – 101.4)	172.4	0.529 ± 0.061	5	4.717
R. dominica	84.4 (73.8 – 90.2)	152.6	0.725± 0.065	5	2.635

Table 1: Fumigant activity of hexane extract of *A. indica* against three stored-grain pests [a]LC_{50} and LC_{90} = µg/l[b]Values in parenthesis represent confidence limits

Further, the hexane extract was highly toxic to mixed age cultures of *S. oryzae, T. castaneum and R. dominica* recorded 85 – 94.2% mortality at dose of 300 µg/l in 24 h exposure (Table 2), whereas 100% mortality was achieved at dose of 300 µg/l in 72 h exposure (Table 2), respectively. Generally, an extended exposure period of 72 h increased mortality in all three species (Table 2). Results of grain protection showed that hexane extracts caused significant reduction in F1 progeny of all the three species in the mixed age culture in both 24 and 72 h exposure (Table 3). This may be due to the exceptionally high tolerance of the eggs of *T. castaneum*, *R. dominica* and *S. oryzae*. In contrast, when essential oils (Biofumigant molecules) of *Artemisia mongolica* and *Artemisia capillarity* were used against *S. zeamais* adults, the mortality was achieved with an LC50 value of 7.35 and 5.31 mg/l, respectively [8]. Whereas methyl bromide and phosphine have fumigant toxicity (24 h) against *S. zeamais* adults with LC_{50} values 0.67 and 0.006 mg/l, respectively [25]. However, considering the currently used fumigants are synthetic insecticides, the fumigant activity of the hexane extracts is quite promising and showing the potential to be developed as possible natural fumigants for the control of stored product insects. In this regard, this report appears to be first on use of *A. indica* as a potent biofumigant against the stored grain insect pests. Further, our study proved that no significant differences between seed germination of control and treated grains (data not shown). The lack of adverse effect of hexane extract of *A. indica* on seed germination is highly desirable for grain protectant.

Hexane extract of A. indica (µg/l)	Mean mortality (% ±SD)a					
	24 h exposed			72 h exposed		
	S. oryzae	R. dominica	T. castaneum	S. oryzae	R. dominica	T. castaneum
25	6.9 ± 1.7a	10.2 ± 2.1a	4.2 ± 0.5a	10.9 ± 2.4a	14.2 ± 4.4a	6.2 ± 1.7a
50	24.1 ± 2.8 b	19.2 ± 2.9b	12.4 ± 1.3b	34.1 ± 4.1 b	23.12 ± 2.9b	37.4 ± 2.7b
100	46.3 ± 5.5c	42.6 ± 3.8c	34.9 ± 3.0c	56.3 ± 3.5c	62.6 ± 3.4c	54.9 ± 2.6c
200	77.3 ± 1.9d	78.3 ± 4.9d	64.3 ± 4.1d	87.3 ± 4.2d	88.3 ± 3.6d	70.3 ± 5.4d
300	88.9 ± 2.8 e	94.2 ± 5.1e	85.9 ± 2.1 e	100 e	100 e	100 e

Table 2: Mortality (%) of mixed age cultures of stored-product insects exposed for 24 h to hexane extract of *A. indica*.

a There were four replicates for each dose (50 g of infested wheat grain or flour per replicate). In untreated controls, the mean numbers of survivors were 194.2 ± 1.8 (*S. oryzae*), 180.8 ± 5.2 (*R. dominica*) and 152.8 ± 2.2 (*T. castaneum*). Values followed by different letters within the vertical columns are significantly different (P < 0.05) by Newman-Keul's multiple range tests.

Dosage (µg/l)	% Reduction in F1 adult emergence					
	24 h exposed			72 h exposed		
	S. oryzae	R. dominica	T. castaneum	S. oryzae	R. dominica	T. castaneum
25	30.9 ± 1.4a	54.2 ± 4.04a	30.2 ± 3.4a	36.3 ± 1a	59.2 ± 2.4a	40.2 ± 4.2a
50	54.1 ± 1.4 b	63.12 ± 1.9b	52.1 ± 2.1b	62.1 ± 2.4 b	73.12 ± 0.9b	59.1 ± 1.8b
100	79.3 ± 1.5c	82.6 ± 1.4c	71.6 ± 4.1c	84.3 ± 1.9c	90.6 ± 3.4c	78.6 ± 5.1c
200	90.3 ± 0.2d	98.3 ± 0.6d	89.5 ± 2.6d	94.3 ± 3.2d	100	92.5 ± 3.6d
300	100 e	100 e	98.9 ± 2.2 e	100 e		100e

Table 3: Grain protection potential of hexane extract of *A. indica*: F1 progeny emergence of Stored- product insects in treated grain*.

Values followed by different letters within the vertical columns are significantly different (P < 0.05) by Newman-Keul's multiple range tests.

The chromatogram of hexane extract of *A. indica* root stock volatiles and the compounds identified by GC-MS are depicted in Table 4. Twelve volatiles were identified viz; 3-Pentanone, 4-hydroxyl-4-methyl (8.37%), Decane (3.53%), 2, 3-Octandione (10.7%), Pantolactone (6.09%), o-Cymol (10.15%), Undecane (2.71%), 2, 4-Decadienoic acid (5.7%), 1-Dodecanol (4.8%), 3-Oxo-α-ionol (7.7%), Tetradecanoic acid (13.3%). Hexadecanoic acid (5.9%), and 9, 12-Octadecadienoic acid (8.35%). Combination of these compounds along with the other components which are yet to be discovered could be the possible reason for insecticidal activity (fumigant toxicity). Further studies are necessary for the synthesis and bioassay of these compounds for confirming their potentiality as a fumigant.

Peak number	Compound	Mass spectral data	Retention time (min)	Relative (%)
1 2 3	3-Pentanone, 4-hydroxyl-4-methyl Decane	31,38,43,53,59,70,83,101,112 43,57,71,85,99,113,142	3.8 5.2 5.92	8.37 3.53%

4	2, 3-Octandione	32,43,55,64,71,79,86,91,99,107,122,142	8.71	10.71 %
5	Pantolactone	15,19,27,31,39,43,51,57,67,71,76,112,	11.55	6.09%
6	o-Cymol	130 ,	21.9	10.15 %
7	Undecane	27,41,51,57,70,77,91,103,119,128,134	26.39	2.71%
8	2,4-Decadienoic acid	29,33,38,43,57,71,85,99,113,127,156	31.2	5.7%
9	1-Dodecanol	29,41,59,67,81,97,111,122,139,151,168	33.9	4.8
10	3-Oxo-α-ionol	36,43,55,69,83,97,111,125,140,156,168,	37.8	7.7
11	Tetradecanoic acid	185	41.9	13.3
12	Hexadecanoic acid	29,43,53,77,91,97,108,117,123,135,152,165,175,193,208	47.0	5.9
	9, 12-Octadecadienoic acid	29,43,54,60,73,85,97,129,143,157,171,185,199,213,228		8.3
		43,60,73,83,97,115,129,143,157,171,185,199,213,220,227,236,256		
		41,55,67,81,95,109,123,137,149,164,182,196,280		

Table 4: Volatile compounds in hexane extract from root stock of *Alocasia indica* identified by gas chromatography-mass spectrometry.

Conclusion

The finding of the present study indicated that *A. indica* could be used as a potential natural grain protectant through its fumigant toxicity which effect on adults as well as various stages of store grain insect pests. A dose of 300 µg/l for a period of 72 h exposure is sufficient to achieve 100% mortality of insect pests. Generally, an extended exposure period of 72 h increased mortality in all three species. Hexane extract of *A. indica* which is biodegradable and because of its ecofriendly properties can be used as a potent biofumigant against stored grain insect pests.

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