A novel natural insecticide molecule for grain protection

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Abstract

We have isolated a novel bioactive molecule from the roots of *Decalepis hamiltonii* that shows insecticidal properties against the stored-product insects *Sitophilus oryzae*, *Rhyzopertha dominica* and *Callosobruchus chinensis* by contact bioassay (LC_{50}: 0.033-0.044 mg/cm^2). This compound proved effective as a grain protectant against stored product insects of wheat and green gram. The compound is a novel insecticidal molecule and a promising grain protectant of natural origin.

Keywords: *Decalepis hamiltonii*, Decaleside- I, Insecticidal activity, Stored-product, insect pests, Grain protection

1. Introduction

Damage to stored grains and their products by insects may amount to 5-10% in the temperate countries to 20-30% in the tropical zone (Nakakita, 1998). Infestation control of stored grain is primarily achieved by the use of synthetic gaseous insecticides such as methyl bromide and phosphine. In several countries including India, mixing of any synthetic insecticide with stored grain is not permitted. Due to environmental concerns and human health hazards, several chemical insecticides have either been banned or restricted for use (Subramanyam and Hagstrum, 1995; Tapandjou et al., 2002). Further, due to the problem of resistance to insecticides, there is an urgent need for safer alternatives to conventional chemical insecticides particularly from natural sources, for the protection of grain against insect infestation. In view of all the aspects in grain protection and these problems have highlighted the urgent need to develop newer ecofriendly safer and effective stored-product insecticide

The highly successful and currently used synthetic pyrethroids were originally derived from the flowers of pyrethrum (Casida et al., 1975). Azadiraechtin, the active principle from the plant *Azadiraechta indica* (Indian neem), is an antifeedant and insect growth regulator but lacks contact toxicity (Isman et al., 1990; Islam and Talukder, 2005). At present, there is no botanical insecticide to replace pyrethrum for protection of stored grain from insect infestation. Bioinsecticides of plant origin often show selectivity to insect species, are biodegradable, have high chance of acceptability and, therefore, it is considered that plants could be the best source of newer chemical structures for development of new, ecofriendly, safer insect control agents (Saxena et al., 1992).

Decalepis hamiltonii Wight & Arn (family: Asclepiadaceae), a wild climber, grows in the hilly forests of peninsular India. The tuberous aromatic roots of *D. hamiltonii* are used in folk medicine and consumed as pickles and as a health drink for the alleged health benefits. Earlier work in our laboratory has shown that roots of *D. hamiltonii* are the source of novel bioactive compounds and more than a dozen compounds have been isolated and identified (Harish et al., 2005, Srivastava et al., 2006a, b, 2007). Previous studies from this laboratory have indicated that roots of *Decalepis hamiltonii* possess biopesticidal properties (George et al., 1999). In this study, we have investigated the insecticidal activity and grain protection potential of a novel bioactive compound isolated from the roots of *D. hamiltonii*.

2. Materials and methods

2.1. Preparation of the bioactive compound

Fleshy outer part of the roots was separated from the inner woody core, dried and powdered. The powder was extracted with organic solvents and the extracts were screened for insecticidal activity. The active extracts showing the insecticidal activity (aqueous extracts) were selected for the isolation of the bioactive compounds. The extracts were subjected to column chromatography using silica gel and eluted with various combinations of solvents (chloroform, ethyl acetate, acetone and methanol). The active fraction was collected and subjected to second round of fractionation on silica gel. Purity of the compound was tested by TLC and by HPLC on a C_{18} column with methanol: water (1:1) and 0.1% trifluoro acetic acid as the mobile phase. Based on NMR and MS data, the structure of the purified compounds was characterized.

2.2. Insects

Cultures of rice weevil, *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), and lesser grain borer, *Rhyzopetha dominica* (F.) (Coleoptera: Bostrychidae), were reared on whole wheat (*Triticium aestivum*) whereas adzuki bean weevil, *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae), were reared on whole green gram (*Phaseolus aureus*). Adult insects were used in the experiments carried out in the laboratory maintained at 27 ± 2°C and 70 ± 5% r.h.

2.3. Insecticidal activity

Toxicity of the purified compound against the stored product insects, S. *oryzae, R. dominica* and *C. chinensis* adults was studied using a residual contact bioassay method (Obeng-Ofori et al., 1998). One ml of solution (0.016- 0.156 mg/cm^2) was applied on Whatman N°1 (9cm) filter paper and placed in a glass Petri dish. The solvent was allowed to evaporate for 10 min prior to the release of 20 adults of the test insect in to each dish. Control filter paper discs were treated with solvent only. Each treatment consisted of four replicates. Insect mortality was recorded after 24 h and percent mortality was determined, while control mortality was corrected as suggested by Abbott (1925).

2.4. Insecticidal effects on the treated grain

The compound dissolved in 2 mL of methanol was applied to 50 g grain at 25, 50, 100 and 125 mg/kg. Controls were treated with solvent alone. Thirty unsexed adults of *C. chinensis, R. dominica* or *S. oryzae,* were introduced into the glass jars (250 mL) containing the 50 g grain. The glass jars were covered with cotton cloth held with rubber bands. Mortality was recorded after 24 h, and 168 h (7 d) post treatment storage period.

2.5. Effect on F_1 progeny

Grains were treated as described above and, after 7 days, the insects (dead and live) were removed and the grains were kept under the same experimental conditions until the emergence of F_1 progeny. Based on the life cycle of the insect species, the counting period of F_1 progeny was established so as to avoid an overlap of generations for each species. At weekly intervals, the F_1 progeny were recorded for 8 consecutive weeks. Percentage reduction in adult emergence of F_1 progeny or inhibition rate (% IR) was calculated as

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

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 (Tapondjou et al., 2002).

2.6. Statistical analysis

The data was analysed using one-way ANOVA ($p < 0.05$) and means were separated by Duncan's multiple range test using Statplus 2007 software. The data were expressed as means ± SE.

3. Results

3.1. Insecticidal activity

Contact toxicity of the compound by filter paper bioassay show that insect mortality was dose dependent at 24 h of exposure (Figure 1). Highest mortality (100%) was observed at dosage 0.156 mg/cm^2 to all the tested insect species. LC_{50} ranged from 0.33 mg to 0.43 mg/cm^2 (Fig. 1).

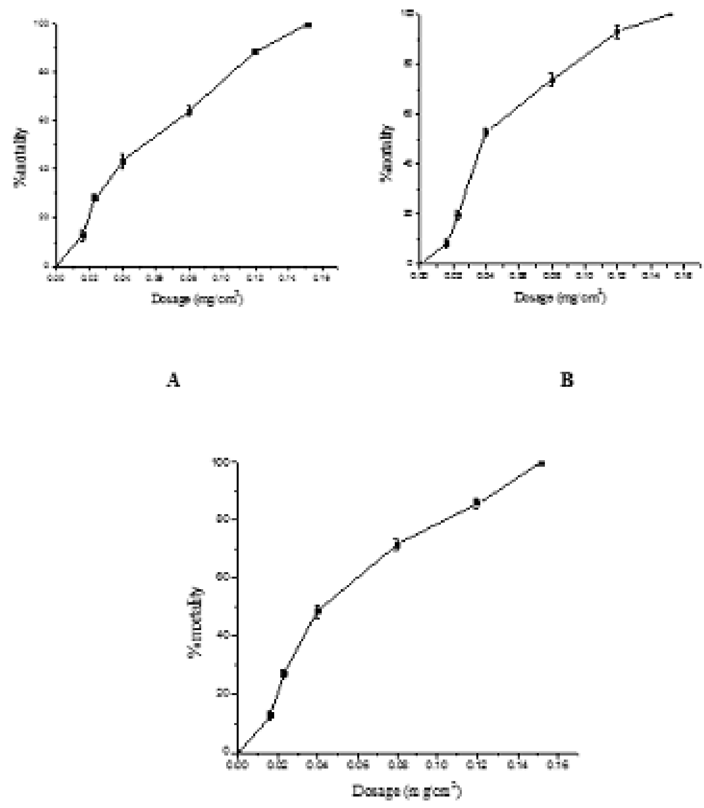

Figure 1 Insecticidal activity of Decaleside-I to **A.** *Sitophilus oryzae*; **B.** *Rhyzopertha dominica* and **C.** *Callosobruchus chinensis* in the contact bioassay. Values are averages of 4 replicates and represents the mean ± SE

3.2 Insecticidal effect on treated grain

Grains treated with the compound at 125 mg/kg (125ppm) produced 90% and 100% mortality at 24 h and 168 h respectively, in all the test species (Table 1).

Table 1 Mortality response of stored product insect pests on wheat grain and green gram treated with Decaleside- I.

Dosage (ppm)	% Mortality (Mean ± SE)					
	S. oryzae		*R. dominica*		*C. chinensis*	
	24h	**7d**	**24h**	**7d**	**24h**	**7d**
25	25.6 ± 1.4a	30.6 ± 1.5a	29.3 ± 1.4a	38.85 ± 1b	26.47 ± 1a	34.6 ± 0.9a
50	42.5 ± 1.7b	48.1 ± 1.7b	50.8 ± 1.4b	62.9 ± 0.8b	44.2 ± 0.9b	48.9 ± 1.6b
100	81.9 ± 1.5c	87.2 ± 2.3c	89.5 ± 1c	94.3 ± 1.6c	80.1 ± 0.9c	86.2 ± 1.2c
125	94.6 ± 1.01d	100d	98.5 ± 2d	100d	94.3 ± 1.5d	100d

Values followed by different letters within the vertical columns are significantly different ($P < 0.05$) by Duncan's multiple range test.

3.3 Effect on F_1 progeny

Significant reduction to total suppression of F_1 progeny of all the insect species in the treated grain was observed (Table 2).

Table 2 Effect of Decaleside I from on adult emergence in F_1 progeny of stored product insects.

Dosage (ppm)	% Reduction in F_1 adult emergence		
	S. oryzae	*R. dominica*	*C. chinensis*
10	12.9 ± 1.4a	10.2 ± 4.04a	15.9 ± 1.7a
25	24.1 ± 1.4b	38.12 ± 1.9b	27.4 ± 1.7b
50	53.3 ± 1.5c	67.6 ± 1.4c	59.4 ± 0.6c
75	77.3 ± 0.21d	75.3 ± 0.6d	84.3 ± 1.4d
100	85.05 ± 0.4e	88.5 ± 0.4e	94.05 ± 0.6e
125	100f	100f	100f

Values followed by different letters within the vertical columns are significantly different ($P < 0.05$) by Duncan's multiple range test.

4. Discussion

With limitations on the use of contact chemical insecticides and fumigants in stored products, and increasing public demand for wholesome and pest-free food products, there is a need for developing biorational pest management technologies in stored products.

Pyrethrin, extracted from flower of *Tanacetum cinerariaefolium,* was considered an almost ideal insecticide. Today, its use is limited because of its high cost. Many studies have reported bioactive compounds from plant extracts with repellent/antifeedant/insecticidal activity against stored-product insect pests (Upasani et al., 2003; Akhtar et al., 2008; Yao et al., 2008) The rhizomes of *Acorus calamus* and the active ingredient (β-asarone) have been investigated for their insecticidal properties but the effort to develop β-asarone as an insecticide received a severe setback with discovery of its mutagenic effect (Abel, 1987). The seeds of *Azadiraechta indica* have been shows insecticidal activity against a variety of insect species and, azadirachtin, the active principle, has exhibits insect antifeedant, moult inhibiting and anti-gonadotropic effects in insects (Schmutterer, 1990). However, its bitter taste and lack of contact toxicity restricts its use and unsuited on stored-products meant for human consumption.

Many plant products and their essential oils have been shown to exhibit insecticidal activity against stored product insect pests (Rajendran and Sriranjini, 2008). At dosage of LD_{50} of 0.21 and 0.17 mg/cm^2, Carvacrol, a compound from *Thujaopsis dolabrata* was toxic to adults of *S. oryzae* and *C. chinesis* (Ahn et al., 1998). Linalool, a bioactive molecule from *Ocimum canum,* killed adults of *S.oryzae* and *R. dominica*, with LD_{50} of 0.42 and 0.428 mg/cm^2 (Weaver et al., 1991). Other natural compounds such as estragole (LD_{50} of 0.066 mg/cm^2) and (+) - fenchone (LD_{50} of 0.092 mg/cm^2) from *Foeniculum vulgare* were toxic against adults of *S. oryzae* (Kim and Ahn, 2001).

Our compound isolated from the roots of *D. hamiltonii* is a novel bioactive molecule as evident from the spectroscopic characterization and shows insecticidal activity against stored product insects by contact bioassay. The residual toxicity on treated grain surface was retained even after several days. Persistence of this toxicity indicates that the compound is stable enough.

The treated grain was free from infestation at the highest dosage (125 ppm) due to cessation of F_1 progeny. Protection of the treated from infestation could be attributed to contact toxicity as well as ovicidal effect. Our study shows that the bioactive compound from *D. hamiltonii* could be a potential grain protectant, which acts by killing various life stages of stored grain insect pests and total suppression of emergence of progeny in treated grain. Furthermore, since the compound derived from an edible source with a long history of human use, it appears to be safe to mammals (Shereen, 2005). Hence, the compound from *D. hamiltonii* could belong to a new class of bioinsecticide and may serve as a promising grain protectant of natural origin

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