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ORIGINAL ARTICLE

Insecticidal activity of the root extract of *Decalepis hamiltonii* against stored-product insect pests and its application in grain protection

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Abstract Root extracts of *Decalepis hamiltonii* were tested for insecticidal activity against the stored products pests, *Rhyzopertha domonica, Sitophilus oryzae, Stigobium pancieum, Tribolium castaneum* and *Callosobruchus chinensis*, in residual and contact toxicity bioassays. Methanolic extract showed LC_{50} value of 0.14 mg/cm^2 for all the test species in a filter paper residual bioassay. The extract was effective as a grain protectant for wheat and green gram. Reduction of F1 progeny was observed in treated grain stored for 3–4 months. The extract did not affect the germination of the treated grains. Our results indicate that methanolic extracts of *D. hamiltonii* has a potential to control stored product pests and could serve as a natural grain protectant.

Keywords *Decalepis hamiltonii* · Methanolic extract · Insecticidal activity · Insect pests · Grain protection

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Introduction

Stored product insect pests reduce the quantity and quality of grain during post-harvest storage. The damage to stored grains and grain products by insects could be in the order of 5–10% in the temperate regions of the world and as high as 20–30% in tropical countries (Shaaya et al. 1997, Nakakita 1998). The use of chemical insecticides including fumigants to prevent or control insect infestation is being practiced for grain protection. Repeated use of chemical insecticides has led to widespread development of resistance and environmental problems due to their effects on non-target organisms and concerns on human health (Subramanyam and Hagstrum 1995). In view of these problems, several chemical pesticides have been either banned or restricted for use in grain protection.

The highly successful and currently used synthetic pyrethroids were originally derived from the pyrethrum plant (Casida et al. 1975). *Azadirachtin,* the active principle from the *Azadiraechta indica* (Indian neem) plant, acts as a insect growth regulator against a number of insects (Isman et al. 1990) and also possesses antifeedant, growth inhibiting properties (Kubo and Klocke 1982, Islam and Talukder 2005). At present, there is no botanical insecticide to replace pyrethrum for the protection of stored grain from insect infestation. In several countries including India, mixing of any synthetic insecticide with stored grain is not permitted. There is an urgent need for safer alternatives to conventional chemical insecticides particularly from natural sources for the protection of grain against insect infestation. Since bioinsecticides of plant origin often exhibit selective toxicity to insects and are ecofriendly, there is a need for the development of new class of biopesticide for insect control. Rich diversity of plant species, especially from the tropical regions of the world could be potential sources of newer bioactive compounds or biopesticides (Saxena et al. 1992).

Decalepis hamiltonii Wight and Arn (Asclepiadaceae), is a wild plant, which grows in the hilly forests of peninsular

India. The roots of *D. hamiltonii* are used in folk medicine and as a substitute for *Hemisdemus indicus* in ayurvedic preparation of ancient Indian medicine (Nayar et al. 1978) and are consumed as pickles and as a health drink. 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. 2006 a, b, 2007). Preliminary studies from this laboratory showed that the root powder of *Decalepis hamiltonii* has biopesticidal properties (George et al. 1999). In this study, we have investigated the insecticidal activity of the organic extracts of the roots of *D. hamiltonii* against important stored grain insect pests and their potential in grain protection.

Materials and methods

Insects: Cultures of rice weevil, *Sitophilus, oryzae* L (Coleoptera: Curculionidae) and lesser grain borer *Rhyzopetha. dominica* F. (Coleoptera: Bostrichidae) were reared on whole wheat (*Triticum aestivum*) whereas adzuki bean weevils, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) were reared on whole green gram (*Phaseolus aureus* Roxb) and the spice beetle, *Stigobium paniceum* F. (Coleoptera: Anobiidae) on coriander (*Coriandrum sativum*) seeds. Cultures were maintained at 30 ± 1°C and 70% RH. Adult insects (3–5 days old for *S. oryzae* and 2–3 days old for the other species) were used for the experiments. Experiments were carried out in the laboratory at 27 ± 2°C and 70 ± 5% RH.

Preparation of the root powder and extracts: Tuberous roots of *D. hamiltonii* (10 kg) procured from a local supplier were washed with water, crushed with a roller and the outer fleshy layer was separated from the inner woody core, dried at 40°C and powdered. Ten kg of roots yielded 1.6 kg of powder. Root powder (100 g) 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. This solution was screened for insecticidal activity using contact toxicity bioassay.

Insecticidal activity of the extracts: Toxicity of various solvent extracts was screened against *S. oryzae* adults using a residual bioassay method (Obeng-Ofori et al. 1998). One ml of stock solution containing 17.5 mg was applied on Whatman No. 1 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 *S. oryzae* into each dish. Control filter paper discs were treated with methanol only. Each treatment consisted of four replicates. Insect mortality was recorded after 24 h exposure and percent mortality was determined (Abbott 1925).

As the methanolic extract was found to be the most active in the preliminary screening, it was chosen for further testing against other stored product insects. Aliquots of the methanol extract ranging from 0.02 to 0.278 mg/cm^2 were used for bioassay against adults of *S. oryzae, S. paniceum*, *C. chinensis* and *R. dominica* at 27 ± 2°C and 70 ± 5% RH. Each treatment group had 4 replicates.

Insecticidal effects on treated grain: The methanol extract (2 ml) was applied to 50 g grain at 25, 50, 100, 150, 200 and 250 mg/kg. Controls were treated with solvent alone. Treated grain was allowed to dry at room temperature (27 ± 2°C). Thirty unsexed adults of *C.chinensis, R. dominica* or *S. oryzae,* were introduced into the glass jars (250 ml) containing 50 g grain. The glass jars were covered with cotton cloth held with rubber bands. Mortality was determined after 1 and 7 days, and the percentage insect mortality was calculated.

Effect on F1 progeny: Grains were treated as described 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 F1 progeny. Based on the life cycle of the insect species, the counting period of F1 progeny was established so as to avoid an overlap of generations for each species. At weekly intervals, the F1 progeny were recorded for 8 consecutive weeks. Percentage reduction in adult emergence of F1 progeny or inhibition rate (%IR) was calculated as

$$\%IR = (C_n - T_n)\,100/C_n$$

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).

Seed germination: Wheat and green gram seeds were treated with methanolic extract at 200 and 400 mg/kg and germination tests were done after 1 and 90 days. Fifty seeds 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. After 5 days, germinated seeds were counted. Percentage germination was calculated as: (number of seeds germinated/total number of seeds) × 100 (Nikpay 2007).

Statistical analysis: The data was analysed using One-Way ANOVA ($p < 0.05$) by Duncan's multiple range test using Statplus 2007 software. Probit analysis (Finney method) was used to determine LC_{50} values and 95% confidence intervals (Finney 1971).

Results and discussion

Insecticidal activity of *D. hamiltonii extracts:* Preliminary screening showed that the methanol extract was most toxic against *S. oryzae,* followed by ethyl acetate, hexane and acetone (Fig. 1). Toxicity of the methanolic extract of *D. hamiltonii* on adults of *S. oryzae, R. dominica, C. chinensis* and *S. paniceum* is shown in Table 1. Based on LC_{50} values and their respective confidence intervals, the extract was found equitoxic to all the 4 insect species tested.

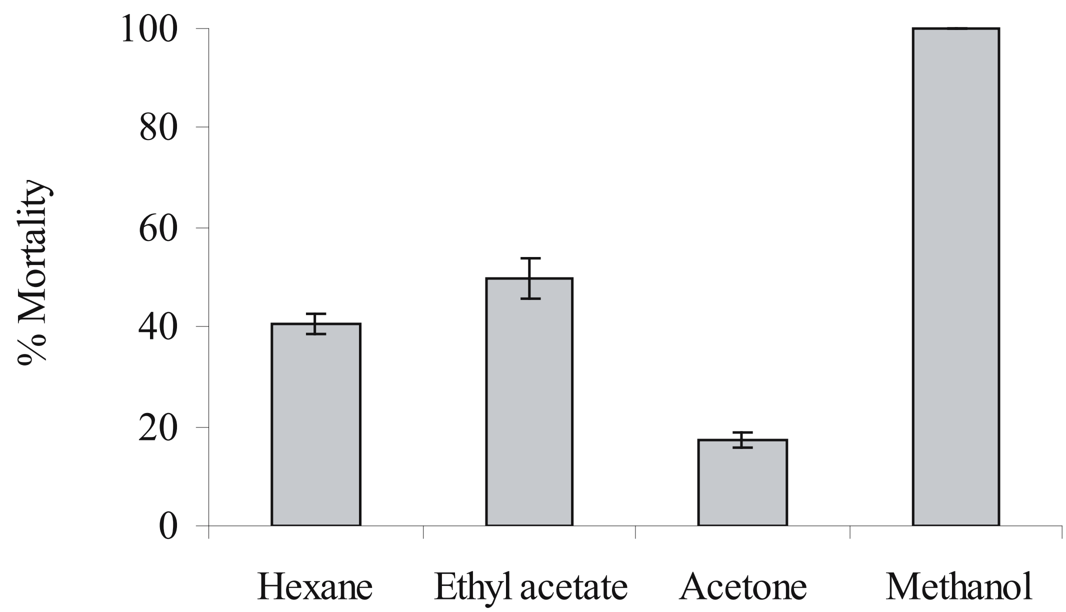

Fig. 1 Insecticidal activity of the solvent extracts of *D. hamiltonii* to *Sitophilus oryzae* in the contact bioassay. The extracts were applied at 0.27 mg/cm^2 (n = 4)

Insecticidal effect on treated grain: Grains treated with methanolic extract of *D.hamiltonii* at 250 mg/kg produced 90% and 100% mortality at 1 and 7 days respectively, in all the test insects (Table 2).

Effect on F1 progeny: The methanolic extract caused significant reduction in F1 progeny of all the insects in the treated grain and the progeny was completely suppressed at 250 mg/kg (Table 3).

Table 1 Insecticidal activity of methanolic extract of *D. hamiltonii* against adults of stored product insects by residual bioassay

Insects	$LC_{50,}$ mg/cm^2
Sitophilus oryzae	0.119 (0.0918, 0.145)
Rhizopertha dominica	0.119 (0.0729, 0.177)
Callosobruchus chinensis	0.115 (0.0742, 0.156)
Stigobium paniceum	0.117 (0.0749, 0.158)

Values in parentheses represent 95% confidence intervals (n = 4)

Seed germination test: Percentage germination of seed in 200 and 400 mg/kg treatments ranged from 86 in the control to 78.9 in the methanol extract treated wheat grain whereas, in green gram, it ranged from 96 in the control group to 93 in the case of methanolic extract. There were no significant differences in germination between the control and treated grains of either species of grain.

The use of plants in the control of stored products insects is an ancient practice (Qi and Burkholder 1981). Plant extracts are seldom used in insect control in spite of their efficacy against all the life stages of insects (Arannilewa et al. 2002, Kim et al. 2003 a, b, Akhtar et al. 2008). Pyrethrin, extracted from flower of *Tanacetum cinerariaefolium,* was considered an almost ideal insecticide due to its broad spectrum of insecticidal activity with swift knock down,

Table 2 Insecticidal effects on stored product pests of wheat grain and green gram treated with methanolic extract of *D. hamiltonii* in contact toxicity bioassay

Dosage, mg/kg	Mortality, %					
	Sitophilus oryzae		*Rhizopertha dominica*		*Callosobruchus chinensis*	
	1 d	7 d	1 d	7 d	1 d	7 d
25	10.9 ± 1.01^a	16.3 ± 1^a	12.4 ± 1.1^a	20.3 ± 1.6^a	5.6 ± 1.5^a	12.3 ± 1.8^a
50	37.4 ± 1.4^b	32.6 ± 1^b	32.1 ± 1.4^b	38.85 ± 1^b	26.47 ± 1^b	34.6 ± 0.9^b
100	40.8 ± 1.6^c	43.9 ± 0.8^c	53.5 ± 1.4^c	62.9 ± 0.87^c	44.2 ± 0.9^c	48.9 ± 1.6^c
150	55.8 ± 1.7^d	77.5 ± 1^d	72.3 ± 1.7^d	79.5 ± 1.06^d	66 ± 1.5^d	73.5 ± 0.9^d
200	74.9 ± 0.9^e	91.3 ± 1^e	83 ± 1^e	94.3 ± 1.6^e	80.1 ± 0.9^e	86.2 ± 1.2^e
250	91.6 ± 0.9^f	100^f	95.5 ± 2^f	100^f	94.3 ± 1.5^f	100^f

d = day (s), Values followed by different superscripts within columns are significantly different (p <0.05) by Duncan's multiple range test (n = 4)

Table 3 Effects of methanolic extract of *D. hamiltonii* on the adult emergence of F1 progeny (49 days) of stored product insects

Dosage, mg/kg	Reduction in F1 adult emergence, %		
	Sitophilus oryzae	*Rhizopertha dominica*	*Callosobruchus chinensis*
25	10.9 ± 1.4[a]	15.2 ± 4.04[a]	12.9 ± 1.7[a]
50	37.4 ± 1.4 [b]	38.12 ± 1.9[b]	37.4 ± 1.7[b]
100	73.3 ± 1.5[c]	67.6 ± 1.4[c]	69.4 ± 0.6[c]
150	87.3 ± 0.21[d]	85.3 ± 0.6[d]	84.3 ± 1.4[d]
200	95.05 ± 0-4 [e]	95.5 ± 0.4 [e]	94.05 ± 0-6 [e]
250	100 [f]	100 [f]	100 [f]

Values followed by different superscripts within columns are significantly different ($p < 0.05$) by Duncan's multiple range test ($n = 4$)

high degree of repellence to insect pests and fast break down and safety to man and animals. Today, its use is limited because of its high cost (Singh 1993). 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 the report of its mutagenic effect (Abel 1987, Rees et al. 1993). The seeds of *Azadiraechta indica* have been screened for insecticidal activity against a variety of insect species (Schmutterer 1990). The active principle, Azadirachtin, has been most intensely investigated and was shown to exhibit insect antifeedant, moult inhibiting and anti-gonadotropic effects in insects. However, its bitter taste restricts its use 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 a dosage of 3.5 mg/cm^2 in a contact bioassay, essential oils of *Foeniculum vulgare, Cinnamomum cassia* and *Brassica juncea* killed all adults of *S.oryzae* and *R.dominica* (Kim et al. 2003 a, b). Hexane extract of *Acorus gramineus* at 0.51 mg/cm ^{2} was toxic to adult *S. oryzae* (Park et al. 2003). Oils of coconut (Singal and Singh 1990) and *Chenopodium ambrosioides* (Tapandjou et al. 2002) were toxic to adult *C.chinensis* and reduced the F1 progeny by 100% at 5 ml/kg, 0.4% and 6.4% respectively on green gram.

Our results show that the methanol extract of the roots of *D. hamiltonii* is a potent insecticidal agent for control of stored product insects. Further, *D. hamiltonii* extract appears to be among the most effective plant extracts reported as evident in its contact toxicity. Our results also indicate that the duration of exposure to treated grain influences the insecticidal efficacy of methanol extract to adults of stored-product insect species.

In our study, the possibility of fumigation effects cannot be ruled out. However, the treated seed surface, even after several days, was toxic to the insects showing the residual effects of the extract. Richards (1978) reported that plant extracts or essential oils of plant origin are highly lipophilic and therefore have the ability to penetrate the insect cuticle. Roots of *D. hamiltonii* have been reported to contain aldehydes, inositols, saponins, amyrins, lupeols and volatile flavour compounds such as 2-hydroxy-4-methoxybenzaldehyde, vanillin, 2-phenylethyl alcohol, benzaldehyde (Nagarajan et al. 2001). Any of these compounds along with other unknown ingredients could be responsible for the insecticidal activity of the extract against stored product insects.

Further, our results show that the number of adults that emerged from the treated grain after 49 days of storage was dose-dependent (Table 3). Methanol extract when applied to the grains exerted insecticidal effects on all the species tested. The reduction in F1 progeny might have resulted from contact toxicity or through feeding on the treated grains as well as ovicidal effects. Our studies show that adult emergence was completely inhibited in grain treated at 250 mg/kg. The treatment of the grain (wheat and green gram) with the extracts did not show significant adverse effect on germination even after 90 days.

Conclusion

The methanolic extract of *D.hamiltonii* could be a potential natural grain protectant through its residual and contact toxicity effects on adults as well as on various life stages of stored grain insect pests. Since the roots of *D. hamiltonii* are edible and in view of the long history of human use, it stands a good chance of being the source of a new ecofriendly bio-insecticide and grain protectant of natural origin.

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References

Abbott WS (1925) Method of computing of effectiveness of an insecticide. J Econ Entomol 18:265–267

Abel G (1987) Chromosomenschadigende Wiruking von β-asarone in menschlichen Lymphocyten. Planta Med 53:251–253

Akhtar Y, Yeoung YR, Isman MB (2008) Comparative bioactivity of selected extracts from Meliaceae and some commercial botanical insecticides against two noctuid caterpillars, *Trichoplusia ni* and *Pseudaletia unipuneta*. Phytochem Rev 7:77–88

Arannilewa ST, Odeyemi OO, Adedire CO (2002) Effects of medicinal plant extract and powder on the maize weevil, *Sitophilus zeamais* Mots. Ann Agric Sci 3:1–10

Casida JE, Ueda K, Gaughan LC, Jao LT, Soderland DM (1975) Structure biodegradability relationships in pyrethriod insecticides. Arch Environ Contam Toxicol 3:491–500

Finney DJ (1971) Probit analysis. 3rd edn. Cambridge University Press, Cambridge, p 333

George J, Ravishankar GA, Pereira J, Divakar S (1999) Bioinsecticide by swallowroot (*D. hamiltonii*) Wight and Arn. protects food grains against insect infestation. Curr Sci 77:81–90

Harish R, Divakar S, Srivastava A, Shivanandappa T (2005) Isolation of antioxidant compounds from methanolic extracts of the roots of *Decalepis hamiltonii* (Wight and Arn). J Agric Food Chem 53:7709–7714

Islam MS, Talukder FA (2005) Toxic and residual effects of *Azadirachta indica, Tagets erecta* and *Cynodon dactylon* extracts against *Tribolium castaneum*. J Plant Diseases Protect 112: 594–601

Isman MB, Koul O, Luczynski A, Kaminski J (1990) Insecticidal and antifeedant bioactivities of neem oil and their relationship to Azadirachtin content. J Agric Food Chem 38:1406–1411

Kim SI, Park C, Ohh MH, Cho HC, Ahn YJ (2003a) Contact and fumigant activities of aromatic plants extracts and essential oils against *Lasioderma serricorne*. J Stored Prod Res 39:11–19

Kim SI, Roh JY, Kim DH, Lee HS, Ahn YJ (2003b) Insecticidal activities of aromatic plant extracts and essential oils against *Sitophilus oryzae* and *Callosobruchus chinensis.* J Stored Prod Res 39:293–303

Kubo I, Klocke JA (1982) Azadirachtin. Insect ecdysis inhibitor. Agric Biol Chem 46:1951–1953

Nagarajan S, Rao MJ, Gurudutt KN (2001) Chemical composition of the volatiles of *Decalepis hamiltonii* (Wight and Arn *).* Flavour Fragr J 16:27–29

Nakakita H (1998) Stored rice and stored product insects. In: Rice inspection technology. Nakakita H (ed), ACE Corporation, Tokyo, p 49–65

Nayar RC, Shetty JKP, Mary Z, Yoganarshimhan (1978) Pharmacognostical studies on the root of *Decalepis hamiltonii* (Wight and Arn) and comparison with *Hemidesmus indicus* (L.) R. Br. Proc Indian Acad Sci 87:37–48

Nikpay A (2007) Insecticidal efficacy of three vegetable oils as post-harvest grain protectants of stored wheat against *Rhyzopertha dominica* (F) (Coleoptera: Bostrychidae). Insect Sci 14: 145–150

Obeng-Ofori D, Reichmuth CH, Bekele AJ, Hassanali A (1998) Toxicity and protectant potential of camphor, a major component of essential oil of *Ocimum kilmanscharicum* against four-stored product beetles. Int J Pest Manag 44:203–209

Park C, Kim SI, Ahn YJ (2003) Insecticidal activity of asarones identified in Acorus gramines rhizome against three coleopteran stored product insects*.* J Stored Prod Res 39:333–342

Qi YT, Burkholder WE (1981) Protection of stored wheat from the granary weevil by vegetable oils*.* J Econ Entomol 74:502–505

Rajendran S, Sriranjini V (2008) Plant products as fumigants for stored-product insect control. J Stored Prod Res 44:126–135

Rees DP, Dales MJ, Golob P (1993) Alternative methods for the control of stored-product insect pests: A bibliographic database. Natural Resources Institute, Chatham, UK

Richards AG (1978) The chemistry of insect cuticle. In: Biochemistry of insects, Academic Press, New York, p 205–232

Saxena RC, Dixit OP, Sukumaran P (1992) Laboratory assessment of indigenous plant extracts for antijuvenile hormone activity in *Culex quinquefasciatum*. Indian J Med Res 95:204–206

Schmutterer H (1990) Properties and potential of natural pesticides from the neem tree, *Azadiraechta indica.* Ann Rev Entomol 35:271–297

Shaaya E, Kostjukovaki M, Eilberg J, Sukprakarn C (1997) Plant oil as fumigants and contact insecticides for the control of stored-product insects. J Stored Prod Res 33:7-15

Singh RP (1993) Botanicals pesticides in developing countries: Current status and future trends In: Pesticides-Their ecological impact in developing countries, Dhaliwal GS, Singh B (eds), Commonwealth Publ, New Delhi, p 236–269

Singal SK, Singh (1990) Studies of plant oils as surface protectants against pulse beetle*, Callosobruchus chinensis* (L.) in chickpea *Cicer arietinum* L. in India*.* Trop Pest Manag 36:314–316

Srivastava A, Harish R, Shivanandappa T (2006a) Novel antioxidant compounds from the aqueous extract of the roots of *Decalepis hamiltonii* (Wight and Arn.) and their inhibition effect on low-density lipoprotein oxidation J Agric Food Chem 54:790–795

Srivastava A, Rao MJ, Shivanandappa T (2007) Isolation of ellagic acid from the aqueous extract of the roots of *Decalepis hamiltonii* Antioxidant activity and cytoprotective effect. Food Chem 103:224–233

Srivastava A, Shereen, Harish R, Shivanandappa T (2006b) Antioxidant activity of the roots of *Decalepis hamiltonii* (Wight and Arn.). LWT- Food Sci Technol 39:1059–1065

Subramanyam B, Hagstrum DW (1995) Resistance measurement and management, In: Integrated management of insects in stored products. Subramanyam B, Hagstrum DW (eds), Marcel Dekker, New York, p 331–397

Tapondjou LA, Alder A, Bonda H, Fontem DA (2002) Efficacy of powder and essential oil from *Chenopodium ambrosioides* leaves as post-harvest grain protectants against six-stored product beetles. J Stored Prod Res 38:395–402