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Toxicity of Three Plants Extracts to *Trogoderma granarium* Everts (Coleoptera: Dermestidae)

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Abstract: Toxicity of aqueous, methanolic and acetonic extracts of three plants: (*Rhazya stricta* Decaisne, *Azadirachta indica* A. Juss and *Heliotropium bacciferum* Forssk.) to the khapra beetle, *Trogoderma granarium* Everts larvae was investigated. All extracts showed remarkable toxicities. The acetonic extract from *R. stricta* has shown a relatively more pronounced toxic effect, having an acute (2-day) and chronic (6-day) LC_{50}s of 391 and 214 ppm compared to 467 and 251; and 576 and 317 ppm, for *A. indica* and *H. bacciferum*, respectively. The toxic effect was found to be dose and exposure-time dependent. The toxic effect observed for acetone extract was about 1.4 fold that observed for either aqueous or methanol extracts. These materials could have promising practical application in protection of stored grains against attack by *T. granarium*.

Key words: *Trogoderma granarium*, extracts, *Azadirachta indica*, *Rhazya stricta*, *Heliotropium bacciferum*.

Introduction

The Khapra beetle *Trogoderma granarium* Everts is one of the most notorious primary insect pests for stored grains (Hill, 1983; Banks, 1977). In addition to direct loss caused by larval feeding, infestation is often followed by colonization by secondary insect pests, fungi, and consequently leading to deterioration in grain characteristics.

Protection of stored grains from insect damage is currently dependent on synthetic pesticides such as fumigation with phosphine or methyl bromide, or dusting with compounds such as pirimiphos-methyl and permethrin, (Singh, 1990; Price and Mills, 1988). The widespread use of such chemicals has significant drawbacks, such as development of strains resistant to insecticides (White, 1995; Zettler and Cuperus, 1990), increased costs, handling hazards, concerns about insecticide residues on grains, and great threats to human health apart from environment. Outbreaks of environmental hazards related to contribution of fumigants such as methyl bromide on the degradation of stratospheric ozone (Taylor, 1994; Noling and Becker, 1994) initiated calls to phase-out methyl bromide by the year 2001. Public awareness of these risks has increased the interest in finding safer insecticides or alternative stored-product protectants to replace dangerous synthetic chemical insecticides (Silver, 1994). One such alternative is the use of natural plant products that have insecticidal activity (Isman, 1994).

Plant extracts of neem tree, *Azadiracta indica* A. Juss(Xie *et al.*, 1995; Mordue and Blackwell, 1993); *Ocimum canum* (Weaver *et al.*, 1991); *Ocimum suave* and *Eugenia caryophyllata* Cloves, were used as grain protectants against stored product Coleoptera. *O. suave* ground leaves and essential oil extract induced mortality and caused significant reduction in progeny of *Rhyzopertha dominica* (Fab.) and *Sitotroga cerealella* (Oliv.) (Bekele *et al.*, 1996). Su (1990) found hexane extracts of *Piper cubeba,* effective against rice and cowpea weevils. Elhag (1998) found that extracts from *Rhazya stricta* Decaisne, *Heliotropium bacciferum* (Forssk.) and neem deterred oviposition by *Callosobruchus maculatus* on chickpea seeds.

Extracts from seeds and leaves of neem tree were found to inhibit the feeding, metamorphosis, fecundity and oviposition and cause diverse behavioral and physiological disorders in many insect species (Chen *et al.*, 1996; Ascher, 1993). Koul (1992) referred to use neem as being both environmentally and economically desirable.

Rhazya stricta Decaisne (Apocyanaceae) and *Heliotropium bacciferum* (Forssk.), (Boraginaceae), are known to possess some biological activity against insects (Elhag *et al.*, 1996), brine shrimps (Al-Yahia *et al.*, 1990), higher animals (Adam, 1998; Hassan *et al.*, 1977), besides medicinal value as being used by local folk medicine practitioners (Al-Yahia *et al.*, 1990). *Rhazya stricta* was shown to be rich in alkaloids such as rhazime, sewarine, strictamine, rhazimal, rhazimol and others (Ahmad *et al.*, 1983; Rahman and Fatima, 1982). Folk medicine practitioners use it for treatment of syphilis, chronic rheumatism and other types of pain (Al-Yahia *et al.*, 1990). *Heliotropium bacciferum* is also practiced in folk medicine for treatment of dog bites and skin diseases (Al-Yahia *et al.*, 1990; Migahid, 1978).

Although plant materials have been used for years, they only recently induced active research to materialize the scientific basis for their use and biologically active constituents and potency. The objective of this study is to test the efficacy of three semi-arid adapted, widely distributed plants on an important primary insect pest of stored grains, under laboratory conditions.

Materials and Methods

The experiment was carried out during 1999 in the Entomology Section of the College of Agriculture, King Saud University at Gassim.

Insects, extracts and grain treatments: Cultures of *T. granarium* were maintained at 25 ± 2°C and 65 ± 5% relative humidity (RH) on wheat, *Triticum aestivum* (L.), cv. Yokora rojo, in a culture room.

Extracts: Plant materials were collected from the central parts of Saudi Arabia. Aerial parts of *R. stricta* and *H. bacciferum*, and neem seed kernels were air dried in laboratory, ground to a fine powder and extracted with warm distilled water, methanol or acetone, as needed, at ambient temperatures. A gentle warming (35-40°C) was sometimes found necessary, especially when the solvents were taken straight from refrigerator. The powdered material was mechanically stirred for 2-3 hrs. with the appropriate solvent and filtered. Solvents were very carefully removed by slow evaporation. All the solvents used in extraction process were from Winlab Ltd. and BDH.

Test procedure: Stock solutions of three extracts were prepared by redissolving the extract in warm distilled water

(0.5 g/ 100 ml) and different concentrations (100, 200, 400, 600, 800 and 1000 ppm) were prepared from the stock solutions. Yecora rojo wheat cv. seeds (10 g) were placed in a Petri dish and sprayed with 10 ml of each of the three concentrations from each extract. Controls were sprayed with distilled water. Groups of ten 3rd instar *T. granarium* larvae were removed from the culture and each group was added to one of the treated groups of seeds. The experiment was laid out in a completely randomized design, with three replications. The treatments were held in the laboratory under ambient conditions which ranged between 20-26°C and 50-65%RH. Larval mortalities were assessed at 2, 4 and 6 days after treatment. Data were analyzed using maximum likelihood procedures and the probit analysis (Finney, 1971), and the effectiveness was expressed as LC_{50} values.

Results and Discussion

The percent mortality of *T. granarium* larvae at 2, 4 and 6 days after treatment are presented in Table 1. The LC_{50} values at 95% confidence limits (CL) are given in Table 2. Significant differences were indicated by failure of 95% CL to overlap. All test materials were toxic to *T. granarium* 3rd instar larvae in a dose dependent manner, although the toxic action was relatively slow for *H. bacciferum* and neem, and their efficacy varied. *R. stricta* was the most effective, where its 1000 ppm aqueous and acetonic extracts caused 70 and 83% mortalities per 48 hr respectively, and the methanolic and acetonic extracts produced 87 and 90% of 6-day mortalities, respectively (Table 1).The range of acute (2 day) and chronic (6 day) LC_{50} valueswere 556-758 and 354-447; 579-849 and 309-485; and 391-576 and 215-317 ppm for aqueous, methanolic and acetonic extracts, respectively (Table 2). Both acetonic and methanolic extracts of *R. stricta* had significantly lower LC_{50}s (6 day) than the aqueous, methanolic or acetonic extracts from both neem and *H. bacciferum* (2 days) as indicated by their confidence limits (Table 2). The acetone extracts of three plant materials were generally more toxic than their aqueous or methanol extracts. Interestingly enough, the acetonic/methanolic or acetonic/aqueous LC_{50} ratios for all materials at all levels were consistently 0.6-0.7 and 0.6-0.8, respectively, indicating that the acetonic extracts were ≈1.4 fold more toxic than either the methanolic or aqueous extracts.

The mechanism whereby the plant constituents caused larval mortality can not be stated from the present study but the antifeeding effect of neem is a well documented fact, especially on stored product insects (Schumutterer, 1990). Thus neem extracts' growth regulating effects on *T. granarium* larvae might have resulted in arrest of further development and survival. It is therefore assumed that the mortality due to neem, observed in our study, is likely a consequence of starvation due to its antifeeding effect, rather than toxic effect. Rembold (1989) reported that azadirachtin is toxic to Mexican bean beetle, *Epilachna varivestis* only if administered at concentrations >1000 ppm. Treating insect food material with neem can disrupt the insect feeding by making the

Table 1: Percentage mortalities of *T. granarium* larvae reared on wheat seeds treated with extracts from three plants.

Plant material	Conc. ppm	Aqueous extract % Mortality after			Methanolic extract % Mortality after			Acetonic extract % Mortality after		
		2d	4d	6d	2d	4d	6d	2d	4d	6d
Rhazya stricta	100	16.7	20.0	23.3	20.0	23.3	26.7	23.3	30.0	33.3
	200	26.7	33.3	36.7	23.3	30.0	36.7	33.3	40.0	46.7
	400	36.7	40.0	46.7	36.7	43.3	53.3	43.3	53.3	60.0
	600	43.3	53.3	63.3	43.3	46.7	60.0	53.3	63.3	73.3
	800	63.3	66.7	70.0	60.0	66.7	73.3	66.7	73.3	83.3
	1000	70.0	73.3	80.0	63.3	76.7	86.7	83.3	86.7	90.0
	Control	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0
Azadirachta indica	100	16.7	23.7	23.0	13.3	23.3	23.3	20.0	26.7	30.0
	200	23.3	33.3	40.0	20.0	26.7	33.3	30.0	36.7	43.0
	400	33.3	40.0	46.7	36.7	40.0	46.7	40.0	53.3	60.0
	600	43.3	50.0	60.0	40.0	43.3	56.7	50.0	63.3	66.7
	800	60.0	63.3	70.0	53.3	60.0	66.7	66.7	70.0	76.7
	1000	66.7	70.0	73.3	66.7	73.3	80.0	73.3	80.0	86.7
	Control	0.0	0.0	3.3	0.0	0.0	3.3	0.0	0.0	0.0
Heliotropium bacciferum	100	13.3	16.7	20.0	10.0	13.3	16.7	16.7	23.3	26.7
	200	23.3	26.7	33.3	16.7	23.3	26.7	26.7	33.3	40.0
	400	30.0	33.3	43.3	23.3	30.0	36.7	36.7	43.3	50.0
	600	36.7	46.7	56.7	36.7	46.7	53.3	43.3	56.7	60.0
	800	53.3	56.7	63.3	46.7	53.3	66.7	63.3	66.7	73.3
	1000	63.3	66.7	70.0	63.3	70.0	73.3	66.7	73.3	80.0
	Control	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0

Table 2: Response of *T. granarium* larvae to three plant extracts.

Plant material	Assay time (days)	LC_{50} (95% confidence limits) and extract * Aqueous	Methanolic	Acetonic
Rhazya stricta	2	555.9 (403-768) (1.4±6.1)**	579.1 (409-822) (1.4±5.9)	391.0 (290-528) (1.5±5.6)
	4	444.4 (325-609) (1.4±0.06)	444.2 (322-614) (1.4±5.5)	277.8 (201-383) (1.5±5.4)
	6	354.3 (259-484) (1.4±5.4)	308.6 (228-419) (1.5±5.5)	214.6 (156-294) (1.8±6.0)
Azadirachta indica	2	619.7 (439-877) (1.4±6.2)	676.9 (479-957) (1.5±6.7)	466.7 (342-637) (1.4±5.7)
	4	475.5 (329-688) (1.2±5.2)	527.9 (368-759) (1.3±5.5)	318.7 (230-441) (1.4±5.3)
	6	360.2 (257-504) (1.3±5.2)	381.6 (281-519) (1.4±5.5)	250.8 (178-352) (1.5±5.4)
Heliotropium bacciferum	2	758.0 (496-1197) (1.2±6.2)	848.8 (582-1240) (1.6±5.3)	575.9 (411-809) (1.4±6.0)
	4	613.7 (428-882) (1.3±6.9)	635.9 (460-880) (1.5±7.7)	411.5 (296-572) (1.3±5.3)
	6	447.0 (321-622) (1.3±6.4)	484.7 (366-642) (1.6±6.3)	317.4 (255-442) (1.4±0.8)

* Significance is indicated by failure of 95% confidence limits to overlap.

** Numbers between right parenthesis are the slopes of regression equation of response (y) on log dose (x) lines.

Table 3: Ratio of LC_{50} of Aqueous or Methanolic extracts to Acetonic

Plant material	Assay time (days)	LC_{50} ratio Aqueous/ Acetonic	Methanolic/ Acetonic
Rhazya stricta	2	1.42	1.50
	4	1.60	1.60
	6	1.65	1.44
Azadirachta indica	2	1.33	1.45
	4	1.50	1.66
	6	1.43	1.43
Heliotropium bacciferum	2	1.32	1.47
	4	1.49	1.54
	6	1.41	1.53

treated material unattractive or unpalatable; and consequently, insect growth, survival and reproduction are adversely affected (Saxena, 1987).

The toxic effect of *R. stricta* was previously reported on mosquito larvae (Elhag *et al.*, 1996), *Agrotis ipsilon* Hufn. and *Hypera brunneipennis* (Elhag *et al.*, 1998), Brine shrimps (Al-Yahia *et al.*, 1990) and wistar rats (Adam, 1998). Although the toxic mode of action of *R. stricta* in insects is not yet known, it might be attributed to its high content of biologically active alkaloids (Rahman and Fatima, 1982).

Heliotropium bacciferum showed somewhat lower toxic effects, compared with neem and *R. stricta*. The highest mortality values, 70, 73 and 80%, were obtained after 6 days at 1000 ppm aqueous, methanolic and acetonic extracts, respectively. Elhag *et al.* (1996) reported $LC_{50} \approx 1000$ ppm of its aqueous extract against 3rd instar *Culex pipiens* L. mosquito larvae. Its aerial parts contain alkaloids, flavonoids, tannins, sterols and/or triterpenes, volatile oils and volatile bases; and is moderately toxic to Brine shrimps (Al-Yahia *et al.*, 1990).

A striking observation on three plant materials investigated in present work was that the length of exposure time of all extracts resulted in increased mortality, indicating that larvae cannot tolerate long exposures to such materials. Jotwani and Sircar (1965) were able to obtain 9-12 month protection against *T. granarium, Sitophilus oryzae* (L.) and *Rhizopertha domonica* (F.) using a mixture of 1-2% neem seed powder with wheat kernels. The materials investigated in this study could have practical application in protection of stored grains against attack by *T. granarium*, by virtue of the merits inherent in plant extracts such as their environmental safety, low mammalian toxicity, low costs and easy handling.

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