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Efficacy of Rhazya stricta leaf and seed extracts against Rhyzopertha dominica and Trogoderma granarium

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Abstract

The protection of stored grains from insect pests depends upon highly toxic synthetic insecticides which cause a number of harmful effects on public health and environment. This necessitates finding eco-friendlier control measures of these stored insects pests. In this study, leaf and seed extracts of the local medicinal plant Rhazya stricta (Apocynaceae) were evaluated for toxicity and repellency to Rhyzopertha dominica (Coleoptera: Bostrichidae) and Trogoderma granarium (Coleoptera: Bostrichidae) under laboratory conditions. The results show an increase in mortality (%) and repellency (%) with an increase in the concentrations of the extracts. After 120 h, the leaf extract caused the highest mortality (61.10 ± 1.20 - 72.11 ± 1.25%) as compared to the seed extract (56.95 ± 3.26 - 69.50 ± 1.04) in both targeted insects. Leaf and plant extracts show maximum repellency (62.50 ± 1.73 - 47.20 ± 1.45%) in R. dominica and T. granarium at higher concentrations in comparison to lower concentration in the 24-hour trial. Our study suggests that leaf extracts of the medicinal plant R. stricta could be used as an effective component of the integrated pest management program against stored grain pests.

Keywords: Insect pests; plant extracts; repellency; stored grains; toxicity.

1. Introduction

Cereal grains are the most important and major component of world food supply. Stored grains require continuous protection to avoid deterioration of quality and weight especially caused by fungal diseases and insects (Padin *et al*., 2013). After harvest, cereal grains are usually stored on farms or in large commercial elevators where different insect pests can infest and damage the grains. Among these, lesser grain borer (Rhyzopertha dominica), Coleoptera: Bostrichidae, and khapra beetle (Trogoderma granarium), Coleoptera: Bostrichidae, cause significant losses (1040%-) to stored grains, including wheat, barley, sorghum, rice, and peas (Toews *et al*., 2000; Jilani *et al*., 1989; Ahmedani *et al*., 2011; Chaubey, 2011). The direct losses are caused by larval feeding. Thereafter, infested grains are susceptible to fungal attack, leading to the deterioration of food grain characteristics (Madkour *et al*., 2012; Ahmedani *et al*., 2011).

R. dominica and T. granarium are usually controlled through heavy use of highly toxic synthetic insecticides. These chemicals are harmful to public health and the environment. In addition, they also cause the development of insecticide resistance in stored grain pests (Tripathi, 1999; Madkour *et al*., 2012). These situations demand a grain storage management strategy which is effective, eco-friendly and one which acts as a deterrent to the development of insecticide resistance in stored grain insect pests.

Plant secondary metabolites having insecticidal properties could be safely used to replace or reduce the amount of hazardous chemicals (Sharifian *et al*., 2012). Studies have shown that plant products/extracts of about 2400 plant species have properties which can kill insects (Liu & Ho, 1999; Tripathi, 2000; Abubakkar *et al*., 2000; Umoetok, 2000; Meera & Mann, 2002; Kanvil *et al*., 2007; Manzoor *et al*., 2016). The plant extracts have been reported to cause mortality and biological changes in insects in several ways. For example, they cause growth inhibition and negatively affect insect reproductive potential and egg fertility (Mulungu *et al*., 2007; Abida *et al*., 2010; Begum *et al*., 2011; Susana *et al*., 2013). Plant extracts have also been tested in many studies to control stored insect pests (Saleem *et al*., 2014; Rajashekar & Tonsing, 2014; Sagheer *et al*., 2013; Sarwar, 2010). These extracts are not only less/non-toxic to non-target organisms but also easily bio-degradable as compared to synthetic chemical insecticides (Dubey *et al*., 2008).

Rhazya stricta (Apocynaceae) is a small medicinal shrub found in Pakistan, India, Saudi Arabia, United Arab

Emirates and many other places (Ali *et al*., 2000; Gilani *et al*., 2007). The plant grows in sandy soil and has a smooth stem, erect shrubs and semi erect branches. Various parts of this plant have been used to cure joint infections, cancer and some other skin and stomach diseases in humans (Khan & Khan, 2007; Marwat *et al*., 2012). The larvicidal and growth retarding effects have been shown on Culex pipiens (El-Hag *et al*., 1996; Al-Doghairi & El-Hag, 2002), Agrotis ipsilon and Hypera brunneipennis (El-Hag *et al*., 1999). Its repellent and toxic effects were found to be effective on the stored grain pest Oryzaephilus surinamensis (Madkour *et al*., 2013) and Trogoderma granarium (El-Nadi *et al*., 2001).

To date, no formal studies have been carried out in Pakistan. This study was conducted in the agro-climate of the Dera Ghazi Khan District in Punjab, Pakistan. The efficacy of R. stricta (Apocynaceae) leaf and seed extracts against two important stored grain pests, R. dominica and T. granarium, was evaluated. The research study aimed to find the effectiveness and dose rate for use against stored grain pests, thereby providing a new, environmentally-benign tool for pest management in stored grains.

2. Materials and methods

The experiment was conducted at the Department of Entomology, College of Agriculture, Dera Ghazi Khan, sub-Campus University of Agriculture, Faisalabad, Pakistan during the year 2013-2014.

2.1. Rearing of insects

The insects were taken from the university's laboratory culture and reared in sterilized glass five jars each containing 2 kg of wheat grains. The wide mouths of the jars were covered with muslin cloth and tied with rubber bands so the insects could not escape. The cultures were maintained in the laboratory for one year at 25+2 ºC. For egg laying, insects were kept in the culture for four days. The insects were shifted to new wheat grain jars by using a camel hairbrush and sieve for the maintenance of culture. The wheat grains and eggs were returned to the same jars. These adults were used for the experiment. Offspring obtained from these stocks were of same size and age (Sagheer *et al*., 2013).

2.2. Preparation of plant extract

Fresh leaves and seeds of R. stricta were collected from the area surrounding the College of Agriculture, Dera Ghazi Khan. The plant was identified by the allelo-chemist working at the college. The plant materials were dried in the shade for a week, and then an electric grinder was used to make them into a fine powder. Extraction was done in 100 ml of acetone by adding 50 gram of powder and continuously shaking in an electric shaker for 24 hours. The solution was then filtered through filter paper (Whatman No. 42). The filtration of plant extracts was repeated three times to obtain maximum filtrate. The obtained plant extracts were used for making the different concentrations.

2.3. Contact toxicity

Four concentrations of the leaf/seed extracts were set in acetone (5%, 10%, 15% and 20%) and a pipette was used to apply the solution on 40 grams of wheat grains in 100 ml jars. The control was treated with only acetone. Each jar was shaken to get a homogeneous mixture of the extract. There were a total of 15 jars for each extract type. The experiments were completely randomized, with three jars for each concentration. After the evaporation of acetone, twenty adults of R. dominica and T. granarium each were put into separate jars. The jar mouths were covered with muslin cloth, which was secured with a band so the insects could not escape. Data about the mortality of insects were recorded after 24, 48, 72, 96 and 120 hours (Awan *et al*., 2012; Sagheer *et al*., 2013). Data were expressed as percent mortality. Mortality data were subjected to analysis of variance (ANOVA), and their means were separated by Tukey's HSD by using Statistix version 8.1 (Analytical software, 2005).

2.4. Repellency test

The experiment was performed using Petri dishes. Whatman filter paper (No. 1) was cut into two equal halves. One half of the filter paper was treated with a specific concentration of extracts, while the other half was kept untreated. Acetone was used as a control treatment. Both halves of filter paper (treated and untreated) were placed into the petri dishes. After drying, twenty adults of R. dominica and T. granarium were introduced into the middle of the petri dish. The dish was then closed firmly. There were a total of 25 petri dishes for each extract type (including control) and insect species. Each concentration was replicated five times. The number of insects present on both treated and untreated halves was recorded after 24 hours (Sagheer *et al*. 2013). Repellency data were expressed as percent repellency. Data were subjected to analysis of variance (ANOVA), and their means were separated by Tukey's HSD by using Statistix Version 8.1 (Analytical Software, 2005).

3. Results and discussion

The current study was carried out to know the mortality and repellency of acetone extracts of R. stricta leaf and seed at different exposure times (24, 48, 72, 96 and 120 hours) against R. dominica and T. granarium. The contact bioassay treatment combination of 20% leaf extract and 120 h post-treatment interval was found to be the best, achieving the highest mortality (72.11 ± 1.25%) against R. dominica. This was followed by a 120 h post-treatment time interval and 15% (48.60 ± 1.70%), 10% (30.54 ± 0.89), 5% (27.83 ± 0.72) leaf extract concentrations. Among the different time intervals and concentrations of seed extracts, the higher concentration (20%) and 120 h post-treatment time achieved the maximum mortality (69.5 ± 1.04%) of R. dominica (Table 1).

R. dominica and T. granarium. Table 3 shows the comparison of mean repellency (%) of tested insects at five concentrations of R. stricta leaf and seed extracts. The repellency of both insects increases as the concentrations increase. Against R. dominica, the leaf and seed extracts showed a maximum repellency of 62.50% and 38.87% respectively at 20% concentration, a finding which can be considered significantly different from the other concentrations. For T. granarium, maximum repellency was recorded in the leaf extract experiment at 20% concentration (60.50 ± 1.73) compared to the remaining concentrations. The seed extracts caused the highest repellency (%) at 20% concentration (47.20 ± 1.45) followed by 15% (27.79 ± 1.45), 10% (19.45 ± 1.20), 5% (13.87 ± 0.88) and 0% (8.33 ± 0.58).

Table 1. Mortality (%) of Rhyzopertha dominica at different concentrations of Rhazya stricta leaf and seed extracts at various time intervals (h).

Extract type	Concentration	Mortality (%)				
		24 h	48 h	72 h	96 h	120 h
Leaf	0%	2.19 ± 0.67 D	4.18 ± 0.58 D	9.71 ± 0.33 D	10.12 ± 0.33 D	10.66 ± 0.88 D
	5%	8.33 ± 0.58 CD	20.83 ± 1.15 C	23.33 ± 0.88 C	25.00 ± 0.58 C	27.83 ± 0.72 C
	10%	13.87 ± 0.88 C	19.66 ± 0.67 C	26.37 ± 0.85 C	29.10 ± 0.58 C	30.54 ± 0.89 C
	15%	38.66 ± 2.40 B	43.33 ± 2.33 B	45.66 ± 2.02 B	47.20 ± 1.89 B	48.60 ± 1.70 B
	20%	50.00 ± 2.31 A	65.29 ± 1.86 A	68.04 ± 1.20 A	69.40 ± 1.45 A	72.11 ± 1.25 A
Seed	0%	2.79 ± 0.33 C	5.55 ± 0.33 D	5.54 ± 0.33 D	6.66 ± 0.45 E	9.66 ± 0.33 E
	5%	5.54 ± 0.29 C	18.04 ± 0.54 C	22.20 ± 0.30 C	33.33 ± 1.20 D	38.87 ± 0.63 D
	10%	12.6 ± 0.58 B	27.79 ± 1.67 B	37.5 ± 1.04 B	40.33 ± 0.88 C	45.00± 0.63 C
	15%	36.13 ± 2.33 A	52.83 ± 1.86 A	61.12 ± 2.01 A	61.12 ± 2.03 B	62.5 ± 2.17 B
	20%	40.29 ± 2.19 A	55.54 ± 1.33 A	59.66 ± 1.45 A	68.11 ± 0.88 A	69.5 ± 1.04 A

Means sharing the same letters in the column are not significantly different (P > 0.05; Tukey's HSD, Statistix 8.1).

For T. granarium, the highest mortality was observed in leaf extract treatments as compared to seed extract ones. After 120 h, the leaf extracts caused maximum mortality (61.101.20±) at 20% concentration followed by 15% (46.70 ± 1.45%), 10% (31.65 ± 2.19), 5 % (25.00 ± 1.73) and 0% (10.33 ± 0.33%) (Table 2). The mortality (%) of T. granarium resulting from the contact bioassay of the seed extracts also followed the same pattern as that of the leaf extracts. The maximum mortality (56.95± 3.26%) was observed at 20% concentration and 120 h post-treatment time. The leaf extract effect was superior to that of the seed extract against both stored grain pests.

Concentrations were significantly different (P=0.05) from each other in terms of their repelling effects against adults of

Table 2. Mortality (%) of Trogoderma granarium at different concentrations of Rhazya stricta leaf and seed extracts at various time intervals (h).

Extract type	Concentration	Mortality (%)				
		24 h	48 h	72 h	96 h	120 h
Leaf	0%	1.37 ± 0.32 C	1.37 ± 0.32 D	6.95 ± 0.33 D	9.70 ± 0.30 D	10.33 ± 0.33 E
	5%	6.95 ± 1.20 BC	19.45 ± 1.8 C	20.83 ± 1.73 C	23.62 ± 1.76 C	25.00 ± 1.73 D
	10%	11.12 ± 1.45 B	20.83 ± 2.20 C	23.66 ± 2.19 C	27.79 ± 1.67 C	31.65 ± 2.19 C
	15%	27.79 ± 2.39 A	37.5 ± 1.04 B	38.87 ± 0.67 B	45.83 ± 0.58 B	46.70 ± 1.45 B
	20%	33.33 ± 1.20 A	44.45 ± 0.88 A	48.62 ± 0.67 A	52.33 ± 0.88 A	61.10 ± 1.20 A
Seed	0%	2.79 ± 0.21 D	5.54 ± 0.73 C	6.95 ± 2.02 D	8.33 ± 0.88 D	12.5 ± 0.76 D
	5%	5.54 ± 0.45 CD	9.70 ± 0.70 BC	16.66 ± 1.76 C	20.83 ± 2.20 C	25.00 ± 2.64 C
	10%	8.33 ± 2.20 C	11.12 ± 1.78 B	18.04 ± 2.09 C	22.20 ± 1.56 C	26.33 ± 2.34 C
	15%	29.16 ± 2.20 B	34.70 ± 2.57 A	37.87 ± 1.73 B	41.66 ± 2.02 B	47.20 ± 1.56 B
	20%	36.12 ± 2.05 A	36.12 ± 2.13 A	44.45 ± 2.35 A	51.37 ± 2.91 A	56.95 ± 3.26 A

Means sharing the same letters in the column are not significantly different ($P > 0.05$; Tukey's HSD, Statistix 8.1).

R. stricta leaf and seed extracts caused significant mortality and repellency against R. dominica and T. granarium. Mortality and repellency were increased with the increase in concentrations and extract exposure time. The toxicity and repellency properties of R. stricta against both insect pests could be attributed to the presence of different glycosides, triterpenes, alkaloids and volatile basis (Rahman & Fatima, 1982; Ahmed *et al*., 1983; Alyahya *et al*., 1990; Hassan *et al*., 1997; Ali *et al*., 2000; Marwat *et al*., 2012). These compounds may interrupt several normal processes in humans and other organisms including insects. For example, Ali *et al*. (1999) reported a decreased ambulatory activity in rats due to R. stricta. According to Sultana & Khalid (2010), compounds present in R. stricta inhibit the lipoxygenase and acetylcholinesterase enzymes, important neurotransmitters in animals. In another study, Saeed *et al*. (1993) reported an arachidonic acid (AA) metabolism inhibition in human by rhazimine, a compound isolated from R. stricta. Tanira *et al*. (1996) also reported anti-inflammatory action and a decrease in blood pressure in rats, mice and rabbits due to different compounds in R. stricta extracts. The various compounds in R. stricta extracts also inhibit the activity of monoamine oxidase (MAO) A and B in rats. In addition, Zaman (1990), El-Hag *et al*. (1996, 1999) and AL-Rajhi *et al*. (1997) found insecticidal, insect growth inhibition and nematicidal activity of R. stricta.

Table 3. Repellency of different concentrations of Rhazya stricta leaf and seed extracts against Trogoderma granarium and Rhyzopertha dominica.

Extract type	Concentrations	Repellency after 24 h	
		R. dominica	T. granarium
Leaf	0%	11.12 ± 0.33 B	9.70 ± 0.67 D
	5%	13.87 ± 0.33 B	16.66 ± 0.58 CD
	10%	16.66 ± 0.58 B	26.37 ± 0.88 BC
	15%	26.37 ± 1.20 B	34.70 ± 0.88 B
	20%	62.50 ± 2.52 A	60.50 ± 1.73 A
Seed	0%	9.70 ± 0.88 C	8.33 ± 0.58 C
	5%	12.50 ± 0.58 C	13.87 ± 0.88 BC
	10%	15.29 ± 0.88 BC	19.45 ± 1.20 BC
	15%	25.00 ± 0.58 B	27.79 ± 1.45 B
	20%	38.87 ± 0.88 A	47.20 ± 1.45 A

Means sharing the same letters in the column are not significantly different ($P > 0.05$; Tukey's HSD, Statistix 8.1).

In a few previous studies, the crude extract of R. stricta showed larvicidal and growth retarding effects against Aedes aegypyti (Zaman, 1990), while the application of an aqueous solution of R. stricta leaves at higher concentrations (100- 500 ppm) showed impaired hatchability, significant larval mortality, and decreased pupation of C. pipiens (El-Hag *et al*., 1996). Similarly, the leaf extract of R. stricta was found to have the toxic and growth retarding effects against the two agricultural pests, i.e. A. ipsilon and H. brunneipennis (El-Hag *et al*. 1999). Madkour *et al*. (2013) exposed Oryzaephilus surinamensis (grub and adult beetles) to the acetonic plant extract of R. stricta. The percentage of mortality was recorded after 2, 4 and 6 days from exposure. The complete mortality was observed at the highest concentration of 500 ppm for both the grub and \ adult beetles, while high repellency (97% at concentration of 500 ppm) was observed against adults. In another study, the toxicity of aqueous, methanolic and acetonic extracts of R. stricta to T. granarium grub was studied. The toxic effect was found to be dose and exposure time dependent. The toxic effect observed for the acetonic extract was 1.4 times that observed for either methanolic or aqueous extracts (El-Nadi *et al*., 2001).

4. Conclusion

Compared to conventional insecticides, plant products/ extracts are quite harmless for warm-blooded animals. They also degraded easily in the environment (Oparaeke *et al*., 2005). Like most other plant products, the concentrations of R. stricta are safer for humans as they are too dilute to be problematic. Moreover, experiments on rats reveal no side effects when extracts of R. stricta were administered orally (Siddiqui and Bukhari, 1972). Based on the results of previous studies and this current study, it is concluded that R. stricta leaf and seed extracts can effectively protect stored grains from pest attack without harming human health. Its extract can be prepared in various solvents, including acetone, ethanol, petroleum ether, hexane or distilled water. The solutions can be applied on grains or on the walls of the storage structures. The solvent should be allowed to evaporate for several hours before grains are deposited in order to avoid harmful effects of the organic solvent on humans and non-targeted organisms. The current study shows that these extracts can be an important, environment friendly and effective alternative to highly toxic insecticides. These products will protect stored grains from small and large pest infestations.

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فاعلية مستخلصات أوراق وبذور نبات الحرمل rhazya stricta ضد حشرة ثاقبة الحبوب الصغرى Rhyzopertha dominica وخنفساء الخابرا Trogoderma granarium

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الملخص

تعتمد حماية الحبوب المخزنة من الآفات الحشرية على مبيدات حشرية اصطناعية عالية السمية تسبب عددًا من التأثيرات الضارة على الصحة العامة والبيئة. وهذا يستلزم إيجاد المزيد من تدابير الحماية الصديقة للبيئة ضد هذه الآفات الحشرية المُخزنة. في الدراسة الحالية، قمنا بتقييم مستخلصات أوراق وبذور نبات طبي محلي وهو الحرمل (Apocynaceae) Rhazya stricta من حيث السمية والنفور نحو حشرة ثاقبة الحبوب الصغرى (Bostrichidae :مغمدات الأجنحة) Rhyzopertha dominica وخنفساء الخابرا (Dermestidae :مغمدات الأجنحة) Trogoderma granarium في المختبر. وأظهرت النتائج زيادة في معدل الوفيات والنفور عند زيادة تركيز المستخلصات. بعد 120 ساعة، تسببت مستخلصات الأوراق في أعلى نسبة وفيات (61.10 ± 1.20 - 72.11 ± 1.25%) مقارنة بمستخلصات البذور (56.95 ± 3.26 - 69.50 ± 1.04) في كلاً من الحشرات المستهدفة. وأظهرت مستخلصات الأوراق النباتية أعلى نسبة نفور (62.50 ± 1.73 - 47.20 ± 1.45%) في R. dominica و T. gran - rium عند تركيزات أعلى مقارنةً بالتركيزات الأقل في فترة تجربة مدتها 24 ساعة. وتقترح دراستنا أنه يمكن استخدام مستخلصات من أوراق النبات الطبي R. stricta كعنصر فعال في برنامج الإدارة المتكامل ضد آفات الحبوب المخزنة.