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INSECTICIDAL AND REPELLENT EFFECT OF SOME INDIGENOUS PLANTextracts Against Saw-Toothed Grain Beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Sivanidae)

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ABSTRACT

Five natural ethanolic plant extracts of *Rhazya stricta*, *Caralluma tuberculata*, *Capparis spinosa*, *Marrubium vulgare* and *Argemone ochroleuca* were tested in the laboratory for their insecticidal and repellent effectiveness against saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.). Four concentrations of each plant extract, 200, 400,600 and 800 ppm were tested. Larvae and adult beetles were exposed to plant extracts for 6 days. Mortality percentage was recorded after 2, 4 and 6 days from exposure. The repellent action of the previous plant extracts was also, studied. All of these extract showed remarkable toxicities. Results showed that complete mortality of *O. surinamensis* was achieved by *C. tuberculata* and *R. stricta* at the concentration of 800ppm for both larvae and adult beetles. The rest of plant extracts increased mortality with increasing of concentrations. Of five plant extracts, the effect of *C. tuberculata* and *R. stricta* were relatively more pronounced with LC_{50} values of 203, 970 and 244, 245 ppm, respectively, two days from treatment. Corresponding LC_{50} values after six days exposure for larva, values were 114, 615 and 117, 775. As for adult, the LC_{50}s values after 2 days were 210.062 and 238.563. After 6days, LC_{50}s were 123.295 and 127.182 respectively.

Moreover, *R. stricta* and *C. tuberculata* exhibited high repellency 100% and 90.08% at concentration of 800 ppm against *O. surinamensis* adults.

The application of these extracts may be promising in protecting of stored date and grains against the attack of *O. surinamensis* specially extracts of *C. tuberculata* and *R. stricta.*

INTRODUCTION

Saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) is one of the most serious and destructive pest of grain and date in bulk condition. This insect feeds on a variety of products including all grains and grain products, dried fruits, fast food, nuts, seeds, yeasts, sugar candy, tobacco, dried meats and all plant products used as human food(Metcalf and Flint,1979). Control of this pest population around the world primarily depend upon applications of organophosphorus and pyrethroid insecticides and fumigants such as methyl bromide, which are still effective for the protection of stored food, feedstuffs and other agricultural commodities from insect infestation (Kim *et al*., 2003 and Park *et al*., 2003). The use of insecticides causes several problems such as environmental pollution, health hazards, pesticide resistance and outbreak of pests due to disrupt biological control and ecosystem (Shah *et al*., 2008). The insecticides have been detected in almost all the food materials including food grains, vegetables, fruits, meat, fish, eggs, milk products and human milk (Das *et al*., 1999). To compact with these problems, there is an urgent need alternative and safe effective methods with no toxic effects on non-target organisms. This has created interest in research of using plant extracts as alternative methods to control pests. Many plant extracts used for protection of stored product pests as they constitute a rich source of bioactive chemicals (Shah *et al*., 2008, Boussaada *et al*., 2008, Ngamo Kundu *et al*., 2007). *Rhazya stricta* Decaisne (Apocyanaceae) and *Caralluma tuberculata* (*Asclepiadaceae*), are herbaceous plant widely distributed in the kingdom of Saudi Arabia (Migahid, 1978) and throughout the semi- arid tropical areas. They are known to possess some biological activity against insects and used in folk medicine. (Elhag *et al*., 1996 Elshanwani, 1996). *Rhazya stricta* was found to be rich in alkaloids of different types, flavonoids, sterols and volatile oil (Ahmad *et al*., 1983; Rahman and Fatima, 1982). *Caralluma tuberculata* possess a strong anti-microbial activity (Elshanawani, 1996). Flavonoides, alkaloids and volatile oils are the main constituents in *C. tuberculata*. Also, *Capparis spinnosa* (Capparridaceae), *Marrubium vulgare* (Labiatae) and *Argemone, ochroleuca* (Papveraceae) were used in folk medicine and the main constituents are flavonoids, glycosides, resins and volatile oils (Elshanwani, 1996). The present research work was under taken to evaluate the toxicity and repellent effects of five indigenous plant extracts against Saw-toothed grain beetle *Oryzaephilus surinamensis***.**

MATERIALS AND METHODS

This study was conducted in the Faculty of Metrology, Environment and Arid Land Agriculture, department of Arid land Agriculture and Faculty of pharmacy, King Abdul Aziz University during 2008.

Rearing Technique

Oryzaephilus surinamensis (L.) was reared in glass Jars at 28-30 C° and 70-75 % RH. Wheat grains and date were used as food for rearing insects. Jars was set up with 90 pairs of adult beetles. The jars were covered with muslin cloth fastened with rubber bands.

Powder preparation

Fresh leaves of *Razya stricta, Caralluma tuberculata, Capparis spinosa, Marrubium vulgar and Argemon ochroleuca* were collected from different parts in Saudi Arabia and kept in the laboratory for air drying. Dust of dried leaves were prepared by using grinder machine. The dusts were passed through a 25 mesh diameter sieve for fine dust.

Extraction preparations

Each plant powder was mixed with ethanol. The mixture was stirred for 30 minutes by magnetic stirrer and left 24 hours. Then, the mixture was filtered. The filtrate (extract) was concentrated using a rotary vacuum evaporator in a water bath at 55˚C according to Chitra *et al*, 1993. The extracts were then freeze dried using a Labconco Freeze Dryer-18 model 75018 for 48-72 hours. Stock solution was prepared from the lyophilized residue of each plant extract.

Test procedure

Five stock solutions of lyophilized concentrated extracts of plants were prepared in distilled water (0.5gm/100ml). Four different concentrations of 200, 400, 600 and 800 ppm were prepared from the stock solutions of different plant extractives used in this experiment. One ml of each concentration was applied to filter papers (whatman No. 9 cm in diameter). After drying, filter papers were placed in the bottom of Petri dish (9 cm) and 50 gm of wheat grains were put inside each of Petri dishes and 30 larvae or adults were released. The mortality percentages were determined at 2, 4 and 6 days after treatment. Control treatment received 1 ml of water only. All treatments were replicated three times. Values of LC_{50} were calculated according to Finney (1971). Data were corrected for control mortality using Abbott's formula (Abbott, 1925).

Repellency test.

Repellency test was conducted according to the method of Talukder and Howse (1994). Petri dishes were divided into two parts, treated and untreated fresh grain portions. One ml solution of each plant extract was applied to one half of the grains of each small petri dish. The treated half was then air-dried. Groups of 20 newly emerged adults of *O. surinamensis* were released at the centre of each Petri dish and covered. Three replications were used for each dose. The numbers of insect present on each portion of the petri dishes were counted at 2 hours intervals. The data were expressed as percentage of repulsion (PR), using the method of Jilani *et al*., (1988). Data (PR) were analyzed using analysis of variance (ANOVA).

RESULTS AND DISCUSSION

The insecticidal activity of five plant extracts against the larvae of *O. surinamensis* at 2,4 and 6 days after treatment are shown in Tables 1and 2. The larval mortality percentages are presented in Table 1. The LC_{50} values and 95% confidence limits are given in Table 2. The data showed that all tested plant extracts were toxic to *O. surinamensis* larvae in a dose dependent manner; although the toxic action was relatively slow for the extract of *M. vulgare* and *A. ochrolecuca* which their toxicity are approx. equal. Both concentrations of *C. tuberculata* and *R. stricta* were the most effective, where 600 and 800 ppm of both plant extracts caused 100% mortality (Table 1). The higher concentration (800 ppm) of *C. spinosa*, *M*. *vulgar* and *A. ochroleuca* extracts were toxic to *O. surinamensis* larvae with percentages of 94.4, 83.3 and 84.4 % mortalities, respectively. The results in Table 2 indicated that *C. tuberculata* and *R. stricta* had significantly lower LC_{50} values (2, 4 and 6 days) than other plant extracts with LC_{50} 114, 127, 203 an, 117, 130, 244 ppm respectively.

The values of LC_{50} after 2, 4 and 6 days assay time for *C. spinosa, M. vulgare* and *A. ochroleuca* were 175, 229, 290; 309, 344, 386 and 251, 291 and 331 ppm, respectively. The mechanism in which the plant extracts caused larval mortality can not be realized stated from the present study,but the antifeeding effect of plant extracts are well documented, especially on stored product insects(Schumutter, 1990). Considerable biological activity relating to toxicity and hindrance of growth and development of larvae of *S. surinamensis* has been observed with the ethanolic extracts of the leaves of the tested plants. Of the five plant extracts, ethanolic extracts of *C. tuberculata* and *R. strecta* were fount to cause highest rate of mortality and lowest values of LC_{50} comparing with with other plant extracts.

Table 1: Percentages mortality of *O. surnamensis* (L.) larvae treated with ethanolic extracts of tested plants.

Ethanolic plant extracts	Concentration (ppm)	Mortality %		
		2d	**4d**	**6d**
Rhazya stricta	200	46.6	67.7	73.3
	400	55.5	73.3	82.2
	600	65.5	82.2	93.3
	800	87.7	100	100
Caralluma tuberculata	200	51.1	66.7	73.3
	400	61.1	77.7	86.6
	600	78.8	93.3	98.8
	800	93.3	100	100
Capparis spinosa	200	44.4	46.6	57.7
	400	51.1	66.7	72.7
	600	65.5	76.6	86.6
	800	81.1	87.7	94.4
Marrubium vulgare	200	36.6	37.7	38.8
	400	45.5	46.7	52.2
	600	58.8	65.5	70.0
	800	72.2	78.8	83.3
Argemone ochroleuca	200	42.2	43.3	46.6
	400	46.7	50.0	55.5
	600	61.1	71.1	77.7
	800	77.7	82.2	84.4
	Cont.	00.0	00.0	2.2

Table 2: LC_{50} values and 95% confidence limits for *O. surnamensis* (L.) larvae reared in media containing ethanolic extractives from tested plants.

Ethanolic plant extracts	Assay times (days)	Slope	LC_{50} (95%CL)
Rhazya stricta	2	0.92	244.25 (205.71- 339.14)
	4	1.37	130.37 (098.00- 207.78)
	6	1.79	117.78 (077.21- 179.29)
Caralluma tuberculata	2	0.83	203.97 (176.23- 284.30)
	4	1.41	127.37 (120.11- 221.22)
	6	1.58	114.61 (096.42- 179.44)
Capparis spinosa	2	1.02	290.58 (226.00- 373.30)
	4	1.49	229.30 (178.32- 294.57)
	6	1.55	175.48 (130.24- 236.13)
Marrubium vulgare	2	1.09	386.73 (312.37- 478.64)
	4	1.68	344.53 (284.69- 416.79)
	6	1.88	309.67 (256.62- 373.52)
Argemone ochroleuca	2	0.51	331.16 (260.85-420.16)
	4	1.78	291.87 (234.00- 363.80)
	6	1.97	251.99 (197.69- 320.95)

The results presented in Table 3 show the percentages of adult mortality endured due to the five plant extracts. The data showed that both concentrations of *C. tuberculata* and *R. stricta* induced 99.7 and 98.6 % mortality with 800 ppm after 4 days of exposure, respectively. The highest percent of mortality achieved was 100 % for both of plant extracts after 6 days from exposure to 800 ppm concentration. The mortality percentage increased after 6 days of exposure for all plant extracts. After 6 days of exposure, *C. spinosa*, *M. vulgar* and *A. ochroleuca* extracts gave 91.1, 82.2 and 77.7 % mortality with 800 ppm, respectively. The lowest mortality was 77.7 % for *A. ochroleuca*. Along 6 days of exposure almost all the plant extracts were superior where the mortalities exceeded 77.7 %. Mortality of control insects was less than 5% along the exposure periods. LC_{50}s and 95 % confidence limits for each plant extract are shown in Table 4. Data were analyzed using the probit analysis, and the effectiveness was expressed as LC_{50} values. The lowest LC_{50}s were for *C. tuberculata* and *R. stricta* after 6 days from exposure which it were 123and 127 ppm, respectively. The respective values of LC_{50}s of the other plant extracts after the same period of exposure were 162, 282 and 310 ppm for *C. spinosa, M. vulgare* and *A. ochroleuca* extracts, respectively. The obtained results showed that the plant extracts of *C. tuberculata* and *R. stricata* were generally more toxic than other plant extracts and possess lower LC_{50}s.

Table 3: Percentages mortality of *O. surnamensis* (L.) adults treated with ethanolic extracts of tested plants.

Ethanolic plant extracts	Concentration (ppm)	Mortality %		
		2d	4d	6d
Rhazya stricta	200	45.5	67.7	68.8
	400	57.7	73.3	81..2
	600	68.8	83.4	95.5
	800	93.2	98.6	100
Caralluma tuberculata	200	47.7	65.2	73.3
	400	58.8	74.6	82.2
	600	78.8	87.7	96.7
	800	94.3	99.7	100
Capparis spinosa	200	43.4	45.6	58.7
	400	52.3	66.7	71.7
	600	64.5	74.4	81.6
	800	80.1	86.7	91.1
Marrubium vulgare	200	35.6	38.7	43.3
	400	44.5	47.7	51.2
	600	57.7	67.7	72.3
	800	73.2	80.0	82.2
Argemone ochroleuca	200	40.0	42.2	44.4
	400	45.5	46.6	50.0
	600	51.1	56.6	58.7
	800	67.7	73.2	77.7
	Cont.	2.2	1.1	1.1

Table 4: LC_{50} values and 95% confidence limits for *O. surnamensis* (L.) adults reared in media containing ethanolic extracts from tested plants.

Ethanolic plant extracts	Assay times(days)	Slope	LC_{50} (95%CL)
	2	0.89	248.65 (200.05- 326.43)
Rhazya stricta	4	1.21	158.11 (120.30- 240.80)
	6	1.62	127.18 (088.91- 189.31)
	2	0.75	210.09 (170.61- 300.30)
Caralluma tuberculata	4	1.31	150.12 (127.66- 229.11)
	6	1.58	123.30 (080.42- 182.21)
	2	0.99	301.96 (231.63- 396.27)
Capparis spinosa	4	1.46	236.04 (184.56- 301.47)
	6	1.63	175.11 (109.90- 238.69)
	2	1.03	389.19 (314.19- 480.13)
Marrubium vulgare	4	1.71	329.28 (287.20- 419.65)
	6	1.84	282.67 (226.28- 377.88)
	2	0.64	415.86 (366.55-556.76)
Argemone ochroleuca	4	1.78	348.77(267.53- 444.25)
	6	1.92	306.89(233.75- 412.03)

The results and statistical analysis of the repellency percentages of tested plant extracts are presented in Table 5. Data demonstrated that *R. stricta* extract had strong repellency action with repellent percentage of 100 % at 800 ppm concentration followed by *C. tuberculata* with 91% for the same concentration. The repellency percentage of other plant extracts were ranged between 50.22 -66.54% at the same concentration which had a moderate repellent action. Statistical analysis showed significant difference between *R. stricta* and other plant extracts. Also, numerically, the repellency percentages of all plant extracts revealed significant deference except between *M. vulgare* and *A. ochroleuca*. These results revealed that the rate of repellency increased with increase of dose level. At 800ppm concentration all plant extracts showed the highest repellency rate. Al- Jaber, 2006 found that essential oil of *Matricaria chamomilla* has strong repellency action against *O. surinamensis*.

Table 5: Repellency of plant extracts to *O. suronamensis*

Ethanolic plant extracts	Repellency at concentrations (%)				Means*
	200	400	600	800	
Rhazya stricta	77.21	84.22	94.3	100.00	88.93a
Caralluma tuberculata	71.03	76.98	88.41	91.08	81.88b
Capparis spinosa	55.65	49.33	44.61	66.54	54.03c
Marrubium vulgare	40.26	41.33	43.27	54.36	44.81d
Argemone ochroleuca	37.61	39.99	42.54	50.22	42.59d

*** Means followed by the same letter(s) are not significantly differ at 5 % level of probability**

The results obtained in this investigation demonstrated the importance of the toxic and repellency of tested plant extracts, especially those of *R. stricta* and *C. tuberculata* for controlling the stored product pests especially *O. surinamensis*. Moreover, application of these extracts is not likely to leave harmful residues in the environment since they are speedly degraded to non-toxic products. In addition *R. stricta* and *C. tuberculata* have been used for years in traditional medicine. Searching for plant extracts to be mixed with date and cereals have many advantages such as serving of finding natural, cheapest and local materials that could be used for prevention of insect infestation and storage date without harmful insects.

Further studies are needed to identify the active components in *R. stricta, C. tuberculata* to evaluate their toxic and biochemical effects to mammals.

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الخواص الإبادية و الطاردة لبعض المستخلصات النباتية على خنفساء التمور المنشارية

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تم دراسة التأثير السام والطارد لخمسه مستخلصات نباتيه هي: *Caralluma tuberculata*, *Capparis spinosa, Marrubium vulagare*, *Rhazya stricta*, و *Argenome ochroleuca* على حشرات خنفساء التمور المنشارية تحت الظروف المعملية. تم اختيار أربع تركيزات وهى 200, 400, 600و800 جزء في المليون حيث تم تعريض اليرقات والحشرات الكاملة المعاملة بهذه المستخلصات لمده 6 أيام. سجلت نسب الموت بعد 2, 4و6 أيام من التعرض. ومن ناحية أخرى تم دراسة التأثير الطارد لمستخلصات النباتات المذكورة أعلاه بنفس التركيزات السابقة على الحشرة الكاملة فقط. توضح النتائج إن كل المستخلصات أظهرت سميه ملحوظة على هذه الحشرة. وأظهرت النتائج نسبه موت 100% لهذه الحشرة في كل من اليرقات والحشرات الكاملة عند المعاملة بتركيز 800 جزء في المليون لكل من مستخلصي *C. tuberculata* و*R. stricta* . أما بقيه المستخلصات فقد زادت لها نسبه الموت مع زيادة التركيز. ومن النباتات الخمسة نجد إن تأثير كل من *C. tuberculata* و*R. stricta* كان واضح حيث كانت الجرعة القاتلة لخمسين في المائة من يرقات هذه الحشرة بعد يومين من المعاملة هي 230.970 و 244.245 جزء في المليون وبعد 6 أيام من المعاملة كانت 114.615 و 117.775 جزء في المليون على التوالي. أما بالنسبة للحشرات الكاملة فكانت 210.062 و238.563 جزء في المليون وذلك بعد يومين من المعاملة وكانت 123.295 و 127.182 جزء في المليون بعد ستة أيام من المعاملة على التوالي مقارنه ببقية المستخلصات النباتية الأخرى. ومن ناحية أخرى بينت نتائج التأثير الطارد أن المستخلص النباتي لكل من *R. stricta* و *C. tuberculata* عند تركيز 800 جزء في المليون كانا أعلى المستخلصات في نسبه الطرد للحشرات الكاملة لخنفساء التمور المنشارية حيث بلغت 100% و 90.08 % على التوالي. ويمكن القول أن هذه النتائج واعده في التطبيق العملي لحماية التمور والحبوب المخزونة من المهاجمة بحشره خنفساء التمور المنشارية وخاصة مستخلصي *C. tuberculata* و *R. stricta* .

قام بتحكيم البحث

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