Repellent and Insecticidal Effects of Some Plant Extracts on Flour Beetle *Tribolium castaneum* Herbst (Coleoptera:Tenebrionidae)

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ABSTRACT

The repellency and insecticidal activity of ethanolic plant extracts *from Rhazya stricta, Caralluma tuberculata , Capparis spinosa, Marrubium vulgare and Argemone ochroleuca* were evaluated against red flour beetle, *Tribolium castanium* in the laboratory. The dosages used were 200, 400, 600 and 800 ppm. Larvae and adult beetle were exposed to the treated wheat flour 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 using the same dosages. All of these extracts showed remarkable toxicities in a dose dependant manner. Results showed that complete mortality of *T. catanium* was achieved by *C. tuberculata* at the concentration of 800 ppm for both larvae and adult beetle. Also, complete mortality was recorded with *R. stricta* extracts for adult of *T. castanium* only at the same concentration. On the other hand, extract of *C. spinosa* was more toxic to larvae (95%) than adult (90%) with the same concentration. The rest of plant extracts mortality was increased with increasing of concentrations. Medium lethal concentrations (LC50) against larvae for *C. tuberculata, R. stricta* and *C. spinosa* were 162.79, 201.25 and 210.64 ppm after 2 days and chronic (6 day) were 112.41, 129.91 and 133.08 ppm. As for adult, it were 231.22 , 286.99 and 307.68 ppm LC50s values after 2 days. After 6 days it was 126.47, 137.14 and 142.54 ppm LC50s, respectively. Other plant extracts were less toxic to larvae and adult of red flour beetle. Moreover, *R. sricta, C. tuberculata* and *C. spinosa* exhibited high repellency 100%, 90.08% and 82.54 % at concentration of 800 ppm against *T. castanium* adult. The application of these plant extracts may be promising in protecting of stored products against the attack of *T. castanium* specially extracts of *C. tuberculata, R. stricta*. and *C. spinosa*

Keywords: *Tribolium castaneum*, Methanolic plant extracts, *Rhazya stricta, Caralluma tuberculata , Capparis spinosa, Marrubium vulgare, Argemone ochroleuca* , Adult and immature insects.

INTRODUCTION

Stored grain insect pests have been damaging our stored agricultural commodities and are responsible for 10-40% of lose worldwide annually (Shukla *et al*., 2008). Simultaneously, the continuous bloom in human population has also posed a great problem of food scarcity. *Tribolium castaneum* is considered the major pest of stored grains (Jabilou *et al*., 2006). Their presence in stored foods affected directly both the quantity and quality of the commodity (Mondal, 1994). In tropical countries like Saudi Arabia, the climate and storage conditions are favorable for insect growth and development. Annual post-harvest losses resulting from insect damages, microbial deterioration and other factors are estimated to be very high all over the world (Matthews, 1993). In such case, protection of stored products and agricultural products from insect infestation is an urgent need. Control of these insects relies heavily on the use of synthetic insecticides and fumigants. But their widespread use has led to serious problems, including development of insect strains resistant to insecticides (white, 1995 and Ribeiro *et al*., 2003), toxic residues on stored grain, toxicity to consumers and increasing cost of applications. There is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly. Considerable efforts have been focused on plant derived materials, potentially useful as commercial insecticides. Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Kundu *et al*., 2007). Insecticidal activity of many plants against several insect pests has been demonstrated (Carlini and Grossi-de-Sa,2002, Kundu *et al*., 2007 and Boussada *et al*., 2008). Moreover, products from several floral species have been demonstrated to act as repellents, toxicants and antifeedants against a number of Coleoptera that attack stored products(Raja *et al*., 2001 and Tapondjou *et al*., 2002). Khanam *et al*.,2006 also reported toxic and repellent properties of sugarcane lignin against some stored grain insect pests including *T. castaneum*. *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 and Elshanwani,1996). *Rhazya stricta* was shown 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.*

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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 (El-shanwani, 1996). The aim of this study is to evaluate the insecticidal and repellent activity of the five ethanolic plant extracts against larvae and adult of *Tribolium castaneum*.

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 AbdulAziz University during 2008.

1. Insects:

Tribolium castaneum (L.) was reared on glass Jars at 28-30 C◦ and 70-75 % RH. This insect was reared on wheat flour powder. Third instars larvae and adult were used in this study. Jars were set up with 90 pairs of adult beetles. The jars were covered with muslin cloth fastened with rubber bands.

2. Plant materials:

Fresh leaves of *Razya stricta*, *Caralluma tuberculata*, *Capparis spinosa*, *Marrubium vulgar* and *Argemon ochroleuca* were collected from different parts in Saudi Arabia. Plants were rinsed with distilled water, dried in an oven at 40C for 48 h. Dust of dried leaves were prepared by using grinder machine. The dusts were passed through a 25 –mesh diameter sieve for fine dust.

3.Extraction preparations

Powder air dried parts of each plant was mixed with solvent (ethanol). The mixture was stirred for 30 m by magnetic stirrer and left 24 hours. Then condensed in a rotary vacuum evaporator of solvent 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.

4. Test procedure:

Five stock solutions of lyophilized extracts of plants were prepared in distilled water (0.5 gm/100 ml). Four different concentrations of 200, 400, 600 and 800 ppm were prepared from the stock solutions of different plants used in these experiments. 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 25 gm of wheat flour were put inside each of Petri dishes. Flour was spread uniformly along the whole surface of the petriplate Thirty 3rd instar larvae or adult were released. Larvicidal and adulticidal properties were determined as mortality percentage at 2, 4 and 6 days after treatment. Control treatment received 1 ml of distilled water only. All treatments were replicated three times. Values of LC50 were calculated according to Finney (1971). Data were corrected for control mortality, Abbott (1925).

5. Repellency test:

Repellency test was conducted according to the method of (Talukder and Howse,1994 and Kundu *et al*., 2007). Petri dishes were divided into two parts, treated and untreated fresh grain portion. Filter papers (Whatman 40) were cut into two half uniformly. One ml solution of each dose from plant extracts was applied to one half with a pipette. The treated half of the papers were then air-dried and attached with the untreated half with a cello-tape at the middle in such a way did not interferer with the free movement of insect from one half to another. Each filter paper was then placed in a Petri dish (9 cm diameter). Groups of 20 newly emerged adults of *T. castanium* were released at the centre of each filter paper and a cover was placed on the Petri dish. Each concentration was replicated three times for each plant extract. The insects present on each half of paper strip were counted at 2 hours interval.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 DISCUSSIONS

The mortality (%) of *T. castanium* larvae treated with five plant extracts in ethanol and their LC50 values and 95% confidence limits are shown in Tables 1 and 2. The mortality (%) of *T. castanium* larvae treated with plant extracts in ethanol is given in Table 1. The data of table 1 showed that all tested plant extracts were toxic to *T. castanium* larvae in a dose dependent manner. Both concentrations of *C. tuberculata* and *R. stricta* were the most effective, where the mortality of beetle larvae ranged from 74.4-100% and 70.00- 97.8 % at 6 days interval respectively. It was observed that the higher concentrations of *C. spinosa*, *M. vulgar* and *A. ochroleuca* extracts showed better performance to the beetle larvae whereas the performance ranged from 70.0-95.6%, 45.5-81.1% and 43.3-77.7% mortality at 6 days post exposure respectively. The results of table 2 indicated that *C. tuberculata* and *R. stricta* had significantly lower LC50 values (2,4 and 6 days) than other plant extracts with 162.78, 137.26, 112.41, 201.25, 160.26 and 129.91 ppm respectively. The values of LC50 for other plant extracts, *C. spinosa*, *M. vulgare* and *A. ochroleuca* after 6 days post exposure were 133.08, 206.83 and 299.92 respectively. Comparing the LC50 values, it was observed the ethanolic extracts of

Table 1. Percentages mortality of T. castanium larvae treated with extracts of five plants

Plant extracts	Concentration (ppm)	Mortality %		
		2d	4d	6d
Rhazya stricta	200	56.6	63.3	70.0
	400	53.3	65.5	80.0
	600	63.3	81.1	92.2
	800	76.7	90.2	97.8
Caralluma tuberculata	200	61.1	67.7	74.4
	400	64.4	70.7	84.4
	600	73.3	90.2	97.8
	800	90.2	97.8	100
Capparis spinosa	200	53.3	61.1	70.0
	400	51.1	63.3	77.7
	600	61.1	80.0	90.2
	800	75.6	88.8	95.6
Marrubium vulgare	200	34.4	38.8	45.5
	400	45.5	47.7	51.5
	600	55.5	66.6	68.8
	800	73.3	76.6	81.1
Argemone ochroleuca	200	36.6	41.1	43.3
	400	42.2	46.6	51.1
	600	46.6	55.5	63.3
	800	70.0	73.3	77.7
	Cont.	00.0	3.30	2.20

Table 2. LC50 values and 95% confidence limits for *T. castnium* larvae reared in media containing ethanolic extracts from five plants materials

Plant extracts	Assay times(days)	Slope	LC55(95%CL)
Rhazya stricta	2	0.98	201.25 (130.25- 282.11)
	4	1.42	160.26(121.39- 280.79)
	6	1.71	129.91(076.61- 205.53)
Caralluma tuberculata	2	0.89	162.77(100.00 - 289.29)
	4	1.56	137.26 (075.31- 250.89)
	6	1.71	112.41(053.19- 189.70)
Capparis spinosa	2	1.08	210.64(230.52- 399.57)
	4	1.51	168.68(130.02- 290.95)
	6	1.63	133.08(078.14- 210.93)
Marrubium vulgare	2	1.09	310.73(250.66- 478.64)
	4	1.72	278.92(155.79- 410.91)
	6	1.90	206.83(132.79- 320.59)
Argemone ochroleuca	2	0.58	410.30(301.05-530.06)
	4	1.79	366.88(260.00- 481.17)
	6	1.97	299.92(200.80- 408.95)

C. tuberculata, R. stricta and C. spinosa showed better performance than other plant extracts.

The efficiency of five ethanolic plant extracts against *T. castanium* adults are presented in Table, 3 and 4. The results presented in Table 3 show the percentage of adult mortality due to exposure to the five plant extracts. The data revealed that concentrations of *C. tuberculata*, *R. strricta* and *C. spinosa* were the most effective plant extracts where as the mortality in adult beetle ranged from 73.3-100 %, 70.0-100% and 64.4-90% after 6 days from treatments respectively. The mortality percentage

increased after 6 days of exposure for all plant extracts. Extracts of *M. vulgar* and *A. ochroleuca* caused 80.0 and 78.9 % mortality with 800 ppm during the 6 days after treatment, respectively. The lowest mortality was for *A. ochroleuca*. Along 6 days of exposure almost all the plant extracts were superior where the mortalities exceeded with increasing exposure time and concentrations. Mortality of control was less than 5% along the exposure periods. LC50s 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 LC50 values. The lowest LC50s were for *C. tuberculata, R. stricta* and *C. spinosa* after 6 days from treatment which it were 126.47, 137. 14 and 142.54 ppm, respectively. The respective values of LC 50s of the other plant extracts after the same period of exposure were 297.74 and 331.49 ppm for *M. vulgare* and *A. ochroleuca* , respectively. The obtained results showed that the plant extracts of *C. tuberculata*, *R. stricata* and *C. spinosa* were generally more toxic than other plant extracts and the possess lower LC50s.

The flavonoids presented in plant extracts possess a catecholic B-ring that seems to be responsible for the toxicant to insects (Onyilagha *et al*., 2004). Also, Jbilou *et al*.,(2006) found insecticidal activity of four medicinal plant extra against *T. castanium*.

The results and statistical analysis of the repellency rate of tested plant extracts are presented in Table 5. Data demonstrated that *R. stricta* had the lead in repellent action against adult of *T. castanium*, where repellent percentage was 100 % at 800 ppm concentration flowed by *C. tuberculata* with 94.% and *C. spinosa* with 82.54% for the same concentration. The repellency rate of other plant extracts were 71.43 and 66.89% for *M. vugare* and *A. chroleuca* 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 rate of all plant extracts revealed significant deference except between *M. vulgare* and *A. ochroleuca*. Finding revealed that the rate of repellency increased with increase of dose level. At the concentration of 800 ppm, all plants extracts showed the highest repellency rate. The present results supports the finding of David *et al*., (1988) and kundu *et al*., (2007), who showed repellent activity of some plant extracts against *T. castanium* and indicated that repellency rate increased proportionally with the increase of concentration of the extract. Also, Abdel-Sattar *et al*., (2009) Showed that the leaf and fruit essential oils of *Schinus molle* L. has insecticidal and repellent effect on *T. castanium* and *Trogoderma granarium*.

Table 3. Percentages mortality of *T. castanium* adults treated with extracts of five plants

Plant extracts	Concentration (ppm)	Mortality %		
		2d	4d	6d
Rhazya stricta	200	46.6	64.4	70.0
	400	55.6	70.0	80.0
	600	71.1	82.2	97.7
	800	84.4	97.8	100
Caralluma tuberculata	200	48.8	66.7	73.3
	400	58.8	71.1	82.2
	600	75.5	87.7	96.7
	800	95.3	99.7	100
Capparis spinosa	200	43.3	47.6	64.4
	400	51.1	64.4	72.2
	600	64.4	75.5	81.1
	800	74.4	78.8	90.0
Marrubium vulgare	200	38.8	40.0	55.6
	400	44.4	55.6	66.7
	600	55.6	64.4	70.0
	800	72.2	77.8	80.0
Argemone ochroleuca	200	37.7	41.1	43.3
	400	41.1	46.6	51.1
	600	53.3	55.5	63.3
	800	67.7	73.3	77.7
	Cont.	2.20	3.30	3.30

Table 4. LC50 values and 95% confidence limits for *T. castanium* adults reared in media containing ethanolic extracts from five plants materials

Plant extracts	Assay times(days)	Slope	LC55(95%CL)
Rhazya stricta	2	0.81	280.99(224.07- 351.13)
	4	1.29	154.22(106.57- 232.88)
	6	1.54	137.14(98.18- 199.31)
Caralluma tuberculata	2	0.79	231.22 (175.61- 310.63)
	4	1.39	148.34(109.87- 224.92)
	6	1.67	126.47(088.19- 181.71)
Capparis spinosa	2	0.92	307.68(241.81- 399.07)
	4	1.34	200.27(132.56- 307.71)
	6	1.69	142.54(101.90- 238.69)
Marrubium vulgare	2	1.08	395.32(305.69- 488.13)
	4	1.83	336.21(272.30- 425.73)
	6	1.92	297.47(226.28- 380.81)
Argemone ochroleuca	2	0.66	413.11(318.33- 535.95)
	4	1.72	380.98(287.56- 493.45)
	6	1.88	331.49(266.12- 428.34)

Table 5. Repellency of plant extracts to *T. castanium*.

Plant extracts	Repellency at concentrations (%)				Means*
	200	400	600	800	
Rhazya stricta	81.29	87.41	96.48	100.00	91.30a
Caralluma tuberculata	74.03	78.75	90.47	94.08	84.33b
Capparis spinosa	60.00	64.39	71.98	82.54	69.71c
Marrubium vulgare	50.00	56.38	63.55	71.43	60.34d
Argemone ochroleuca	48.69	51..96	59.64	66.98	56.82d

*****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, repellency influence of the extracted plant materials, specially *R. stricta* and *C. tuberculata* for controlling the stored product pests specially *T. castanium*. Moreover, application of these materials are not likely to leave harmful residues in the environment since they are naturally occurring among the local flora. Some of them such as *R. stricta* and *C. tuberculata* have been used for years in traditional medicine. Searching for plant extracts to be mixed with storing materials have many advantages such as serving of finding natural, cheapest and local materials that could be used for prevent of insect infestation to stored products. We can conclude that this study suggest that ethanolic extracts of *C. tuberculata, R. stricta and C. spinosa* plants possesses toxic and repellent principles with significant insecticidal effect and could be a promising a potential grain protectant against *T. castanium*.

These results suggest that there may be different compounds in extracts possessing different bioactivities. Further studies are required, in order to isolate, identify and assess the bioactivity of insecticide compounds present in these plants against pests of stored products.

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الملخص العربي

التأثيرات السامة والطاردة لبعض المستخلصات النباتية على خنفساء الدقيق الصدئية

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تم تقييم النشاط السام والطارد للمستخلص الإيثانولي لخمسة نباتات وهى: الحرمل, الكنديثة, الشفلح, الزقوم والأرجيمون وذلك على اليرقات والحشرات الكاملة لخنفساء الدقيق الصدئية تحت الظروف المعملية. ومن المعروف أن هذه النباتات من البيئة السعودية وتستخدم في الطب الشعبي. وقد تم اختبار أربع تركيزات وهى 200,400,600 و800جزء في المليون وذلك لمدة 6 أيام. تم تقدير النسب المئوية للموت بعد 2,4 و6 أيام من المعاملة. هذا بالإضافة إلى تقدير الفعل الطارد لهذه المستخلصات النباتية بنفس التركيزات السابقة. وأظهرت النتائج أن كل المستخلصات أعطت سميات ملحوظة على كل من اليرقات والحشرات الكاملة لخنفساء الدقيق الصدئية وذلك على حسب التركيز. أوضحت النتائج أيضا حدوث نسبه100% موت لكل من اليرقات والحشرات الكاملة عند تعرضها لتركيز 800 جزء في المليون لمستخلص الكنديثة بعد 6 أيام من المعاملة. أيضا بينت النتائج أن المستخلص الإيثانولي لنبات الحرمل أعطى نسبه موت 100% وذلك عند تعرض الحشرات الكاملة لنفس التركيز السابق. ومن ناحية أخرى أظهر المستخلص الإيثانولي لنبات الشفلح نسبه موت 95% وذلك على اليرقات أما الحشرات الكاملة فكانت نسبه الموت فيها 90% عند نفس التركيز السابق. أما بقيه المستخلصات النباتية فزادت السمية لليرقات والحشرات الكاملة بزيادة التركيز. وجد أن قيم الLC50s عند معامله يرقات الحشرة بمستخلصات الكنديثه, الحرمل والشفلح هي على التوالي 162.79 , 201.25 و 210.64جزء في المليون. أما بعد 6 أيام من المعاملة فكانت 112.41, 129ز91 و 133.08 جزء في المليون. وبالنسبة للحشرة الكاملة فكانت النتائج هي 131.22,286.99 و 307.78 جزء في المليون وذلك بعد يومين من المعاملة. أما بعد 6أيام من المعاملة فكانت 126.47,137.14و142.54 جزء في المليون لمستخلصات النباتات الثلاثة السابقة في حين بقيه المستخلصات كانت سميتها أقل على اليرقات والحشرات الكاملة.

من ناحية أخرى أظهرت مستخلصات كل من الكنديثه, الحرمل والشفلح نسبه طرد عاليه للحشرات الكاملة لخنفساء الدقيق حيث كانت النسب هي100%, 90.58 و82.54% على التوالي عند تركيز 800 جزء في المليون.

ومن النتائج يمكن القول أن استخدام المستخلصات النباتية خصوصا مستخلصات كل من الكنديثة, الحرمل والشفلح ربما تكون واعده في حماية المواد المخزونة من الإصابة بخنفساء الدقيق الصدئية.