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Repellency of Five Indigenous Plant oils against Red Flour Beetle, *Triboliumcastaneum*

Syedaazra Tariq[1], M. F. Khan[2], Hinazafer[1*], Waseem Abassi[3]

[1] *Vertebrate Pest Control Institute, Southern-zone Agricultural Research Centre, Pakistan Agricultural Research Council, Old Blocks 9-10, University of Karachi, Karachi-75270*

[2]*Department of Zoology,* [3]*Department of Botany, University of Karachi, Karachi-75270*

Corresponding Author:** ***Hinazafer, *Vertebrate Pest Control Institute, Southern-zone Agricultural Research Centre, Pakistan Agricultural Research Council, Old Blocks 9-10, University of Karachi, Karachi-75270*

Abstract: *Laboratory studies were carried out to evaluate the repellency of essential oils of Azadirachtaindica (neem), Valerianaofficinalis(valerian), Acoruscalamus(sweet flag), Curcuma longa (turmeric) andSaussurealappa(costus)against red flour beetle, Triboliumcastaneum. Saussurealappa (costus) was found the best and the most persistent repellent among all the plants tested by achieving 88.67% repellency in first week which increased in second (88.79%) and fourth week (88.92%). Acoruscalamus (sweet flag) revealed 71.15% repellency at 0.100% in first week which decreased to 63.60% in second, 62.17% in fourth and 60.39% in eighth week. Azadirachtaindica (neem) showed 64.16% in first week which remain same in second but decreased to 58.87% in fourth and 45.72% in eighth week. Curcuma longa (turmeric) exhibited 60.45% repellency at 0.100% in first week which decreased to 49.02% in eighth week. At all other concentrations turmeric proved it a weak repellent by showing less than 40% repellency except at 0.050% in first week (42.75%). Valerianaofficianalis(valerian) could not prove it a promising repellent.*

Keywords: *Triboliumcastaneum, Azadirachtaindica, Valerianaofficinalis, Acoruscalamus, Curcuma longa, Saussurealappa, repellency.*

1. INTRODUCTION

Insect pests inflict heavy losses to stored grains during storage. Food and Agriculture Organization has reported 10- 25% losses of the world's harvested food annually by insects and rodent pests (Anonymous, 1980). Chaudhry (1980) reported 2%to 6%overall lossesof stored grains and 3.24% post-harvest loss of wheat in Pakistan every year by insect pests.Ahmad (1983) evaluated 2.5% post-harvest losses of stored food commodities. Similarly Baloch *et al.* (1994) estimated 4% storage losses to wheat in public sector. Red flour beetle (*Triboliumcastaneum*) is a major stored grain pest causing enormous losses to grain and grain products. Conventional pesticide pose detrimental effects on environment, other animals and human (Pitasawat*et al*., 2003). To avoid such hazards various plants are being explored against insect's pests. There is a need for development of effective, safe, environment friendly, convenient and inexpensive methods for protection of the stored grains. The trend for use of botanical insecticides throughout the world has led to conduct this research on plant oils against the red flour beetle. Higher plants contain a wide spectrum of secondary metabolites such as phenols, flavonoids, quinones, tannins, essential oils, alkaloids, saponins and sterols. Such plant-derived chemicals are biodegradable and do not leave toxic residues or by-products. Essential oils have been screened for their medicinal and pesticide activities but detailed studies on their repellence have not been done. Therefore, there is an urgent need to discover the potential of different essential oils for control of post-harvest bio-deterioration of food commodities and thereby enhancing their shelf lives. Researchers have discovered many biological functions such as, attractants (pigments and scents), deterrents (repellents and antifeedents) or Insect growth regulators from plants (El-nahal, 1989;Deshmukh&Renapurkar, 1987; Schmidt &Risha,1989;Chander, 1990; Risha *et.al*, 1990; Su, 1991;Bhathal *et.al*, 1993;Rahman& Schmidt, 1999; Sharma, 1992,Raguraman& Singh, 1997; Khan *et.al*, 2000, Tariq &Qadri, 2001;Kim *et.al*, 2003; Kostyukovsky *et.al*, 2005; Jillani*et.al*, 2006; Jillani*et.al*, 1988;Jilani& Su,1983 ; Ullah*et al.* (1990); Ibrahim (1997) Liang *et al*. (2013); Liu *et al.*

(2012);Seo*et al*. (2008);Khattak*et al*. (2009); Kumar *et al*. (2007);Spurr& McGregor (2003) andNazli*et al*. (2003),

In this study, oils of five plants, namely,*Azadirachtaindica* (neem),*Valerianaofficinalis* (valerian), *Acoruscalamus* (sweet flag), *Curcuma longa* (turmeric)and *Saussurealappa* (costus) have been tested to evaluate their repellence against red flour beetle (*Triboliumcastaneum*).Thisstudy may be useful in utilization of indigenous plants for management ofred flour beetleand other insect pestsin stored wheat, flourand stored grains. Use of plant oils can be a better solution for safe, economical and environment friendly storage of the food grains. The findings of this study may be an addition for integrated pest management (IPM) models for the end-users.

2. Materials and Methods

2.1. Rearing/ Culturing technique of Test Insects

Insects were reared in laboratory at 30ºC ±2ºC temperature with a relative humidity of 60% ±5% on whole wheat grain as food medium contained in glass jars covered with muslin cloth. Ten days old adults of red flour beetle, Triboliumcastaneum (Herbst) (Coleoptra: Tenebrionidea) were used in the trials. To prepare insects of known age, sub-cultures of the insect from parental cultures were prepared by repeating raring/culturing procedure.

2.2. Test Materials

Five plants oils Azadirachtaindica (neem), Valerianaofficinalis (valerian), Acoruscalamus (sweet flag), Curcuma longa (turmeric) andSaussurealappa (costus) were tested to estimate their repellency. The plant oils were obtained by extracting plant powders with n-hexane on Soxhlet's extraction apparatus.

2.3. Treatment Method for Repellency of the Plant Oils

Repellence tests were conducted by following McDonald et.al (1970) and Jillani& Su (1983).For the purpose, filter paper strips (What man No. 1, measuring, 10×5cm) were treated separately with the plant oils with 0.100%, 0.050%, and 0.025%concentrations.The treated filter paper strips were attached lengthwise, edge to edge to untreated filter paper strips of similar size with cellophane tape, after the evaporation of the solvent. On the center of the joined paper, a glass ring (2.5cm high with 7.0 cm internal diameter) was placed. Ten, 10-day old, laboratory reared Triboliumcastaneum starved for 24 hours were released in the middle of the test ring. After one hour and six hours, the settled insects were counted for five consecutive days. The repellency was observed continuously up to second, fourth and eighth week. Fresh insects were used in all tests, using the same treated filter paper arena. Percentage repellency was calculated by deducting the percentage of insects on the treated half from the insects on the untreated half divided by total number of insects, multiplied by hundred. Evaluation of weekly repellency of all the plant oils was carried out by comparing the results.

3. Results

3.1. Repellency of Neem Oil

The maximum repellency, 49.20±1.50 % was observed at 0.025% concentration which remain same in second week but increased up to 51.21±1.03% in fourth week. The repellency was decreased to 44.11±2.03% up to eighth week. The oil at 0.100% concentration triggered 64.16±1.10% repellency which remain same during the second week after that declined a little bit to 58.87±2.06% in fourth week and finally to 45.72±2.21% in eighth week of the study (table 1). Two way ANOVA was applied to know the repellency of plant oils against red flour beetle. The results were found highly significant for time (LSD 0.05 = 2.76) and concentration (LSD 0.05 = 2.39). The interaction of time and concentration was also found highly significant ($p<0.001$, Appendix 1).

3.2. Repellency of Valerian Oil

The minimum repellency was observed as much as 39.90±1.19% in first week at 0.025% which remain up to 31.56±1.49% in second, 26.19±1.33% in fourth and 19.44±2.50% in eighth week of the trials. Maximum repellency was observed as much as 48.29±2.36% in first week, 37.41±1.89% in second, 31.36±2.05% in fourth and again 37.56±1.37% in fourth week of the study (table 2). Repellency of the plant was found highly significant ($p<0.001$) for the time period (LSD 0.05 = 2.10) whereas concentration was found non-significant ($p>0.05$, LSD 0.05 = 2.59). However the interaction of time period and concentration was highly significant ($p<0.001$, Appendix 1).

3.3. Repellency of Sweet Flag Oil

Repellency of sweet flag was 61.23±2.20% in first week which decline up to 52.08±2.39% in eighth week of the study at 0.025% concentration. At 0.100% concentration the repellency was 71.15±3.61% which decline up to 60.39±2.03% (table 3). Repellency was found significant ($p<0.05$) for time period (LSD 0.05 = 5.23), whereas highly significant ($p<0.001$) for concentration (LSD 0.05 = 4.55). However the interaction of time and concentration was found non-significant (Appendix 1).

3.4. Repellency of Turmeric Oil

Turmeric oil showed 38.64±3.77% repellency in first week which decreased up to 39.33±1.99% in fourth week at 0.025% concentration. Maximum repellency, 60.45± 1.45% was observed at 0.100% concentration in first week which remain 60.41±1.46% in second and 60.43±1.45% in fourth week. After which it declined up to 49.02± 2.08% in eighth week (table4). The oil was found highly significant ($p<0.001$) for time period (LSD 0.05 = 3.97) and concentration (LSD 0.05 = 3.44), but the interaction of time and concentration was nonsignificant ($p>0.05$, Appendix 1).

3.5. Repellency of Costus Oil

Costus oil showed highest 88.15±0.10% repellency at 0.100% concentration in first week which increased up to 88.79±0.96% in second week and up to 88.92±1.96% in fourth week. The repellency decreased to 83.56±2.51% only during eighth week of the study. At the lowest concentration (0.025%) the repellency was found 71.59±3.07% in first week, 71.81±2.24% in second, 68.99±1.02% in fourth and 67.16±1.17% in eighth week of the study (Table 5). Findings were highly significant ($p<0.001$) for time period (LSD 0.05 = 3.31) and concentration (LSD 0.05 = 2.87). The interaction among time and concentration was nonsignificant ($p>0.05$, Appendix 1).

4. DISCUSSION

Repellents are materials that offer some vapors which discourage the insects. Plants Oils, twigs hangings and their smoke omitted by burning is a common practice in villages for repellence against insect pests. Conventional pesticide pose detrimental effects on environment, other animals and human (Pitasawatet al., 2003). To avoid such hazards various plants are being explored against insects pests. Keeping the magnitude of insect pest problem in grain storage this study was designed to utilize some indigenous plants besides the well documented neem plant. For the purpose all the five plants (neem, valerian, sweet flag, turmeric and costus) were tested for their repellency against T. castaneum. Plant materials gave very promising results for repellency (both in filter paper and mixing of extract/powder in medium) (Tables 63 to 77). Most of the plant materials proved them as promising repellents (above 40% repellency is considered as promising).

All plant oils proved them as promising repellents (above 40% repellency is considered as promising). The results are depicted in tables 1-5 whereas the plant oils significance as repellant is shown in appendix 1. Among different concentrations, 0.100% was the most effective for all the plant oils generally.Neem oil showed 49.20±1.50% repellency at 0.025% concentration in first week of the study which remain same in second week then increased up to 51.21±1.03% in fourth week. After that the neem oil repellency was decreased to 44.11±2.03% in eighth week. Satti&Elamin (2012) reported that the efficacy of neem seed oil increased up to 92.5% on the third week of experience which is in agreement with the present trials on neem oil for its repellence against red flour beetle. The oil at 0.100% concentration triggered 64.16±1.10% repellency which remain same during the second week after that declined a little bit to 58.87±2.06% in fourth and finally to 45.72±2.21% in eighth week of the study. Repellent effect was inconstant according to the dose like this study. The time factor (weeks) as well as concentration proved highly significant for time whereas their interaction was also found highly significant. Valerian oil revealed 39.90±1.19% repellency in first week at 0.025% which remain up to 31.56±1.49% in second, 26.19±1.33% in fourth and 19.44±2.50% in eighth week of the trials. Maximum repellency was observed as much as 48.29±2.36% in first week, 37.41±1.89% in second, 31.36± 2.05% in fourth and again enhanced as 37.56±1.37% in eighth week of the study. Time period was found very significant whereas concentration was non-significant for the repellence activity of the plant; however the interaction of time period and concentration was highly significant. Sweet flag oil showed 61.23±2.20% repellence in first week which decline to 52.08±2.39% in eighth week of the study at 0.025% concentration. At 0.100% concentration the repellency was 71.15±3.61% which decline to 60.39±2.03%. For sweet flag time period and concentration were found significant. However their interaction found non-significant. Turmeric oil showed 38.64±3.77% repellency in first week which decreased to 39.33±1.99% in fourth week at 0.025% concentration. Maximum

repellency, 60.45±1.45% was observed at 0.100% concentration in first week which remain 60.41±1.46% in second and 60.43±1.45% in fourth week. After which it declined to 49.02± 2.08% in eighth week. Time period and concentration were found highly significant but their interaction was nonsignificant. Like these studies Jilani&Saxena, (1990) observed promising repellent activity of oils of turmeric, Curcuma longa (L.), sweet flag, Acoruscalamus (L.), neem, Azadirachtaindica and Margosan-O (neem product) against the lesser grain borer, Rhyzoperthadominica (F.) for eight weeks.

Costus oil proved the best by showing highest 88.15±0.10% repellency at 0.100% concentration in first week which increased up to 88.79±0.96% in second week and up to 88.92±1.96% in fourth week. The repellency decreased only to 83.56±2.51% during eighth week of the study. At the lowest concentration (0.025%) the repellency was found 71.59±3.07% in first week, 71.81±2.24% in second, 68.99±1.02% in fourth and 67.16±1.17% in eighth week of the study. Findings were highly significant for the repellence activity of the plant. Time period and concentration were significant for the repellence activities however their interaction was nonsignificant. Like the present studies Kanvilet al. (2006) assessed repellency of V. officianalis, P. harmala, S. lappaand A. indicaoils against T. castaneum and discovered that V. officianalis most effective by showing maximum (80.83%) repellency all through the first week at 1000 µg/cm2. Their findings are in disagreement with the results of this study in which S. lappashowed maximum 88.15±0.10% repellency at 0.100% concentration in first week which increased up to 88.79±0.96% in second week and up to 88.92±1.96% in fourth week; however they quoted 58.83% repellency imposed by A. indica oil which is in agreement with this study in which neem oil showed 51.21% repellency in first week on 0.050% concentration. Offered amount of 1,000 µg/cm2 was the most successful followed by 500 and 250 µg/cm2. Petroleum ether extract of V. officianalis comprised 49.25% and 42.25% at 1,000 and 500 µg/cm2 and S. lappa having 47.75% average repellency were capable repellents against T. castaneumadults over eight weeks' time, respectively, as against 56.63% in A. indica oil. In these studies costus, sweet flag and neem oils gave very promising results for repellency. This study maycontribute in to the use of non-hazardous, economic and environment friendly promising plant oils as repellent against the stored grain pests.

Table1. *Repellency of neem oil against Triboliumcastaneum in choice test arena*

Concentrations (%)	**Mean (%) Repellancy after treatment up to eight weeks**			
	1st Week	**2nd Week**	**4th Week**	**8th Week**
0.025	49.20±1.56	51.21±1.03	51.22±2.37	44.11±2.05
0.050	60.76±1.13	63.22±1.45	58.42±1.53	59.81±1.60
0.100	64.16±1.53	64.16±1.10	58.87±2.06	45.72±2.21
LSD*0.05		Time	2.76	
		Concentrations	2.39	

Table2. *Repellency of valerian oil to Triboliumcastaneum in choice test arena*

Concentrations (%)	**Mean (%) Repellancy after treatment up to eight weeks**			
	1st Week	**2nd Week**	**4th Week**	**8th Week**
0.025	39.90±1.19	31.56±1.49	26.19±1.33	19.44±2.50
0.050	46.59±2.49	39.50±1.34	32.63±1.88	17.95±1.21
0.100	48.29±2.36	37.41±1.89	31.36±2.05	37.56±1.37
LSD*0.05		Time	2.10	
		Concentrations	2.59	

Table3. *Repellency of sweet flag oil to Triboliumcastaneum in choice test arena*

Concentrations (%)	**Mean (%) Repellancy after treatment up to eight weeks**			
	1st Week	**2nd Week**	**4th Week**	**8th Week**
0.025	61.23±2.20	58.76±3.08	54.62±1.99	52.08±2.39
0.050	63.89±3.36	65.46±3.49	66.94±4.84	60.50±4.43
0.100	71.15±3.61	63.60±2.03	62.17±3.07	60.39±2.03
LSD*0.05		Time	5.23	
		Concentrations	4.55	

Table4. *Repellency of turmeric oil against Triboliumcastaneum in choice test arena*

Concentrations (%)	Mean (%) Repellancy after treatment up to eight weeks			
	1st Week	2nd Week	4th Week	8th Week
0.025	38.64±3.77	42.55±1.89	38.78±3.67	39.33±1.99
0.050	42.75±1.96	38.04±3.63	39.33±2.34	31.36±1.33
0.100	60.45±1.45	60.41±1.46	60.43±1.45	49.02±2.08
LSD*0.05		Time	3.97	
		Concentrations	3.44	

Table5. *Repellency of costus oil against Triboliumcastaneumin choice test arena*

Concentrations (%)	Mean (%) Repellancy after treatment upto eight weeks			
	1st Week	2nd Week	4th Week	8th Week
0.025	71.59±3.07	71.81±2.24	68.99±1.02	67.16±1.17
0.050	82.67±2.46	76.87±2.71	76.06±2.27	70.98±1.86
0.100	88.15±0.10	88.79±0.96	88.92±1.26	83.56±2.51
LSD*0.05		Time	3.31	
		Concentrations	2.87	

All values are mean of five replicates±Standard Error, *Fisher's Least Significant Difference

Percent repellency = (Nc - Nt) x 100 / NT

Where,

Nc =Number of insects found in untreated arena

Nt =Number of insects found in treated arena

NT =Total number of insects in untreated arena

Appendix1. *Two-way ANOVA for repellency of the plant oils against Triboliumcastaneum*

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Time	Neem	811.5544133	3	270.5181378	19.21990792	2.51629E-08
	Valerian	3312.245112	3	1104.081704	66.46762528	4.09797E-17
	Sweetflag	468.60726	3	156.20242	3.089144007	0.035755556
	Turmaric	547.8919383	3	182.6306461	6.253357536	0.001136982
	Costus	390.0303517	3	130.0101172	6.40598344	0.000972296
Concentration	Neem	1511.250893	2	755.6254467	53.68605456	5.71263E-13
	Valerian	51.50082333	2	25.75041167	1.550219252	0.222637671
	Sweetflag	768.87661	2	384.438305	7.602860993	0.001354055
	Turmaric	4714.266413	2	2357.133207	80.7093279	4.41999E-16
	Costus	3104.231543	2	1552.115772	76.47733994	1.18969E-15
Time * Concentration	Neem	555.3003867	6	92.55006444	6.575543255	4.07192E-05
	Valerian	1622.602443	6	270.4337406	16.28057821	4.07103E-10
	Sweetflag	234.04087	6	39.00681167	0.771419921	0.596172791
	Turmaric	332.8053867	6	55.46756444	1.899234983	0.100245151
	Costus	125.7534033	6	20.95890056	1.03270709	0.415966776
Error	Neem	675.59484	48	14.0748925		
	Valerian	797.31932	48	16.61081917		
	Sweetflag	2427.11772	48	50.5649525		
	Turmaric	1401.85028	48	29.20521417		
	Costus	974.16512	48	20.29510667		
Total	Neem	191087.0232	60			
	Valerian	75273.6085	60			
	Sweetflag	232556.4398	60			
	Turmaric	128976.9875	60			
	Costus	369280.1463	60			

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