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Repellency of Petroleum Ether Extract of Some Indigenous Plants Against *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract.- Petroleum ether extracts of *V. officianalis*, *P. harmala*, *S. lappa* and *A. indica* oil were studied for their repellent effect against *T. castaneum*. Out of these plants, *V. officianalis* showed highest (80.83%) repellency during the first week at 1000 μg/cm^2, which was significantly higher than 58.83% in *A. indica* oil (used as standard). Plant extracts showed highest repellency during the first week which significantly decreased in the second and fourth weeks and completely exhausted after the eighth week. Application rate of 1000 μg/cm^2 was the most effective followed by 500 and 250 μg/cm^2. Petroleum ether extract of *V. officianalis* having 49.25 and 42.25% at 1000 and 500 μg/cm^2 and *S. lappa* having 47.75% average repellency were promising repellents against *T. castaneum* adults over eight weeks period, respectively, as against 56.63% in *A. indica* oil.

Key words: *Saussurea lappa*, *Peganum harmala*, *Valeriana officianalis, Azadirachta indica*, *Tribolium castaneum*, repellency.

INTRODUCTION

Several species of storage insect pests occurred in granaries and other food storage structures in ancient times (Levinson and Levinson, 1985). Insect pests cause heavy food grain losses in storage, particularly at farm level which are 5-10% in developing countries. Storage losses are more significant at farm level where more than 60% grain is stored. Small farmers cannot afford to purchase expensive chemicals and their storage structures do not have proper air tightness required for fumigation.

In Pakistan grain storage losses by insects have been estimated to be 2.5% in six months storage (Ahmed, 1983). *Tribolium castaneum* is a major pest of stored products causing both qualitative and quantitative losses. Management of insect pests in stored grains mainly consists of the use of grain protectants and fumigants. Use of chemicals is responsible for undesirable effects on non-target organisms, and environmental and health concerns (White and Leesch, 1995). However, Aluminium phosphide is still used for protection of stored food, feedstuffs, and other agricultural commodities from insect infestation. EPA (2001) has proposed elimination of production of methyl bromide by 2005 because of its ozone depletion potential. Additionally, some stored product insects are found to have developed resistance to methyl bromide and phosphine (Champ and Dyte, 1977; Subramanytum and Hagstrum, 1995).

These problems have highlighted the need for development of new types of selective insect control alternatives. Plants may provide potential alternatives to currently used insect control agents because they constitute a rich source of bioactive chemicals (Wink, 1993). These are often active against a limited number of insect species including specific target insects, biodegradable to non-toxic products and potentially suitable for use in integrated pest management. They could lead to the development of new classes of safer insect-control agents. Jilani *et al.* (1984) evaluated powder and petroleum ether extracts of 30 local plants including *P. harmala, S. lappa*, *V. officianalis* and *A. indica* for repellent properties against *T. castaneum.* Five plants namely; *Acorus calamus*, *V. officianalis*, *Xanthoxylum armatum*, *Artemesia maritima* and seeds of *A. indica* were found to be promising and showed persistent repellency against the test insect. Jilani *et al.* (1989) tested repellent effect of ten plant extracts against *T. castaneum*. Out of these n-hexane extracts of *Neslia apiculata* leaves, *Limonium cabulicum* whole plant and *Achillea millefolium*

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flowers were promising. Results of first two plants showed higher repellency during the first week which decreased to lower levels in the second, fourth and eighth week while leaf extract of the third plant was persistent repellent even after 8 weeks showing 41% repellency. Jilani *et al.* (1993) reported that among the plant materials tested for grain protectant qualities as repellents, n-hexane extracts of *Agriophylum latifolium* whole plant, *Ferula oopoda* stem and leaves and *Stocksia brahuica* whole plant against red flour beetle were promising repellents providing promising repellency over 8 weeks. The remaining seven materials provided moderate to low level of repellency indicating low potential as repellents. Nazli *et al.* (2003) evaluated repellency of neem seed oil of Karachi, Hyderabad, Dokri, Shikarpur and Faisalabad against *T. castaneum*. All the samples proved promising repellent at 600 $\mu g/cm^2$. Highest average repellency was 52.25% after 8 weeks in Hyderabad sample followed by 50.13 and 44.75% in Shikarpur and Dokri samples, respectively.

MATERIALS AND METHODS

Collection and preparation of plant materials

Roots of 'Kuth' *Sassurea lappa,* seeds of 'Harmal' *Peganum harmala* and roots of 'Balchar' *Valeriana officianalis* were purchased from an ayervedic shop in Rawalpindi. The plant materials were ground to fine powder and preserved in glass jars to be used for solvent extraction. "Neem oil", *Azadirachta indica* extracted from seeds of neem with petroleum ether in the laboratory was used as standard for comparison.

Extraction of plant materials

Each plant powder was extracted separately with petroleum ether (60-80ºC) on Soxhlet's extraction apparatus for at least 8 hours. The extracts were concentrated on rotary evaporator by removing the excess solvent under vacuum. The concentrated extracts were finally made solvent free in a vacuum desiccator.

Insect culture

Culture of the red flour beetle, *Tribolium castaneum* was reared in the laboratory under controlled conditions of temperature, humidity and light. Rearing temperature was 29±1°C and relative humidity 65±5% with 12 hours light. Diet medium consisted of 50% wheat flour, 45% corn meal and 5% brewer's yeast.

Repellency study

Repellency was evaluated by the Filter Paper Strip Method used by Jilani *et al.* (1988). There were 3 plants each tested at 1000, 500 and 250 $\mu g/cm^2$. Each treatment was replicated four times. For preparation of different application rates of plant extracts, stock solution of each extract was made by mixing 426.4 mg of each plant extract in 8 ml acetone separately. Individual Whatman No. 1 filter paper strips measuring 10 × 4 cm were dipped in the stock solution to achieve 1000 $\mu g/cm^2$ deposit of the test plants; 3 ml of the solution was utilized (A single filter paper strip absorbed 0.75 ml of stock solution). To the remaining solution 5 ml of acetone was added and filter paper strips were dipped in this solution to achieve 500 $\mu g/cm^2$ deposit of the extract. Again 3 ml was utilized and 7 ml acetone was added to the remaining solution to get 250 $\mu g/cm^2$ deposit of the test materials. The strips treated with acetone alone served as control. Treated and untreated strips were allowed to dry for 3 days at room temperature. After the acetone had evaporated, each treated strip was attached lengthwise, edge-to-edge to a control strip with cellulose tape on the reverse side. A glass ring (2.5 cm high, 7 cm diameter) was placed over the two matched strips such that the joined edge bisected the ring. Fifteen days old ten adults of *T. castaneum* were released in the middle of the test arena within the glass ring at 0800 hours. Individuals that settled on treated and control halves were counted at 0900 and 1600 hours daily for five consecutive days in a week. Tests were repeated during the second, fourth and eighth weeks after paper treatment using fresh individuals each time. Average insect counts of each 5-day period were converted to percent repellency by deducting the percentage of individuals on treated half from those on the control half of the test arena. Weekly repellency and persistence up to 8 weeks of different treatments were compared. Data were statistically analysed using Analysis of Variance. Duncan's Multiple Range Test (Duncan,

1951) at 5% probability was applied if the number of means were more than six, otherwise LSD test was applied.

RESULTS

Effect of type of plant

Percent average repellency of test plant extracts in petroleum ether and neem oil (standard) is presented in Table I. Out of three plants, *V. officianalis* showed the highest repellency value of 41.71% which was non-significant from 44.17% in standard (neem oil) and 35.67 in *S. lappa* whereas *P. harmala* was not a promising repellent showing 18.96% average repellency.

Table I.- Percent average repellency of petroleum ether extract of plants to *Tribolium castaneum* adults provided with a choice of treated and control filter papers.

Plant	Average % repellency
V. officianalis	41.71 a
P. harmala	18.96 b
S. lappa	35.67 a
A. indica oil	44.17 a

Means followed by the same letter are not significantly different (P>0.05) using LSD Test; each value is average of 4 weeks, 3 application rates; 4 replications with 10 insects per replicate.

Effect of plant extract with passage of time

Persistence of repellent effect of plant extracts against *T. castaneum* is presented in Table II which revealed that plant extracts generally showed highest repellency (66.42%) in the first week which significantly decreased to 56.79% in the second week and 21.67% in the fourth week. After eight weeks, plant extracts completely lost their repellent action; rather slight attraction was indicated in some cases.

Effect of different doses

Effect of different doses of plant extracts that were tested for repellency against *T. castaneum* is given in Table III. Among different concentrations, 1000 μg/cm^2 was the most effective showing 45.03% average repellency which was significantly higher than 31.47 and 28.88% in 500 and 250 μg/cm^2, respectively.

Table II.- Percent average repellency of petroleum ether extract of plants upto eight weeks to *Tribolium castaneum* adults at given time intervals provided with a choice of treated and control filter papers

Weeks	Average % repellency
1st	66.42 ^{a}
2nd	56.79 ^{b}
4th	21.67 ^{c}
8th	-4.375 ^{d}

Means followed by the same letter are not significantly different (P>0.05) using LSD Test; each value is mean of 4 plants; 3 application rates; 4 replications with 10 insects per replicate.

Table III.- Percent average repellency of various application rates of plants extracts against *Tribolium castaneum* adults provided with a choice of treated and control filter papers

Rate of application (μg/cm^2)	Average % repellency
1000	45.03 a
500	31.47 b
250	28.88 b

Means followed by the same letter are not significantly different (P>0.05) using LSD Test; each value is mean of 4 plants; 4 weeks; 4 replications with 10 insects per replicate.

Comparison of effect of different plant extracts

Percent repellency during different weeks by petroleum ether extracts of different plants and neem oil against *T. castaneum* is presented in Table IV. The highest repellency of 80.83% was shown by *V. officianalis* during the first week, which was significantly higher than 58.83% in *A. indica* oil (standard) and 46.83% in *P. harmala*. *S. lappa* was next in order showing 79.17% repellency which was significantly higher than that of *P. harmala* but not from *A. indica* oil. After the second week, although repellency decreased but it was non-significant among the plant extracts except *V. officianalis* which maintained its repellency at 65.00% followed by 60.50% in *S. lappa* which was non-significant from 60.83% in *A. indica* oil. In case of *P. harmala*, repellency was 40.83%, which was significantly lower than 65.00% in *V. officianalis*. After the fourth week, the same trend was observed in plant extracts. Repellency generally decreased and it was non-significant among all the test plants except *P.*

harmala which had lost its repellent value to 3.17%. After eight weeks, all the plant extracts completely lost their repellent action except the standard *A. indica* oil which still had significantly higher repellency of 20.50%.

Table V shows average repellency of plant extracts at different doses. It indicated that *V. officianalis* at 1000 and 500 µg/cm^2 and *S. lappa* at 1000 µg/cm^2 were the promising repellents. *P. harmala* did not show promising repellency at any application rates. However, *A. indica* oil showed promising repellency at 1000 and 500 µg/cm^2. Repellency values of promising plant extracts were not significantly different from one another.

Table IV.- Interaction effect of plant extracts and weeks on percent average repellency against *Tribolium castaneum* adults provided with a choice of treated and control filter papers

Plant	Avg.% repellency in week			
	1st	2nd	4th	8th
V. officianalis	80.83 ^{a}	65.00 ^{abc}	32.50 ^{ef}	-11.50 ^{g}
P. harmala	46.83 ^{cde}	40.83 ^{def}	-3.167 ^{g}	-8.667 ^{g}
S. lappa	79.17 ^{ab}	60.50 $^{a-d}$	20.83 ^{f}	-17.83 ^{g}
A. indica oil	58.83 ^{bcd}	60.83 $^{a-d}$	36.50 ^{ef}	20.50 ^{f}

Means within column and rows followed by the same letter are not significantly different (P>0.05) using DMR Test; each value is mean of 4 application rates and 4 replications with 10 insects per replicate.

Table V.- Interaction effect of plant extracts and application rates on percent average repellency against *Tribolium castaneum* adults provided with a choice of treated and control filter papers

Plant	Rate of application (µg/cm^2)		
	1000	500	250
V. officianalis	49.25 ^{ab}	42.25 $^{a-d}$	33.63 $^{b-e}$
P. harmala	26.50 ^{def}	12.50 ^{f}	17.88 ^{ef}
S. lappa	47.75 ^{abc}	29.50 $^{c-f}$	29.75 $^{c-f}$
A. indica oil	56.63 ^{a}	41.63 $^{a-d}$	34.25 $^{b-e}$

Means within column and rows followed by the same letter are not significantly different (P>0.05) using DMR Test; each value is mean of 4 weeks and 4 replications with 10 insects per replicate.

Effect of different doses of plant extracts *viz.* 1000, 500 and 250 µg/cm^2 administered for different periods of time against *T. castaneum* is presented in Table VI. The highest repellency of 74.25% was obtained at 1000 µg/cm^2 in the first week which decreased to 62.88% in the second week. In the fourth week, significant decrease of repellency to 39.50% was recorded while after eight weeks, almost no repellent action was observed. At 500 µg/cm^2, 56.88 and 58.88% repellency was recorded in the first and the second weeks respectively but in the fourth week the repellency significantly decreased to 18.13% which further decreased to -8.00% in the eighth week. At 250 µg/cm^2, 68.13% repellency was recorded in the first week which significantly decreased to 48.63% in the second week and finally showing no repellent effect in the fourth and the eighth week. At higher application rates persistence of repellency was better than at lower application rates.

Table VI.- Interaction effect of weeks and application rates on percent average repellency against *Tribolium castaneum* adults provided with a choice of treated and control filter papers

Plant	Rate of application (µg/cm^2)		
	1000	500	250
1st	74.25 ^{a}	56.88 ^{ab}	68.13 ^{a}
2nd	62.88 ^{ab}	58.88 ^{ab}	48.63 ^{bc}
4th	39.50 ^{c}	18.13 ^{d}	7.38 ^{de}
8th	3.50 ^{de}	-8.00 ^{e}	-8.63 ^{e}

Means within column and rows followed by the same letter are not significantly different (P>0.05) using DMR Test; each value is mean of 4 plants and 4 replications with 10 insects per replicate.

Comparison of repellency shown by petroleum ether extract of different plants and neem oil against *T. castaneum* during the first, second, fourth and eighth week after treatment is given in Table VII. Generally, the plants extract showed higher repellency during the first week which gradually decreased during the second and the fourth week with majority of the extracts having no repellency during the eighth week. On the basis of eight weeks average repellency, *V. officianalis* and *A. indica* oil at 1000 and 500 µg/cm^2 and *S. lappa* at 1000 µg/cm^2 were promising repellents as these extracts showed more than 40% repellency against *T. castaneum*. Repellency values of both the above plants, although higher but were statistically non-significant from those of the standard. During first

Table VII.- Repellency of petroleum ether extract of plants to *Tribolium castaneum* adults at given time intervals in a test arena provided with a choice of treated and control filter papers.

Plant	Rate of application ($\mu g/cm^2$)	Average % repellency in week				
		1st Week	2nd Week	4th Week	8th Week	Average
V. officianalis	1000	81.00 a	78.00 a	53.50 a	-15.50 c	49.25 ab
	500	80.50 a	56.00 ab	48.00 a	-15.50 c	42.25 a-d
	250	81.00 a	61.00 a	-4.00 cd	-3.50 bc	33.63 b-e
P. harmala	1000	62.00 ab	47.00 ab	4.00 cd	-7.00 bc	26.50 def
	500	27.00 bc	43.50 ab	-12.50 d	-8.00 bc	12.50 fg
	250	51.50 ab	32.00 ab	-1.00 d	-11.00 c	17.88 ef
S. lappa	1000	84.00 a	60.50 a	49.00 a	-2.500 bc	47.75 abc
	500	67.00 ab	68.00 a	13.00 bcd	-30.00 c	29.50 c-f
	250	86.50 a	53.00 ab	0.500 cd	-21.00 c	29.75 c-f
A. indica oil	1000	70.00 ab	66.00 a	51.50 a	39.00 a	56.63 a
	500	53.00 ab	68.00 a	24.00 abc	21.50 ab	41.63 a-d
	250	53.50 ab	48.50 ab	34.00 ab	1.00 bc	34.25 b-e
Control	0	9.00 c	14.00 b	-4.500 cd	-18.00 c	0.1250 g

Means within column followed by the same letter are not significantly different (P>0.05) using DMR Test; each value is mean of 4 replications with 10 insects per replicate.

week, *P. harmala* was the only plant extract which was not promising repellent at 500 $\mu g/cm^2$. All other extracts at all application rates were promising repellent. After the second week, repellency generally decreased but it was non-significant in case of *S. lappa*, *V. officianalis* and *A. indica* at all the application rates. However, in case of *P. harmala* repellency was not maintained at par with other plants and the standard even at 1000 $\mu g/cm^2$. After the fourth week, only *V. officianalis* showing 53.50 and 48.00% at 1000 and 500 $\mu g/cm^2$ and *S. lappa* having 49.00% at 1000 $\mu g/cm^2$ could maintain repellency at par with *A. indica* oil having 51.50% repellency at 1000 $\mu g/cm^2$. After four weeks all the plant extracts completely lost repellent action except the standard, still showing 39.00% repellency. Control having acetone treated papers did not show repellent or attractant action.

DISCUSSION

Results of present studies have shown that the test plants *V. officianalis* and *S. lappa* have potential to be used as grain protectants. Their extracts in petroleum ether have strong repellent action against *T. castaneum*, a major pest of stored products. These plants have a range of chemicals which can be isolated and used for pest control. Out of the tested materials, petroleum ether extract of *V. officianalis* at 1000 and 500 $\mu g/cm^2$ and *S. lappa* at 1000 $\mu g/cm^2$ were promising repellents against *T. castaneum*. These results are in conformity with those of Jilani *et al.* (1984) who also rated petroleum ether extract of these plants as promising repellents. Similarly, Harish *et al.* (2000) showed that acetone extract of *S. lappa* was not a promising repellent against *T. castaneum*. This confirms our findings in the sense that petroleum ether extracts compounds, having lower polarity, are highly volatile and good repellents whereas acetone extracts compounds, having comparatively higher polarity, are not better than petroleum ether as repellents.

Petroleum ether extracts, majority of the low polarity compounds, remain effective as repellent upto 4 weeks especially at higher application rates of 1000 and 500 $\mu g/cm^2$ in case of *V. officianalis*. This might be due to the presence of biologically active diterpenes actinidine ($C_{10}H_{13}N$; MW147) and Chatinine ($C_{10}H_{22}N_2O_2$; MW202). Actinidine having lower molecular weight of 147 might have shown higher repellency due to faster volatilization rate during the first and second week supplemented by

Chatinine during the second and the fourth week due to slightly higher molecular weight of 202. However, *P. harmala* was not a promising repellent. Although, it had shown repellency but for limited period of 2 weeks only at the highest application rate of 1000 μg/cm^2. The reason for this could be the presence of biologically active alkaloids of low molecular weight such as Deoxy-4-oxo-peganine ($C_{11}H_{10}N_2O$; MW 186) and traces of Harmaline ($C_{13}H_{12}N_2O$) of comparatively higher molecular weight of 214. These compounds might not be good repellents because of having comparatively lower volatilization rate. Similarly, in case of *S. lappa*, the extract was effective for two weeks even at higher application rates. Petroleum ether might have extracted some traces of triterpene Costunolide ($C_{15}H_2O_2$: MW 232). This compound having comparatively higher molecular weight might have persisted upto 4 weeks. However, the extract was promising repellent only at 1000 μg/cm^2.

This study will lead to the isolation of promising repellent fractions/compounds from the promising repellent plants for future use. Such chemicals are expected to be environment friendly because all the three test plants have been traditionally used for medicinal purpose since centuries. Therefore, their use will be non-hazardous and non-toxic for human and animal health.

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