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Repellency of Acetone Extract of Some Indigenous Plants Against *Tribolium Castaneum* (Herbst) (Coleoptera: Tenebrionidae)-II

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Abstract.- Repellency of acetone extracts of three plants *viz. Saussurea lappa*, *Peganum harmala* and *Valeriana officianalis* was tested against *Tribolium castaneum* in a free choice Filter Paper Strip Method for 8 weeks. *Azadirachta indica* oil was used as standard in the study. All the three plant extracts showed < 25 % average repellency except the standard *A. indica* oil showing 44.21 %. None of application rates of 1000, 500 and 250 µg/cm^2 of the test plant extracts showed promising repellency. After one week of treatment, acetone extract of *P. harmala* showed 88.00, 84.00 and 74.50 % repellency at 1000, 500 and 250 µg/cm^2, respectively. However, repellency decreased to -30.00 % at 500 µg/cm^2 after four weeks while it was -26.50 % at 250 µg/cm^2 indicating no repellent effect. Generally, the acetone extracts of the test plants were not promising repellents.

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

INTRODUCTION

In Pakistan, grain storage losses by insects have been estimated to be 2.5 % in six months storage (Ahmed, 1983). The red flour beetle, *Tribolium castaneum,* has been observed as a common pest of stored wheat and other cereals all over the world. Since, both quality and quantity are seriously damaged by it (Hussain *et al*., 1996). Plants may provide potential alternatives to currently used insect control agents because they constitute a rich source of bioactive chemicals (Wink, 1993). In fact, some of the oldest known insecticides originated from plants including rotenone from derris roots, nicotine from tobacco leaves, ryania from the ground stem wood of the ryania shrub and pyrethrins from chrysanthemum flowers (Ware, 1980).

Many plant extracts are known to possess repellent activities against storage insect pests (Jilani and Malik, 1973; Jilani *et al*., 1984; Saim and Meloan, 1990; Mohyuddin *et al*., 1993; Abubakar *et al*., 2000; Dwivedi and Kumari, 2000; Ulubelen *et al*., 2001; Padin *et al*., 2002; Nazli *et al*., 2003). Sundararajan and Kumuthakalavalli (2000) described biologically active components of *S. lappa* (Kuth), *P. harmala* (Harmal) and *V. officianalis* (Balchar) which may be successful for repellent and growth inhibiting effect against *T. castaneum.* Little work has been done to manage stored-product insects by using aromatic medicinal plants despite their excellent pharmacological actions (Tang and Eisenbrand, 1992; Namba, 1993). Therefore, in the present studies, medicinal plants were selected because they had a number of biologically active compounds and evaluated for their repellent effects against *T. castaneum.*

MATERIALS AND METHODS

Collection and preparation of plant materials

Roots of "Kuth" (*Saussurea 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 acetone on Soxhlet's extraction apparatus for at

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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). Acetone extracts of the test plants and neem oil were evaluated for their repellent effect against *T. castaneum* at 1000, 500 and 250 $\mu g/cm^2$. Each treatment was replicated four times. For preparation of different application rates of plant extract, 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 plants

Overall repellency of acetone extracts of plants against *T. castaneum* is shown in Table I. It revealed that all the test plant materials showed < 25 % average repellency in 8 weeks except standard *A. indica* oil showing 44.21 percent.

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

Plant	Average % repellency
V. officianalis	15.56 ^{b}
P. harmala	14.46 ^{b}
S. lappa	22.88 ^{b}
A. indica oil	44.21 ^{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 weeks

Table II revealed that higher repellency of 48.50 and 44.96 percent was showed in the first and the second week, respectively, which decreased significantly to 16.69 percent in the fourth week while in the eighth week, no repellent action was observed.

Effect of application rates

Effect of different application rates at which acetone extract of plants were tested against *T. castaneum* is presented in Table III. None of the application rates of 1000, 500 and 250 $\mu g/cm^2$

showed promising average repellency. However, it was 29.92 and 28.00 percent at 1000 and 500 µg/cm^2 respectively, which was significantly higher than 14.91 percent at 250 µg/cm^2. In the study, repellency was not dose dependent.

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

Weeks	Average % repellency
1st	48.50 ^{a}
2nd	44.96 ^{a}
4th	16.69 ^{b}
8th	-13.04 ^{c}

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	Average % repellency
1000	29.92 ^{a}
500	28.00 ^{a}
250	14.91 ^{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.

Interaction effect of plants and weeks

There was no general trend of gradual decrease in repellency with time (Table IV). The highest repellency of 82.17 percent was shown by *P. harmala* in the first week which abruptly decreased to -0.16 in the second week. The negative values in the fourth and the eighth week showed that test plant exhausted and had lost its repellent action. *V. officianalis* was next in order showing 54.83 percent repellency in the first week which decreased to 38.83 percent in the second week while in the fourth and the eighth week, plant extract did not show promising repellency. *S. lappa* showed very low repellency in the first week while in the second and the fourth week it showed 68.50 and 43.00 percent repellency. In the eighth week, *S. lappa* had also lost its repellent action. The standard *A. indica* oil showed a persistent promising average repellency upto four weeks but decreased to 11.17 percent in the eighth week. The above situation revealed that acetone extract of *P. harmala* was not promising repellent. However, it had shown repellency only for a limited period of one week after which the extract was rather slightly attractant. This type of insect behavior to *P. harmala* extracts might be due to the presence of some biologically active alkaloids having lower molecular weight and being more volatile were highly effective repellent only for one week and could not persist beyond that.

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	54.83 ^{bc}	38.83 ^{c}	2.92 ^{de}	-34.33 ^{g}
P. harmala	82.17 ^{a}	-0.16 ^{def}	-20.67 ^{efg}	-4.17 ^{def}
S. lappa	4.83 ^{de}	68.50 ^{ab}	43.00 ^{c}	-24.83 ^{fg}
***A. indica* oil**	52.17 ^{bc}	72.67 ^{ab}	40.83 ^{c}	11.17 ^{d}

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.

Interaction effect of plants and application rates

A comparison of 1000, 500 and 250 µg/cm^2 application rates of acetone extracts of each plant is presented in Figure 1. Infact, plant and application rates interaction was non-significant, however, all the test plants showed < 35 % repellency at all the application rates except the standard neem oil showing 51.00 and 48.63 percent repellency at 1000 and 500 µg/cm^2 whereas at 250 µg/cm^2, it showed 33.00 percent repellency. Moreover, percent average repellency in case of all the test plants was not proportional to application rates.

Interaction effect of weeks and application rates

Table V revealed the persistency of various application rates of acetone extracts of plants upto 8 weeks. At 1000 µg/cm^2, higher repellency of 51.63%

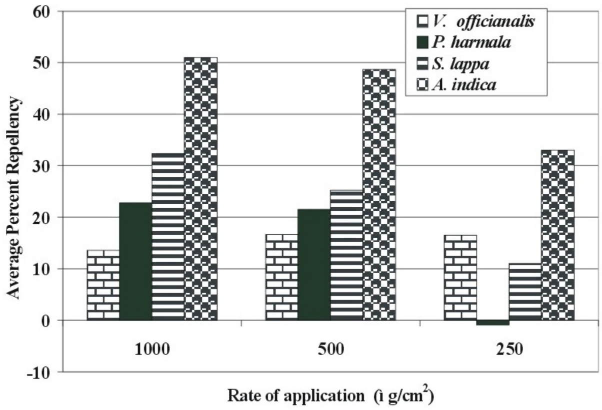

Fig. 1. Interaction effect of plants and application rates on percent average repellency against *Tribolium castaneum* in a test arena provided with a choice of treated and control filter paper.

Table V.- 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	38.38 ab	54.38 a	52.75 a
2nd	51.63 a	49.63 a	33.63 ab
4th	36.00 ab	18.63 b	-5.00 c
8th	-6.75 c	-10.63 c	-21.75 c

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.

was observed in the second week which decreased to 36.00 and -6.75 percent in the fourth and the eighth week, respectively. The repellency was 38.38 percent in the first week which was quite low. This can be due to the volatilization of those active components in the first week which have low molecular weight but have no or less repellent action. Volatilization of those active components having comparatively heavier molecular weight with repellent action which persisted for 8 weeks. At 500 µg/cm^2, 54.38 percent repellency was recorded in the first week which decreased non-significantly to 49.63 percent in the second. Subsequently it decreased significantly to 18.63 percent in the fourth week, respectively. After eight weeks, plants had lost repellent action. At 250 µg/cm^2, plant extract showed 52.75 percent repellency in the first week which decreased to 33.63 percent in the second week while in the fourth and the eighth week, plant had no repellent action. Repellent action was not dependent on application rates.

Average repellency

Percent repellency shown by acetone extract of different plants and neem oil against *T. castaneum* during first, second, fourth and eighth weeks after treatment is given in Table 6. It revealed that no plant extract was promising repellent as they had < 40 % average repellency after 8 weeks. In case of *S. lappa* repellency was very low after first week which drastically increased after the second week; remained high after the fourth week and again decreased abruptly after the eighth week. During the first week, highest repellency of 88.00 and 84.00 percent was recorded in *P. harmala* at 1000 and 500 µg/cm^2, respectively. It was followed by 83.00 percent in *V. officianalis* and 74.50 percent in *P. harmala* both at 250 µg/cm^2. All the above repellency values were statistically non-significant from those of neem oil having 49.50, 60.00 and 47.00 percent at 1000, 500 and 250 µg/cm^2, respectively. During second week, *S. lappa* extract had the highest repellency values of 74.50, 68.00 and 63.00 at 1000, 500 and 250 µg/cm^2 which were statistically non-significant from neem oil exhibiting 74.50, 66.50 and 77.00 percent at respective application rates and 52.50 percent in *V. officianalis* at 1000 µg/cm^2. *P. harmala* at all application rates and *V. officianalis* at 500 and 250 µg/cm^2 had significantly lower repellency than other plant extracts and the standard. During fourth week an abrupt decrease in repellency was observed in all the plant extracts except *S. lappa* and neem oil having 77.00 and 69.00 percent respectively both at 1000 µg/cm^2 which were statistically similar. During the eighth week, repellency further deteriorated to very low level. However, neem oil still had the highest repellency of 26.00 percent at 500 µg/cm^2. Control papers did not show any significant repellency.

Table VI.- Repellency of acetone extract of plants against *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 (µg/cm^2)	Average % repellency in week				
		1st Week	2nd Week	4th Week	8th Week	Average
V. officianalis	1000	24.50 $^{b-e}$	52.50 ^{ab}	12.25 ^{bc}	-35.00 ^{cd}	12.25 ^{bcd}
	500	57.00 $^{a-d}$	35.00 ^{abc}	14.50 ^{bc}	-40.00 ^{d}	16.38 ^{bcd}
	250	83.00 ^{ab}	29.00 ^{bc}	-18.00 ^{cd}	-28.00 ^{bcd}	15.00 ^{bcd}
P. harmala	1000	88.00 ^{a}	5.00 ^{c}	-13.00 ^{cd}	11.00 ^{ab}	22.00 ^{bc}
	500	84.00 ^{a}	29.00 ^{bc}	-30.00 ^{d}	3.000 ^{abc}	21.75 ^{bc}
	250	74.50 ^{abc}	-34.50 ^{d}	-17.00 ^{cd}	-26.50 ^{bcd}	23.00 ^{bc}
S. lappa	1000	-8.50 ^{e}	74.50 ^{a}	77.50 ^{a}	-14.00 ^{bcd}	33.63 ^{bc}
	500	16.50 ^{cde}	68.00 ^{ab}	48.00 ^{ab}	-31.50 ^{cd}	26.13 ^{bc}
	250	6.50 ^{de}	63.00 ^{ab}	3.50 ^{cd}	-29.00 ^{bcd}	10.25 ^{cd}
***A. indica* oil**	1000	49.50 $^{a-e}$	74.50 ^{a}	69.00 ^{a}	11.00 ^{ab}	51.00 ^{a}
	500	60.00 $^{a-d}$	66.50 ^{ab}	42.00 ^{ab}	26.00 ^{a}	48.50 ^{a}
	250	47.00 $^{a-e}$	77.00 ^{a}	11.50 ^{bc}	-3.500 $^{a-d}$	34.25 ^{ab}
Control	0	4.00 ^{de}	6.500 ^{c}	13.50 ^{bc}	-26.50 ^{bcd}	-2.50 ^{d}

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.

DISCUSSION

The result of preliminary investigations suggest that all the test plants have potential for repellent action but the acetone extracts of these plants were not promising repellents at any application rate. This means that test plants contained those active components which were not completely extractable with acetone solvent. So, other organic solvents either with low polarity like petroleum ether or with high polarity like ethanol should be used for extraction. The above finding was in conformity with Dwivedi and Kumari (2000) who also reported that acetone extract of *T. procumbens*, *R. communis*, *A. nilotica*, *L. inermis* and *D. sissoo* were less potent than petroleum ether extracts whereas *P. hysterophorus* extracts in both (acetone or petroleum ether) solvents exhibited the highest repellent action against the storage insect (*C. chinensis*). On the basis of eight weeks average repellency, none of the plant extracts in acetone was a promising repellent except the standard neem oil which showed 51.00 and 48.50 percent average repellency. *P. harmala* showed very high level of 88.00, 84.00 and 74.50 percent repellency at 1000, 500 and 250 µg/cm^2 only after one week of treatment which decreased with passage of time. However, repellency decreased to -30.00 percent at 500 µg/cm^2 and after eight weeks it was -26.50 at 250 µg/cm^2 indicating some attractant action rather than being repellent. The above findings are also reported by Jilani and Saxena (1990) who tested turmeric oil or sweetflag oil in a free choice test, Filter Paper Strips against *T. castaneum*. Both plants repelled insects at 800, 400 and 200 µg/cm^2 during first 2 weeks; thereafter, repellency decreased more rapidly than with neem oil or Margosan-O. Similarly, Jilani *et al*. (1989) also reported that out of ten plant extracts tested against *T. castaneum*, n-hexane extracts of *Neslia apiculata* leaves, *Limonium cabulicum* whole plant and *Achillea millefolium* flowers were promising repellent, results of first two plants showed higher repellency during first week which decreased to lower levels in second, fourth and eighth week. This also reconfirms our finding for the test plants.

The above situation revealed that acetone mainly extracts medium polarity compounds and traces of low polarity compounds which are not persistent repellents. Therefore, repellent effect of

V. officianalis faded away very fast within one week. However, in case of *S. lappa*, its acetone extract did not perform well by showing very low repellency. In the first week no repellency was observed, however, it boosted up in the second and fourth weeks. This might be due to the volatilization of compounds of lower molecular weight in the first week which was not repellent. Later on, volatilization of active components of medium and traces of higher polarity compounds proved highly repellent and persisted upto four weeks. *P. harmala* had shown repellent effect upto two weeks at higher application rates of 1000 and 500 µg/cm^2. This might be due to the repellent compounds of low polarity such as dl-peganine/L-peganine ($C_{11}H_{12}N_2O$; MW 188), 1-deoxy-4-oxo-peganine ($C_{11}H_{10}N_2O$; MW 186) as well as medium polarity such as Harmine ($C_{13}H_{12}N_2O$; MW 212), Harmaline ($C_{13}H_{12}N_2O$; MW 214), Tetrahydro-harmine ($C_{13}H_{16}N_2O$; MW 216). Almost all the active components of *P. harmala* have lower molecular weight than those of *S. lappa* (Costunolide, $C_{15}H_{20}O_2$; MW 232) and *V. officianalis* (Actinidine, $C_{18}H_{22}NO$; MW 268) as reported by Raffauf (1970).

Therefore, the extracts of *P. harmala* being more volatile were effective repellents only for one or two weeks and could not persist up to four or eight weeks. This property of the active components resulted into lower level of average repellency. The above results are in conformity with those of Jilani *et al.* (1984) where petroleum ether extracts of 30 local plants, including *V. officianalis*, *S. lappa* and *P. harmala* were studied for their repellent effect against *T. castaneum.* Fumigants such as methyl bromide and phosphine are still the most effective for protection of stored food, feedstuffs, and other agricultural commodities from insect infestation. Repeated use of chemicals, however, has resulted in the development of resistance (Szlendak *et al*., 2000), has had undesirable effects on non-target organisms, and has fostered environmental and human concerns (Hays and Laws, 1991). Therefore, alternate strategies, especially those utilizing renewable resources and/or traditionally experiences are needed. This has been recommended by UNCED Agenda 21 and endorsed by the 22nd FAO Regional Conference for Asia and the Pacific (Manilla, 3-7 October 1994). Pakistan has been a wheat exporting country, for the last two years looking for the international markets. For international trade very strict standards are to be maintained. In this respect upcoming challenges of WTO and Agreement on application of sanitary and phytosanitary measures are to be maintained. These measures have triggered the revision of International Plant Protection Convention of which Pakistan is a signatory. To meet the objective of 'discipline in trade', effective and environment friendly pest control strategies are needed for which present investigation were undertaken. To achieve the goal of having pest and pesticide free grains, these results are expected to be useful. However, further research is needed on the isolation of active fractions/ compounds for preparing formulations to be used by the farmers and traders. Effective application methodology of the grain treatment / bag treatment need to be developed.

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