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

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Abstract: Laboratory studies were carried out to evaluate the repellency of essential oils of Azadirachta indica (neem), Valeriana officinalis (valerian), Acorus calamus (sweet flag), Curcuma longa (turmeric) and Saussurea lappa (costus) against red flour beetle, *Tribolium castaneum*. Saussurea lappa (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%). Acorus calamus (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. Azadirachta indica (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%). Valeriana officinalis (valerian) could not prove it a promising repellent.

Keywords: *Tribolium castaneum*, Azadirachta indica, Valeriana officinalis, Acorus calamus, Curcuma longa, Saussurea lappa, 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 losses of 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 (*Tribolium castaneum*) 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.
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(2012); Seo et al. (2008); Khattak et al. (2009); Kumar et al. (2007); Spurr & McGregor (2003) and Nazli et al. (2003).

In this study, oils of five plants, namely, *Azadirachta indica* (neem), *Valeriana officinalis* (valerian), *Acorus calamus* (sweet flag), *Curcuma longa* (turmeric) and *Saussurea lappa* (costus) have been tested to evaluate their repellence against red flour beetle (*Tribolium castaneum*). This study may be useful in utilization of indigenous plants for management of red flour beetle and other insect pests in stored wheat, flour and 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 30°C ±2°C 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, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) 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 *Azadirachta indica* (neem), *Valeriana officinalis* (valerian), *Acorus calamus* (sweet flag), *Curcuma longa* (turmeric) and *Saussurea lappa* (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 (Whatman 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 Tribolium castaneum 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 oil 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 (table 4). 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 (Pitasawat et 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 the repellence activity of the plant; however the interaction of time period and concentration was 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 repellancy 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 remained 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, Acorus calamus (L.), neem, Azadirachta indica 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 Kanvile et al. (2006) assessed repellency of V. officinalis, P. harmala, S. lappa and A. indica oils against T. castaneum and discovered that V. officinalis 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. lappa showed 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. officinalis 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 may contribute in to the use of non-hazardous, economic and environment friendly promising plant oils as repellent against the stored grain pests.

Table 1. Repellency of neem oil against Tribolium castaneum 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 |

Table 2. Repellency of valerian oil to Tribolium castaneum 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 |

Table 3. Repellency of sweet flag oil to Tribolium castaneum 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 |
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### Table 4. Repellency of turmeric oil against *Tribolium castaneum* 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 | Concentrations |
|------|----------------|
| 3.97 | 3.44 |

### Table 5. Repellency of costus oil against *Tribolium castaneum* in choice test arena

| Concentrations (%) | Mean (%) | Repellancy after treatment up to 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 | Concentrations |
|------|----------------|
| 3.31 | 2.87 |

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

Percent repellency = \( \frac{(N_c - N_t) \times 100}{N_T} \)

Where,

\( N_c \) = Number of insects found in untreated arena

\( N_t \) = Number of insects found in treated arena

\( N_T \) = Total number of insects in untreated arena

### Appendix 1. Two-way ANOVA for repellency of the plant oils against *Tribolium castaneum*

| 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 | 0.03575556 |
|        | Turmaric          | 547.891938              | 3  | 182.630461  | 6.253357536 | 0.001136982 |
|        | Costus            | 390.030317              | 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 | 76.4773994  | 1.18969E-15 |
| Time * Concentration | Neem              | 1511.250893             | 6  | 255.5006444 | 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          | 332.805386              | 6  | 55.46756444 | 1.899234983 | 0.100245151 |
|          | Costus            | 125.7534033             | 6  | 20.95890056 | 1.03270709 | 0.415966776 |
| Error   | Neem              | 675.59484               | 48  | 14.0748925 | 56.53006444 | 4.07192E-05 |
|          | Valerian          | 797.31932               | 48  | 16.61081917 | 16.28057821 | 4.07103E-10 |
|          | Sweetflag         | 2427.11772              | 48  | 50.5649525  | 0.771419921 | 0.596172791 |
|          | Turmaric          | 1401.85028              | 48  | 29.20521417 | 1.899234983 | 0.100245151 |
|          | Costus            | 974.16512               | 48  | 20.29510667 | 1.03270709 | 0.415966776 |
| Total   | Neem              | 191087.0232             | 60  | 32.35006444 | 16.28057821 | 4.07192E-10 |
|          | Valerian          | 75273.6085              | 60  | 12.50006444 | 16.28057821 | 4.07103E-10 |
|          | Sweetflag         | 232556.4398             | 60  | 12.50006444 | 16.28057821 | 4.07103E-10 |
|          | Turmaric          | 128976.9875             | 60  | 12.50006444 | 16.28057821 | 4.07103E-10 |
|          | Costus            | 369280.1463             | 60  | 12.50006444 | 16.28057821 | 4.07103E-10 |
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