

EFFECTIVENESS OF FOUR MEDICINAL PLANT ESSENTIAL OILS AS FEEDING DETERRENT TOWARDS DIFFERENT STRAINS OF STORED GRAIN INSECT PESTS

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Stored products can be protected by applying essential oils as antifeeding agent. Essential oils extracted from *Datura stramonium*, *Eucalyptus camaldulensis*, *Moringa oleifera* and *Nigella sativa* were examined for feeding deterrent action against different strains of *Tribolium castaneum*, *Trogoderma granarium* and *Cryptolestes ferrugineus*. Test insects were allowed to feed on treated food with concentrations of 5, 10, 15 and 20% by keeping constant temperature of 30±2°C and 65±5% relative humidity. Significant reduction in weight loss of treated food as compared with untreated was observed due to reduced feeding of insects. *D. stramonium* was the most active antifeedant with higher feeding deterrence index (FDI, 37.37%). Feeding deterrence index was found to increase from 22.01 to 43.70% with the increase in concentrations of essential oils from 5 to 20%. Among strains, FDI (%) of essential oils was recorded highest against SWL (Sahiwal) strain of *T. castaneum* and *T. granarium* while no significant difference was observed among strains of *C. ferrugineus*. Essential oils possessed anti-feeding/deterrent properties and must be used as grain protectants in future.

Keywords: Essential oils, phago-deterrent, anti-feedant, medicinal plants, stored grain insect pests.

INTRODUCTION

Food safety and security is a key issue now a day, especially in the circumstances of day by day rapidly increasing population of the world (Tubiello *et al.*, 2007). Pakistan has a population of 177 million which is increasing with 2.07% growth rate. Postharvest storage of grains is facing 10 to 40% losses throughout the world (Rajashekar *et al.*, 2010). Saving the produced grains after harvesting can be helpful to meet the food requirements of increasing population. Quality and quantity of stored commodity is directly affected by the presence of insect pests in stored food (Mondal, 1994). Insect pests can develop large populations in short period of time and contaminate the products by feeding on it, leaving their faeces, hairs, dead bodies and casted skins and produce some toxins which ultimately results in grain losses (Arlian, 2002). Earlier reports by scientists declared *T. granarium* (Mark *et al.*, 2010), *T. castaneum* (Danahaye *et al.*, 2007) and *C. ferrugineus* (Mason, 2003) as most serious and economic loss causing insects.

Currently drawbacks associated with the use of conventional control strategies (synthetic insecticides) such as effect on non-target organisms (Rajendran and Sriranjini, 2008),

human health concerns (Isman, 2006), environmental pollution (Ogendo *et al.*, 2003), ozone depletion and development of insect resistance and pest resurgence (Sousa *et al.*, 2009) have necessitated to find out some biodegradable, safe and environmental friendly sources of pesticides.

Insecticides from plant sources (Essential oils) having novel mode of action against insect pests can provide an opportunity to design target specific molecules which may also be useful to overcome the problem of cross resistance (Liu *et al.*, 2011). Numerous types of insecticidal activities of essential oils have been documented (Hasan *et al.*, 2006). Along with feeding deterrent effects (Suthisut *et al.*, 2011; Ko *et al.*, 2010) essential oils may be used as insect repellent (Mishra and Tripathi, 2011), fumigant (Theou *et al.*, 2013), contact insecticide (Gallardo *et al.*, 2012;) and insect growth regulators (Jbilou *et al.*, 2006).

For insects, accepting any product as food is easier in the absence of feeding deterrents, than in the presence of attractants (Jermey, 1983). Extensive research work has proved the potential of plant derived essential oils as feeding inhibitors but there is not a single product commercialized to protect stored commodities (Isman, 2006). Different type of inter related complex behavioral responses (Isman, 1993),

rapid adaptive behavior of insect toward antifeedants (Akhtar *et al.*, 2003) and lack of toxicity when ingested by insects (Bernays, 1990, 1991) are major hurdles in commercialization of antifeeding products (Isman, 2002). Keeping in view the role of plant extracts against insect pests present studies were carried out to find their efficacy at different concentrations and exposure times against serious damage causing insect pests of stored grain.

MATERIALS AND METHODS

Plant materials: Plant parts from the locally grown medicinal plants, *Datura stramonium* (Dhatora), *Eucalyptus camaldulensis* (Sofaida), *Moringa oleifera* (Sohanjana) and *Nigella sativa* (Kalwanji) were collected from different parts of Faisalabad, Punjab, Pakistan. The fresh plant parts were brought to laboratory (Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad. Latitude: 31° 30' 0" N; Longitude: 73° 6' 0" E; Altitude: 184 m) and dried in shade. Dried plant parts were ground to powder using stone electric grinder (Machine No. 20069, Pascall Engineering Co. Ltd.). The grinded material sieved through 40 mesh sieve to obtain fine powder.

Extraction: Soxhelt extraction apparatus (Model WHM12295, DAIHAN Scientific Co., Ltd.) was used to prepare essential oils. Soxhelt thimble was filled with 50 g of fine botanical powder and placed in flask. Acetone was used as solvent in bottom flask. This process of extraction oil from all plant powders was repeated many times to achieve enough quantity of essential oil. Extracted essential oil was then purified by evaporating solvent by using electric rotary evaporator. The pure extracted essential oils were preserved in glass vials at 4°C and then used to prepare appropriate concentrations of 5, 10, 15 and 20% by mixing acetone as solvent. The concentrations were used for subsequent experiments.

Test insects: Infested grain samples of wheat from selected districts of central Punjab, Pakistan {Faisalabad (FSD), Sahiwal (SWL) and Toba Tek Singh (TTS)} were collected and brought to the Laboratory. Test insect populations of *Cryptolestes ferrugineus*, *Tribolium castaneum* and *Trogoderma granarium* were sorted out separately from the collected samples of each district.

Collected insects were kept in the jars of wide mouths (9.5 cm) and covered with the muslin cloths. Rearing of the insects was performed in the laboratory to achieve uniform population. Insects were regularly checked for their growth and sieved and transferred to new un-infested wheat grain or wheat flour diet. Temperature at 30±2°C and relative humidity at 65±5% was maintained for insect maximum growth by using incubator (Model MIR-254, SANYO). Test insects of equal size and age from the new jars were used for subsequent experiments.

Bioassay: Weight loss method was adopted for the evaluation

of antifeedant activities of essential oils. Weighed amount (5g) of grain (for *T. granarium*) and wheat flour (for *T. castaneum* and *C. ferrugineus*) was treated with 2 ml of all concentrations (5, 10, 15 and 20%) of each essential oil. Control was treated with acetone alone. Treated diet was kept in air and allowed to evaporate the solvent (acetone) for 10 min. Twenty test insects were released in each jar on treated diet. Jars were kept in incubator (Model MIR-254, SANYO) at 25±2°C and 65±5% relative humidity after covering with a cloth to avoid insect escape. Four replications were performed for each concentration of the essential oils. Data regarding weight loss were recorded after 15, 30, 45 and 60 days of treatment.

The weight loss (%) of wheat flour by *T. castaneum* and *C. ferrugineus* in the treated and control sets was calculated by using the formula suggested by Parkin (1956).

$$\text{Weight loss (\%)} = \frac{W_i - W}{W_i} \times 100$$

where, W_i = Weight of wheat flour before the experiment and W = Weight of wheat flour after the experiment.

Feeding deterrence was calculated by using the feeding deterrent index following Isman *et al.* (1990).

$$\text{FDI (\%)} = \frac{C - T}{C} \times 100$$

where, C = Weight loss in the control diet and T = Weight loss in the treated diet.

Statistical analysis: The collected data was subjected to Analysis of Variance (ANOVA) under CRD using Statistica software (Stat Soft, 8.0). Means were separated by the Tukey's multiple range test when results was significant ($p < 0.05$)

RESULTS

Assessment feeding deterrence index by observing weight loss in commodities treated with the essential oils showed that essential oils significantly reduced weight loss in the treated grain samples when test insects, *T. castaneum* ($F_{(3, 720)} = 84.056, p = 0.000$), *T. granarium* ($F_{(3, 720)} = 125.80, p = 0.000$) and *C. ferrugineus* ($F_{(3, 720)} = 73.630, p = 0.000$) were allowed to feed on treated diet. Feeding deterrence index of *D. stramonium* was found higher against *T. granarium* (20.0%) and *C. ferrugineus* (53.2%) (Fig. 1) proving it most effective among essential oils. While against *T. castaneum* Feeding deterrence of *D. stramonium* (38.9%) and *N. Sativa* (39.2%) was found statistically at par. Mean overall Feeding Deterrence Index (FDI) activities of essential oils were observed the highest against *C. ferrugineus* (45.35%) moderate against *T. castaneum* (37.35%) and the lowest against *T. granarium* (17.56%).

Weight loss caused by *T. castaneum* ($F_{(4, 720)} = 5604.2, p = 0.0000$), *T. granarium* ($F_{(4, 720)} = 2773.7, p = 0.00$) and *C. ferrugineus* ($F_{(4, 720)} = 1052.3, p = 0.0000$) was significantly reduced with the increase in concentration of essential oil applied (Fig. 2). Feeding deterrence index (FDI) of higher concentration (20 %) was also found higher i.e., 60.78, 25.58 and 78.79 % against *T. castaneum*, *T. granarium* and *C. ferrugineus*, respectively (T_4 , Table 1).

Table 1. Percentage feeding deterrence index (Mean \pm SE) of *T.castaneum* ($F_{(9,576)} = 72.969$, $p = 0.000$), *T. granarium* ($F_{(9, 576)} = 37.104$, $p = 0.000$) and *C. ferrugineus* ($F_{(9, 576)} = 70.044$, $p = 0.000$) against different exposure time and concentrations of essential oils.

Treatments	<i>T.castaneum</i>	<i>T.granarium</i>	<i>C.ferrugineus</i>
15hr \times 5%	32.48 \pm 1.13gh	7.11 \pm 0.70l	27.26 \pm 3.29ij
30hr \times 10%	39.46 \pm 0.71de	12.03 \pm 0.41ij	46.08 \pm 2.33de
45hr \times 15%	53.24 \pm 0.91b	19.81 \pm 0.60de	75.30 \pm 2.14ab
60hr \times 20%	60.78 \pm 0.77a	25.58 \pm 0.53ab	78.79 \pm 2.01a
15hr \times 5%	17.40 \pm 0.76j	9.06 \pm 0.55k	31.83 \pm 1.22i
30hr \times 10%	28.94 \pm 0.56i	16.26 \pm 0.43gh	48.17 \pm 0.99d
45hr \times 15%	39.37 \pm 0.69de	23.03 \pm 0.52c	62.75 \pm 1.12c
60hr \times 20%	46.14 \pm 0.59c	27.03 \pm 0.46a	72.44 \pm 1.00b
15hr \times 5%	30.22 \pm 0.31hi	10.76 \pm 0.29jk	25.87 \pm 0.68j
30hr \times 10%	33.63 \pm 0.39fg	13.55 \pm 0.40i	31.94 \pm 0.84i
45hr \times 15%	38.25 \pm 0.41e	17.53 \pm 0.44fg	40.10 \pm 0.87fg
60hr \times 20%	41.55 \pm 0.34d	20.48 \pm 0.37d	45.72 \pm 0.77de
15hr \times 5%	29.45 \pm 0.37i	15.52 \pm 0.36h	27.25 \pm 0.73ij
30hr \times 10%	32.38 \pm 0.43gh	18.28 \pm 0.37ef	32.15 \pm 0.80hi
45hr \times 15%	35.38 \pm 0.47f	21.01 \pm 0.38d	36.93 \pm 0.93gh
60hr \times 20%	38.92 \pm 0.36de	23.96 \pm 0.33bc	42.98 \pm 0.64ef

Means within the same column followed by the same letter are not significantly different. ANOVA, HSD ($P > 0.05$).

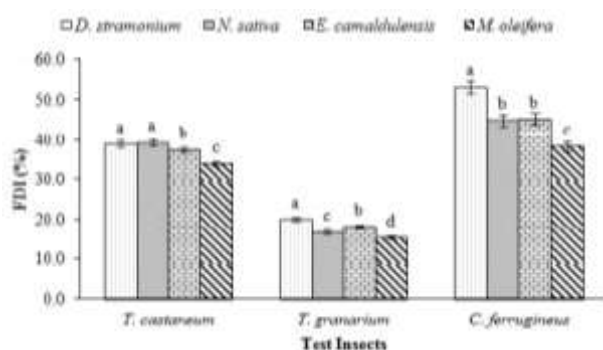


Figure 1. Feeding deterrence index (FDI) of *C. ferrugineus*, *T.castaneum* and *T. granarium* against four essential oils.

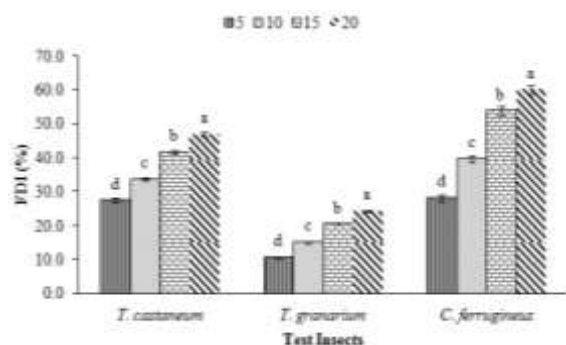


Figure 2. Feeding deterrence index (FDI) of *C. ferrugineus*, *T.castaneum* and *T. granarium* against different concentrations of essential oils.

Persistency of essential oils as antifeedant was also evaluated. The results depicted significant effect of exposure time on the feeding deterrence of essential oils against *T. castaneum* ($F_{(3, 576)} = 487.78$, $p = 0.000$), *T. granarium* ($F_{(3, 576)} = 102.00$, $p = 0.000$) and *C. ferrugineus* ($F_{(3, 576)} = 520.64$, $p = 0.000$). Feeding deterrence index was found the highest (46.5, 22.3 and 56.9%) when recorded after 15 days (first exposure time) of treatment but deterrence effectiveness found to decrease with increase in time period and it reduced to 34.0, 15.1 and 34.8% after 60 days of treatment against *T. castaneum*, *T. granarium* and *C. ferrugineus*, respectively (Fig. 3).

Antifeeding effect of essential oils against *T. castaneum*: Weight loss caused by *T. castaneum* was significantly reduced ($F_{(3, 576)} = 78.286$, $p = 0.000$) with the application of the essential oils. Feeding deterrence index of *N. sativa* (39.2%) was found the highest followed by *D. stramonium* (38.9%), *E. camaldulensis* (37.5%) and *M. oleifera* (33.8) (Fig. 1). Three strains of *T. castaneum* significantly affected weight loss ($F_{(2,576)} = 4.7695$, $p = 0.00882$). Maximum feeding deterrence index (37.8%) was recorded against SWL strain followed by TTS (37.5%) and FSD (36.8%) strain (Fig. 4).

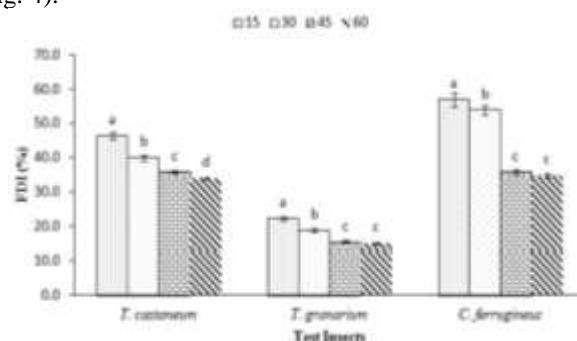


Figure 3. Feeding deterrence index (FDI) of *C. ferrugineus*, *T.castaneum* and *T. granarium* against essential oils at different exposure times.

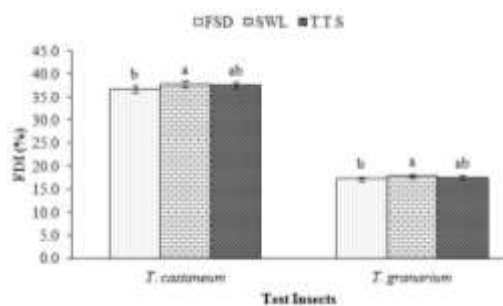


Figure 4. Feeding deterrence index (FDI) of different strains of *T.castaneum* and *T. granarium* at different locations.

Antifeeding effect of essential oils against *T. granarium*: *T. granarium* was found tolerant test insect which caused the highest weight loss as compared to *T. castaneum* and *C.*

ferrugineus. *D. stramonium* was recorded as most effective (FDI 20.0%) feeding deterrent essential oil against *T. granarium* followed by *E. camaldulensis* (18.0%), *N. sativa* (16.8%) and *M. oleifera* (15.4%) (Fig. 1). Effect of strain was also found significant ($F(2, 576) = 3.3770, p = 0.03483$). Feeding deterrence index was found higher in SWL (17.9%) strain, while the lowest was recorded in FSD strain (17.2%) (Fig. 4).

Antifeeding effect of essential oils against *C. ferrugineus*: Maximum reduction in feeding was observed in *C. ferrugineus*. Maximum feeding deterrence (78.79%) was exhibited by *D. stramonium* when applied with 20% concentration (T_4 , Table 1). The effect of different strains of *C. ferrugineus* did not significantly affect feeding deterrence ($F(2, 576) = 1.1087, p = 0.330$).

DISCUSSION

Essential oils of *D. stramonium*, *E. camaldulensis*, *M. oleifera* and *N. sativa* were found efficacious against *T. castaneum*, *T. granarium* and *C. ferrugineus* as antifeeding agent. Feeding efficiencies of the test insects declined as compared to control when they were allowed to feed on essential oil treated diet. Among essential oils *D. stramonium* was found the strongest anti feedant against *T. granarium* and *C. ferrugineus* while *T. castaneum* was affected the most with the application of *N. sativa*. Results of the present study confirm the conclusion drawn by Abbasipour *et al.* (2011) who reported feeding inhibition activities of *D. stramonium* against *T. castaneum* following flour disk method. They found maximum feeding deterrence index (FDI) of 97.21% which is higher as compared to the results of present study (60.78%). The difference in FDI may be because of difference in diet material i.e., Abbasipour *et al.* (2011) made flour disks whereas flour (powder) was used as diet in present study to assess weight loss. Recently, Ebadollahi (2011) examined efficacy of *Lavandula stoechas* and *Eucalyptus globulus* grain protector by reducing feeding potential of *T. castaneum* adults. Data regarding weight loss of flour (diet) taken after 48 hr of treatment indicated that both essential oils significantly reduced feeding as compared to control. In some other studies feeding inhibiting activities of essential oils have been investigated by Cis *et al.* (2006), Liu *et al.* (2011) and Suthisut *et al.* (2011) against *T. castaneum*, *T. granarium* and *C. ferrugineus*.

Feeding inhibition was significantly increased with increase in concentration of essential oil applied. Feeding deterrence index at the lowest concentration (5%) recorded was 27.4, 10.6 and 28.1% which remarkably increased to 46.8, 24.3 and 60.0% at the highest (20%) concentration against *T. castaneum*, *T. granarium* and *C. ferrugineus*, respectively. These findings are in accordance with earlier reports made with essential oil of *L. salicifolia* against *S. zeamais* and *T. castaneum* (Ko *et al.*, 2011). Feeding deterrence index at the

lowest concentration (4%) of *L. salicifolia* against *T. castaneum* was documented 53.58% which increased to 84.62% with the application of the highest (10%) concentration of essential oil. Similar trend was also observed by Abbasipour *et al.* (2011) who reported Feeding Deterrence Index of *D. stramonium* against *T. castaneum* increased from 34.93% to 97.21% with the increase in concentration from 947 to 3007 mg/l. Recent investigation carried out by Jaya *et al.* (2012) to determine feeding deterrence index of *Coleus aromaticus* against *T. castaneum* resulted in the same effect of concentration i.e., FDI at 250 ppm was 56.39% which increased to 72.31% at 500 ppm and achieved 100% FDI at 1000 ppm concentration. These results are also in agreement with that of Sarwar (2010).

Strains of *T. castaneum* and *T. granarium* significantly, while of *C. ferrugineus* did not significantly affect the feeding inhibition properties of essential oils. According to susceptibility of strains, order observed was SWL (37.8 and 17.9%) > TTS (37.5 and 17.6%) > FSD (36.8 and 17.2%) for *T. castaneum* and *T. granarium*, respectively. This stands in accordance with previous report of Nukenineet *et al.* (2010) dealing with antifeedant activity of *Plectranthus glandulosus* against Cameroon and German strains of *S. zeamais*. *P. glandulosus* reduced grain damage and weight loss, 7.2 and 3.5 fold, respectively, against Cameroon strains and 2.3 and 2.5 fold, respectively, against German strains of *S. zeamais*.

Conclusion: Weight loss caused by insect pests was considerably decreased with application of essential oils. Moreover, this investigation proved the phago deterrent activities of essential oils which can be helpful to ensure safe storage of commodities. *D. stramonium* was concluded as most effective feeding deterrent among tested essential oils which is suggested to be applied on jute bags and after air drying jute bags can be filled with grain for safe storage.

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