

INSECTICIDAL AND REPELLENT ACTIVITIES OF ESSENTIAL OILS OF THREE MEDICINAL PLANTS TOWARDS INSECT PESTS OF STORED WHEAT

Ch. M. Sh. HANIF¹, M.-UL-HASAN², M. SAGHEER², Sh. SALEEM*³, S. AKHTAR⁴ and M. IJAZ⁵

¹ *Bahauddin Zakariya University, Department of Agri. Entomology, Multan, Punjab, Pakistan*

² *University of Agriculture, Department of Entomology, Faisalabad, Pakistan*

³ *COMSATS Institute of Information Technology, Department of Biosciences, Sahiwal 57000, Pakistan*

⁴ *Department of Agriculture Entomology, Islamia University Bahawalpur, Pakistan*

⁵ *Bahauddin Zakariya University, Department of Agronomy, Multan, Punjab, Pakistan*

Abstract

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The present study was carried out to assess the efficacy of essential oils of some indigenous plants *Melia azadarach* (Bakain), *Azadirachta indica* (Neem) and *Datura stramonium* (Datura) for their potential repellent and mortality efficiencies against three most important insect pests of stored grain (*Tribolium castaneum*, *Rhyzopertha dominica* and *Trogoderma granarium*). Experiment was performed at Grain Research, Training and Storage Management Cell of the Department of Agri. Entomology, University of Agriculture Faisalabad. Three concentrations viz., 5%, 10% and 15% of essential oils with three replications were applied. The results advocated *Azadirachta indica*, the most effective repellent against *Tribolium castaneum* and *Rhyzopertha dominica* with maximum of 77.66% and 81.48% repellency respectively. While *Datura stramonium* depicted highest repellency (76.43%) against *Trogoderma granarium*. For mortality assay, data was collected after 24, 48h and 72hr of treatment with plant oils. The highest mortality of *Tribolium castaneum* and *Trogoderma granarium* was observed against *Datura stramonium* which was 28.82 and 24.30% respectively. While in case of *Rhyzopertha dominica* observed maximum mortality was 25.45% against *Azadirachta indica*. Results of the study strongly recommend the use of essential oils as stored grain protectant.

Key words:

Introduction

Wheat (*Triticum aestivum* L.) is main cereal crop with the major area under cultivation in Pakistan which plays a vital role in improving the economic stability of the country. Wheat is used as staple food by Pakistan's 188.2 million of population which is increasing day by day with a growth rate of 1.95% (GOP, 2013-14). Food with better quality is always required for a nation to cherish people and to become stronger among the world society (Ahmad, 2009). Its importance is

understood by the reality that it contributes 10.3% to the value added in agriculture and 2.2% to GDP (GOP, 2013–14).

In field fortunately this crop has no insect enemy. But during storage heavy qualitative and quantitative damage by insects is recorded (Parsad et al., 1977). A valuable loss of 15.3% is also been observed by Chaudhary (1980). The post-harvest losses of wheat grain have been reported to 10–15% in Pakistan (Ahmad et al., 1994). Wheat grain losses due to insect pest infestation have been estimated up to 10 to 20% (Ramzan et al., 1991; Khan et al., 2010). *Sitotroga cerealella*,

*Corresponding author: shahzadsaleem@ciitsahiwal.edu.pk

Tribolium castaneum, *Rhyzopertha dominica* and *Sitophilus spp.* were the most commonly found live insects in the 75% of the sample taken (Khan et al., 2010)

It is recorded that there are almost 1660 insect pest species which attack on stored agricultural products after harvesting during storage period (Hagstrum and Subramanyam, 2009). While *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitotroga cerellella*, *Trogoderma granarium* and *Sitophilus spp.* were recorded as most commonly found live insects in the 75% of the sample taken (Khan et al., 2010). Stored wheat is damaged by a number of stored grain insect pests out of which *Trogoderma granarium* is one of the most destructive pests of stored grain throughout the world (Burgess, 2008; Mark et al., 2010). Red flour beetle (*T. castaneum*) is a major secondary pest which feed on processed or already damaged grains by primary pests and causes economic loss throughout the world (Danahaye et al., 2007). A survey carried out by Irshad and Talpur (1993) showed *Rhyzopertha dominica* F, *Tribolium castaneum* (Herbst) and *Sitotroga cerellella* most commonly found and economic damage causing insect pests of stored wheat in Pakistan.

From last many decades various types of fumigants and synthetic insecticides are mostly used for the control of stored grain pests but the frequent use of these pesticides has made the pest strains resistant against these pesticides (Subramanyam and Hagstrum, 1995).

Methyl bromide proved itself as an efficient fumigant against insect pests of stored grains (Le Goupil, 1932). Moreover nonflammable, noncorrosive and insignificant contaminations to commodities were the properties which made it more suitable for insect control (Bond, 1984). Later on in a meeting in 1992 it was agreed to reduce its use and to find replacement because of its active role in ozone depletion (Shaaya and Kostyukovsky, 2006). On the other hand many important insect pests of stored grain (*R. dominica*, *T. castaneum* and *O. surinamensis*) have developed resistance against phosphine by reducing the respiration rate for minimum phosphine uptake (Pimentel et al., 2007). In this scenario it was necessary to search and evaluate better alternative control strategies (essential oils) to control these economic loss causing insect pests of stored grain.

Volatile compounds produced by higher plants are responsible for plant to plant interaction, defense mechanism and for attracting pollinators (Batish et al., 2008). These volatile compounds (essential oils) have also shown their insecticidal efficiencies against stored grain insect pests (Bakkali et al., 2008). Diverse effects have been exhibited by essential oils against insect pests of stored products (Papachristos and Stamopoulos, 2002). Some of these essential oils showed acute toxicity against insects (Weaver et al., 1994) while oth-

ers have anti feeding (Huang et al., 1997), fumigant (Shaaya et al., 1997), repellent (Ndungu et al., 1995), development inhibition or may interact with biochemical and physiological processes of insect pests (Talukder, 2006).

Present investigation aimed to assess the insecticidal activities of essential oils, towards *Tribolium castaneum*, *Trogoderma granarium* and *Rhyzopertha dominica*.

Materials and Methods

Collection and rearing of insects

Tribolium castaneum, *Trogoderma granarium* and *Rhyzopertha dominica* populations was collected from godowns of flour mills and grain market from Faisalabad, Pakistan. After collection, the insect population was kept in the jars and was covered with muslin cloths to prevent escape. Insect population was regularly checked for their growth, and was sieved and transferred to new uninfested wheat flour diet. Temperature $30 \pm 2^\circ\text{C}$ and relative humidity at $65 \pm 5\%$ was maintained using incubator (Model MIR-254, SANYO) in for maximum insect growth. Homogenous population of equal size and age was sieved out which later on was used for bioassay studies.

Preparation of plant extracts and essential oils

Weighed amount of fully mature fresh plant leaves of each botanical (*Azadirachta indica*, *Melia azadarach* and *Datura stramonium*) were grinded separately after shade drying at room temperature. For extraction of oil, grinded samples were run on Soxhlet's apparatus (Model WHM12295, DAIHAN Scientific Co., Ltd.) with acetone as solvent in the flask. Solvent was evaporated leaving the essential oil. Different concentrations of 5, 10 and 15% were prepared by adding acetone for subsequent experiments.

Contact toxicity of essential oils

Experiment was carried out in 90 mm Petri dish lined with whatman No 1 filter paper. Different concentrations (5, 10 and 15%) of essential oils of *Datura stramonium*, *Azadirachta indica* and *Melia azadarach* were applied on the filter paper and left the filter paper to air dry. Acetone alone was applied on filter paper as control. Twenty adults of *T. castaneum* and *R. dominica* and grubs of *T. granarium* were released separately in the Petri dish and covered it. Each treatment was replicated three times. Completely Randomized Design (CRD) was followed for the experiment. Percent mortality was recorded after 24, 48 and 72 hours. The surviving individuals of each treatment were transferred into the new clean jars containing sterilized wheat flour and grains.

The mortality (%) was corrected by Abbott's formula:

$$\text{Corrected Mortality} = (M_o - M_c / 100 - M_o) \times 100,$$

where, M_o = Observed mortality, M_c = Control mortality.

Repellent impact of essential oils

Area Preference Method was followed for the evaluation of repellent activities of the essential oils (Caballero-Gallardo et al., 2011). Filter papers were cut in two halves. Different concentrations (5, 10, and 15%) of solution were applied to half a filter paper disc uniformly with a micropipette. The other half of the filter paper was treated with acetone alone as a control. The treated and untreated halves were air dried and attached to their opposite sites using adhesive tape and placed in Petri dishes. Twenty adults of each insect species were released separately at the center of each filter paper. The dishes were then covered and placed in dark. Three replications were used for each concentration. Observations on the number of insects present on treated and untreated halves were recorded after 24 hours in mild light to evaluate the repellency of these oils.

The following formula for the calculation of percentage repellency (PR) was used as described by Asawalam et al. (2006):

$$\text{PR} = \frac{(N_c - N_t)}{(N_c + N_t)} \times 100,$$

where N_c = number of insects on the control (untreated) area;
 N_t = number of insects on the treated area.

Statistical analysis

Statistical analyses of all collected data of percent mortality were subject to analysis of variance using Statistica-8 software. Means of significant treatments were compared using Tuckey HSD test at $\alpha = 5\%$.

Results

Repellent effect of plant essential oils

Data regarding percent repellency of *T. castaneum*, *T. granarium* and *R. dominica* against *Melia azadarach*, *Azadirachta indica* and *Datura stramonium* revealed that percentage repellency of *T. castaneum* was concentration dependent i.e increase in concentration resulted in higher repellency percentage. It is evident from the Figure 1 that repellency significantly increased with the increase in concentration of essential oil. *Azadirachta indica* was found more effective showing maximum repellency (77.66%) at 15% (highest) concentration, while in case of *D. stramonium* and *M. azadarach* highest repellency observed was 70.80% and 74.25% at 15% concentration respectively. Similarly in case of *Rhyzopertha dominica* most effective essential oil was *Azadirachta indica* (Figure 2) showing highest repellency (81.48%) at

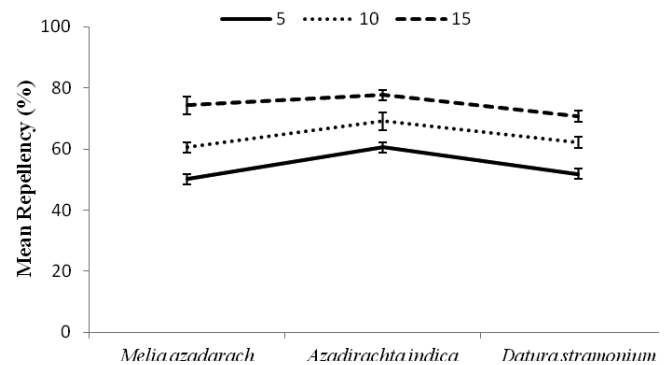


Fig. 1. Comparison of the means of the data regarding repellency percentage of *Tribolium castaneum* at different concentrations of oil of *Melia azadarach*, *Azadirachta indica* and *Datura stramonium*

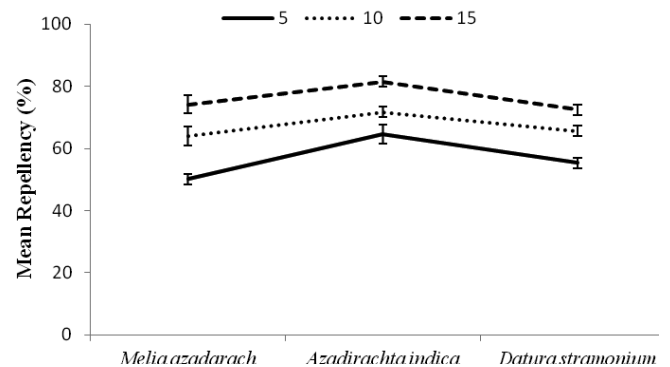


Fig. 2. Comparison of the means of the data regarding repellency percentage of *Rhyzopertha dominica* at different concentrations of oil of *Melia azadarach*, *Azadirachta indica* and *Datura stramonium*

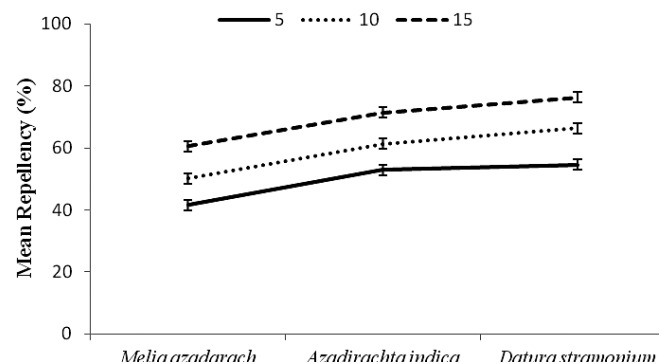


Fig. 3. Comparison of the means of the data regarding repellency percentage of *Trogoderma granarium* at different concentrations of oil of *Melia azadarach*, *Azadirachta indica* and *Datura stramonium*

15% concentration, followed by *Melia azadarach* (74.23%) and *Datura stramonium* (72.51%) at 15 % concentration. While *T. granarium* was most effectively repelled against *Datura stramonium* (76.43%) and least (60.48%) against *Melia azadarach* (Figure 3)

Insecticidal efficiencies of plant essential oils

All essential oils studied in this experiment showed significant toxicity against *T. castaneum*, *T. granarium* and *R. dominica*. Mean toxic effect of *D. stramonium* was higher than other essential oils against *T. castaneum*, *T. granarium*

Table 1

Percent Mortality of adult of *Tribolium castaneum* (Herbst) against different essential oil concentrations of *Melia azadarach*, *Azadirachta indica* and *Datura stramonium*

Time (h)	Concentration (%)	Mean Mortality \pm S.E*		
		<i>M. azadarach</i>	<i>A. indica</i>	<i>D. stramonium</i>
24	5	3.42 \pm 2.93 d	5.17 \pm 1.72 e	5.10 \pm 2.93 e
24	10	6.81 \pm 1.69 cd	12.07 \pm 2.98 cd	10.18 \pm 1.69 de
24	15	11.89 \pm 1.69 bcd	18.96 \pm 1.72 bc	13.57 \pm 1.69 cde
48	5	8.51 \pm 2.93 bcd	10.35 \pm 1.72 cd	13.57 \pm 2.93 cde
48	10	13.59 \pm 2.93 abcd	15.52 \pm 1.72 bcd	18.65 \pm 2.93 bcd
48	15	16.98 \pm 1.69 abc	24.14 \pm 3.44 ab	18.65 \pm 2.93 bcd
72	5	16.98 \pm 1.69 abc	15.52 \pm 1.72 bcd	23.74 \pm 3.05 bc
72	10	18.67 \pm 2.93 ab	24.52 \pm 1.72 ab	27.13 \pm 1.69 ab
72	15	23.75 \pm 2.93 a	34.48 \pm 1.72 a	35.60 \pm 1.69 a

Table 2

Percent Mortality of adult of *Rhyzopertha dominica* against different essential oil concentrations of *Melia azadarach*, *Azadirachta indica* and *Datura stramonium*

Time (h)	Concentration (%)	Mean Mortality \pm S.E*		
		<i>M. azadarach</i>	<i>A. indica</i>	<i>D. stramonium</i>
24	5	5.10 \pm 1.69 e	3.42 \pm 1.69 e	5.10 \pm 1.69 e
24	10	10.18 \pm 1.69 cd	13.59 \pm 2.93 bcd	10.18 \pm 1.69 cd
24	15	15.26 \pm 1.69 bc	16.98 \pm 1.69 bc	15.26 \pm 1.69 bc
48	5	11.87 \pm 1.69 cd	8.50 \pm 2.93 cd	11.87 \pm 1.69 cd
48	10	16.96 \pm 1.69 bc	18.67 \pm 2.93 bc	16.96 \pm 1.69 bc
48	15	23.74 \pm 2.93 b	23.75 \pm 2.93 ab	23.74 \pm 2.93 ab
72	5	16.96 \pm 1.69 bc	15.28 \pm 4.48 bcd	16.96 \pm 1.69 bc
72	10	22.04 \pm 1.69 b	25.45 \pm 1.69 ab	22.04 \pm 1.69 b
72	15	32.21 \pm 1.69 a	35.61 \pm 1.69 a	32.31 \pm 1.69 a

Table 3

Percent Mortality of adult of *Trogoderma granarium* against different essential oil concentrations of *Melia azadarach*, *Azadirachta indica* and *Datura stramonium*

Time (h)	Concentration (%)	Mean Mortality \pm S.E*		
		<i>M. azadarach</i>	<i>A. indica</i>	<i>D. stramonium</i>
24	5	5.12 \pm 1.69 f	1.72 \pm 1.72 e	6.00 \pm 0.34 e
24	10	8.50 \pm 1.72 de	6.89 \pm 1.69 de	8.49 \pm 2.93 e
24	15	16.98 \pm 1.69 bcd	12.07 \pm 2.98 cd	11.87 \pm 1.69 cd
48	5	10.20 \pm 1.69 d	6.89 \pm 1.72 de	10.18 \pm 1.69 cd
48	10	15.28 \pm 1.69 c	12.07 \pm 2.98 cd	15.26 \pm 1.69 bcd
48	15	23.75 \pm 1.72 ab	18.96 \pm 1.72 bc	18.65 \pm 2.93 bc
72	5	15.28 \pm 1.69 c	13.79 \pm 1.72 bcd	18.65 \pm 2.93 bc
72	10	20.36 \pm 1.69 bc	20.69 \pm 1.72 b	23.74 \pm 2.93 ab
72	15	30.52 \pm 1.69 a	29.31 \pm 1.72 a	31.52 \pm 1.69 a

while *A. indica* was observed to kill the highest percentage of *R. dominica*. Higher concentrations of essential oils were found more lethal as increase in concentration significantly increased mortality against all test insects. Similar trend was observed in exposure time. It was obvious from results that contact insecticidal efficiency was significantly higher when insects were exposed to essential oils for longer period of time. All the essential oils expressed significant lethal actions against *T. castaneum*. It is clear from the Table 1 that application of highly concentrated (15%) *D. stramonium* produced maximum mortality (35.60%) after 72 h of treatment while minimum mortality was produced by *M. azadarach* (3.42%) at lowest concentration of 5%. Insecticidal efficiencies of all tested essential oils were also significant against *R. dominica*. Highest mean mortality (35.61%) was exhibited by *A. indica* (Table 2) followed by *D. stramonium* (32.31%), and *M. azadarach* (32.21%). Effect of different essential oils was found significant against *T. granarium* too as 31.52, 30.52 and 29.31% mean mortality was exhibited by *D. stramonium*, *M. azadarach* and *A. indica* respectively. It is obvious from Table 3 that mortality was considerably high when test insects were exposed to essential oils for longer period of time.

Discussion

Studies were carried out to evaluate toxic and repellency effectiveness of plant essential oils against stored grain insect pests. Data depicted considerable repellency and toxic bio activities of three indigenous essential oils against three most important economic loss causing insect pests of stored grain to possess potential as alternative compounds to currently used insect-control agents. Similar bio activities of these essential oils have also been documented by Shaaya et al., 1991; Huang et al., 2000; Rajendran and Sriranjini, 2008; Batish et al., 2008; Sahaf et al., 2008 and Cosimi et al., 2009. All the essential oils assessed in this study exhibited significant, time and concentration dependent mortality against adults of *Tribolium castaneum*, *Trogoderma granarium* and *Rhyzopertha dominica*, however *Azadirachta indica* was found more effective with 35.61% mortality against *Rhyzopertha dominica* while *Datura stramonium* was more effective against *Tribolium castaneum* and *Trogoderma granarium*. Previous research testified *Azadirachta indica* as potential for the field evaluation as grain protectant (Rahim, 1998; Lale and Mustapha, 2000; Khan and Marwat, 2003). Similar trend was also observed by Manzoor et al. (2011) who studied the toxic effects of *Melia Azedarach*, *Mentha longifolia*, *Myrtus communis*, *Cymbopogon citratus* and *Datura stramonium* and found *D. stramonium* most toxic with 21.43% mortality among all essential oils. Mortality in all populations of tested insect pests was significantly affected by dose rate. Insecticidal potential calculated was directly proportional to concentration. Moreover Percentage of mortality recorded was significantly high when insects were kept in contact with essential oil for longer period of time. Studies carried out by Mahfuz and Khanam (2007) were also in agreement this experiment who reported decrease in lethal dose (0.681 to 0.289 mg/cm²) of *D. stramonium* extract against *T. confusum* with increase (24 to 72 h) in exposure time.

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