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# EVALUATION OF ESSENTIAL OILS OF DIFFERENT CITRUS SPECIES AGAINST *TROGODERMA GRANARIUM* (EVERTS) (COLEOPTERA: DERMESTIDAE) COLLECTED FROM VEHARI AND FAISALABAD DISTRICTS OF PUNJAB, PAKISTAN

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**ABSTRACT**

In the present study, an attempt was made to evaluate the impact of essential oils of four citrus species (*Citrus paradisi*, *Citrus sinensis*, *Citrus aurantium* and *Citrus reticulata*) on two different strains (Faisalabad and Vehari) of *T. granarium.* Grubs of *T. granarium* were exposed for 3, 6 and 9 days to grains treated with 2, 4 and 8% concentrations of citrus peel essential oil under laboratory conditions. At concentration of 8% *C. aurantium* essential oils were highly effective against *T. granarium* than other citrus species with respect to percent mortality (27.30%), followed by *C. reticulate* (26.65%), *C. sinensis* (22.36%) and *C. paradisi* (20.00%). Our data reveals that Faisalabad strain is susceptible than Vehari strain. At 8% concentration $F_1$ progeny failed completely to develop in case of *C. sinensis* application. Data regarding grain loss caused by *T. granarium* showed that minimum grain loss occurred in case of *C. aurantium* treated grains, followed by *C. paradisi*, *C. sinensis* and *C. reticulate.* It could be concluded that citrus essential oils possess excellent insecticidal, growth inhibition and antifeedant activities against *T. Granarium.*

**Keywords:** *Trogoderma granarium*, essential oil, growth inhibition, $F_1$ progeny

## INTRODUCTION

Khapra beetle *Trogoderma granarium* Everts is considered as one of the most severe stored grain and cereal insect pest worldwide (Ramzan and Chahal, 1986). The infestation of khapra beetle most likely confined to grain storage facilities, food processing plant, warehouse or other buildings containing suitable host material (Bank, 1977). When attack of this pest is very serious in grain, it makes grain unable to germination or unmarketable because larvae consume specific nutrients during feeding on grain (Jood and Kapoor, 1993). Losses that occur due to *T. granarium* infestation range from 0.2 to 2.9 % over a period of 1 to 10.5 months (Irshad *et al.,* 1988).

The efficient control of stored grain from insect pests is dependent on synthetic insecticide such as fumigation with phosphine or methyl bromide or dusting with compound as primiphos methyl and permethrin (Price and Mills, 1988). Methyl bromide is used to control insect as a space fumigant in flour and feed mills. Methyl bromide acts rapidly, controlling insects in less than 48 h in space fumigation but this chemical was banned in 2005 in developed countries because it depletes ozone layer (Field and White, 2002). It has been observed that different development stages of *T. granarium* has developed resistance against phosphine (Sharma and Kalra, 1988).

However due to these above constraints, there is a dire need to use safe insecticides especially botanical for stored grain protection. The botanical insecticides are best suited for use in organic food production. It can play important role in the production and post harvest safety of food grains against insect pests (Isman, 2006). The use of plant based products for the control of stored grain is best suited on a very large scale. It has been studied that essential oils are good repellents, growth inhibitors, biodegradable and less toxic to non target organisms as compared to synthetic chemical insecticides (Dubey *et al*., 2008; Sagheer *et al.,* 2011).

The objective of the present study was to determine insecticidal, growth inhibition and antifeedent activities of various citrus peel essential oil against *T. Granarium.*

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## MATERIALS AND METHODS

The experiment was carried out in the grain research laboratory in the department of Agri. Entomology, University of Agriculture, Faisalabad, during the year 2009-2010.

### Insect collection and rearing

The mixed age insects of two different strains *Trogoderma granarium* were collected from granaries of Faisalabad and Vehari district. The insects, thus collected were kept in wide mouth plastic jars cover with muslin cloth and kept in the laboratory at 27±3 $^0$C and 55±5 RH for two month for rearing. Wheat grains that were used for insect rearing were kept in an oven at 60 $^0$C for 6hrs to disinfest the seeds from any prior infestation before using them as a substrate for insect rearing. Fifty pairs of the adults of *T. granarium* were placed in 1-litre glass jar containing 20 g seeds. The jar was then covered with nylon mesh that was held in place with rubber band. The adults were sieved out after an oviposition time of 10-13 days. Then the seeds were kept under laboratory condition until $F_1$ progeny, which emerged after 10-15 days, were sieved out and used for the experiment.

### Extraction of Citrus Essential Oils

Citrus fruits peels of different citrus species were collected from orchards and local markets of Faisalabad and Sargodha. Plant materials were cleaned with distal water and dried under shade in the laboratory. Dried peel was grounded into powder using Mortal and Pestle. Essential oil from grounded peel of different citrus species like *C. sinensis* (Musambi), *C. aurantium* (bitter orange), *C. paradisi* (grape fruit) and *C. reticulate* (kinnow) were obtained by subjecting plants material into soxlets apparatus. An average yield of 7.4 ml oil was collected from 1 kg of orange peels. The oil was kept in refrigerator for later use.

### Solution preparation in percentage

Solutions were prepared in percentage (2% 4% and 8%) for larvicidal trial, from the different citrus peel oils in acetone. These solution were prepared on volume/volume basis; 2 ml of oil in 98ml of acetone made 2% solution, 4ml of oil was used for 4% and 8ml for 8% solution.

### Assessment of toxicity, progeny development and grain loss:

Experiment was conducted in randomize complete block design and each treatment was replicated three times. 20 g of disinfected wheat grains were kept into each plastic jar of 1 L capacity and spray with three different concentration ( 2% 4% and 8%) of each oil. Each jar was shaken mechanically for two minutes to allow for effective permeation of the seed with oil. Control was sprayed with acetone only. The treated grains were allowed to dry. Then one week old, 10 larvae of *T. granarium* were released in each glass jar. The jars were covered with nylon mesh and held in place with rubber bands. The treated jars were kept at ambient temperature of 28±2 $^0$C in the laboratory throughout the course of study. The insect mortality data was recorded after 3, 6 and 9 days against different concentrations of citrus essential oil. Further, the individual which survived were released on fresh untreated wheat grains. The data was recorded for grain loss and development of $F_1$ progeny after 35days.

### Statistical analysis

The obtained data were subjected to statistical analysis using two way Anova and least significant difference (L.S.D.) was also used to detect the significant differences between and within treatments using Statistica 6 software.

## RESULTS

The essential oils of *C. sinensis, C. aurantium, C. paradisi* and *C. reticulata* were evaluated against the larvae of *T. granarium* at 2, 4, and 8% concentrations at three exposure periods. The results (Table-1) revealed that highest mortality affect was observed in case of *C. aurantium* oil, followed by *C. reticulate*, while mortality was lowest in *C. sinensis* treated larvae.

The mean value of mortality (Table-1) in *C. aurantium* treated larvae, with respect to different exposure periods was highest (27.30%) at 8% concentration after 9 days, and was minimum 5.18% after 6 days of treatment application, followed by 6.67% after 3 days exposure period at 2% concentration. Similarly, *C. reticulate* showed highest mortality 26.85% (Table-1) after 9 days of treatment application at 8% concentration while no mortality was observed at 2 and 4% concentrations after 3 days of exposure period. In *C. sinensis* treated larvae, highest mortality was 22.36% and lowest mortality was 1.33% at 8% concentration after 9 and 3 days, respectively. While no mortality was recorded at 2% concentration after 3 days of treatment and mortality was 8.55% after 9 days at same concentration. Mortality values in case of *C. paradisi*

Treated larvae were 6.66% and 20.00% at 8% concentrations, after 3 and 9 days of treatment application, respectively (Table-1). Mortality was 3.33 and 6.67% at 2% concentration after 6 and 9 days of treatment of larvae, respectively. A direct dose mortality response was also in all observed in all test plant oils.

Table-2 shows the impact citrus essential oils on $F_1$ progeny of two strains of *Trogoderma granarium.* The response of the plant oils varied significantly on adult emergence. The essential oil of the *Citrus sinensis* proved highly effective as compared to other plant oils to inhibit the adult emergence rate of *T. granarium*, while *C. paradisi* oil revealed least effective. The results indicate that lower doses of essential oils proved less effective as in these treatments there was high rate of adult emergence, while with increase in concentrations the minimum adult emergence was reported. Maximum concentration of *C. sinensis* was proved most effective against Faisalabad and Vehari strains of test insects where 0.0% adult emergence was recorded. The minimum dose (2.0%) of *C. paradisi* resulted in maximum adult emergence (9.33%) in both strains of test insects. Moderate adult emergence activities were obtained by the essential oils of the plants of *C. reticulata* and *C. aurantium.* From these results it

was concluded that with increase in dose $F_1$ progeny decreases and these essential oils respond more effectively to Faisalabad strain as compared to Vehari strain.

**Table 1**
Toxicity of different concentrations of citrus essential oils against grubs of *Trogoderma granarium* (Everts) at various exposure times.

| Plant Essential Oils | Concentration (%) | Mean mortality (%) | | |
|---|---|---|---|---|
| | | Exposure periods (Days) | | |
| | | 3 days | 6 days | 9 days |
| *Citrus reticulata* | 8 | 1.66±3.45 | 17.18±2.76 | 26.85±2.58 |
| | 4 | 0.00±0.00 | 15.45±3.18 | 19.57±2.31 |
| | 2 | 0.00±0.00 | 6.83±2.67 | 10.69±3.26 |
| *Citrus sinensis* | 8 | 1.33±1.33 | 12.00±2.31 | 22.36±3.53 |
| | 4 | 0.00±0.00 | 6.83±2.67 | 15.45±3.18 |
| | 2 | 0.00±0.00 | 3.38±2.18 | 8.55±1.72 |
| *Citrus paradise* | 8 | 6.66±3.33 | 13.33±2.33 | 20.00±2.88 |
| | 4 | 3.33±1.33 | 8.33±1.67 | 15.00±0.00 |
| | 2 | 0.00±0.00 | 3.33±1.67 | 6.67±1.61 |
| *Citrus aurantium* | 8 | 18.36±3.33 | 21.07±4.22 | 27.30±3.38 |
| | 4 | 10.00±3.33 | 12.26±1.78 | 16.38±1.91 |
| | 2 | 6.67±3.33 | 5.18±0.73 | 7.19±3.92 |

**Table 2**
Development of $F_1$ progeny of *Trogoderma granarium* treated with different concentrations of essential oils of various citrus species.

| Essential oils | Concentrations (%) | Mean $F_1$ Adults | |
|---|---|---|---|
| | | Strain | |
| | | Faisalabad | Vehari |
| *Citrus reticulata* | 8 | 6.33±2.66 | 7.33±2.88 |
| | 4 | 7.33±1.85 | 7.66±0.33 |
| | 2 | 8.00±1.20 | 8.66±0.66 |
| | 0 | 9.66±1.20 | 9.66±0.66 |
| *Citrus sinensis* | 8 | 0.00±0.00 | 0.00±0.33 |
| | 4 | 0.33±0.00 | 0.00±0.00 |
| | 2 | 0.00±0.00 | 0.33±0.00 |
| | 0 | 8.33±0.33 | 9.00±0.00 |
| *Citrus paradisi* | 8 | 7.66±0.33 | 8.33±0.33 |
| | 4 | 8.33±2.40 | 8.66±1.20 |
| | 2 | 9.33±1.15 | 9.33±0.33 |
| | 0 | 1.00±1.20 | 10.00±0.66 |
| *Citrus aurantium* | 8 | 5.33±0.33 | 5.33±0.88 |
| | 4 | 5.66±0.33 | 5.66±0.33 |
| | 2 | 6.00±0.57 | 6.33±0.33 |
| | 0 | 9.33±0.33 | 9.66±0.33 |

Table 3 exhibits that the impact of essential oils of test plants on grain weight loss caused by two strains of *Trogoderma granarium* was highly significant. The response of the plant oils varied significantly on grain weight loss. The essential oil of the *C. aurantium* evidenced highly effective against Faisalabad strain and *C. paradisi* showed the effective results against Vehari strain as compared to other plant oils to lower down the grain weight loss. The result indicates that lower doses of essential oils proved less effective where high grain weight loss was resulted, while with increase in concentrations the minimum grain weight loss was reported. Maximum concentration of *C. aurantium* was proved most effective against Faisalabad strain of test insect where 2.05g grain weight loss was recorded while against Vehari strain *C. paradisi* showed minimum grain weight loss that was 3.47g. From these results it was concluded that with increase in concentrations of essential oils grain weight loss decreases and these essential oils respond more effectively to Faisalabad strain as compared to Vehari strain.

## DISCUSSION

The results show that *Citrus aurantium* had highly significant effect on the mortality of *T. granarium* grubs. These results are consistent with those of the previous findings. Don-Pedro (1985) checked the toxicity of powdered sun-dried orange and grapefruit peels to *Callosobruchus maculates* and *Dermestes maculates* was evaluated in the laboratory. $LD_{50}$ values of the ground peels on adults *D. maculates* were much higher.

The extent to which the citrus essential oil affected the survival of subsequent progeny was found to vary among them. This indicates that the active ingredients of citrus essential oils which are responsible for the toxicity of the plant kill the insect gradually; the current findings are similar to the results of Tripathi *et al*. (2003) who has also reported oviposition reduction effect of citrus peel oil against *Tribolium castaneum* by 94.5%. Similarly Sharaby (1988) also reported reduce oviposition and egg hatching of potato tuber moth expose to 220 micro L of the oil.

The results of grain loss are similar as those obtained by Sharma and Verma (1971), when they examine that the neem seed powder in wheat grain against *T. granarium.* The damage was considerably low after 8 month as compare to control treatment, by the work Onu and Sulyman (1997) checked the efficacy of powdered citrus peel for control of *Callosobruchus maculates* (F). Dried powdered peel from four citrus fruits was tested, using two application rates. Powdered grapefruit peel on cowpea seed discourages oviposition, suppressed emergence of the F1 generation, and substantially reduced damage.

The results of the current study suggested that essential oil derived from *Citrus sinensis*, *Citrus aurantium*, *Citrus paradise* and *Citrus reticulata* may be used as wheat grain protectants against *T. granarium* for small scale farmer. Therefore, investigation on incorporating, improving and adapting for the control of stored product insect need to be investigated.

**Table 3**

Grain losses caused by grubs of *Trogoderma granarium* survived the exposure of essential oils of various citrus species

| Essential oils | Concentration (%) | Mean grain weight loss (grams) | |
|---|---|---|---|
| | | Strains | |
| | | Faisalabad | Vehari |
| *Citrus reticulata* | 8 | 2.56±0.26 | 3.33±0.32 |
| | 4 | 3.60±0.23 | 3.66±0.17 |
| | 2 | 4.73±0.14 | 5.00±0.26 |
| | 0 | 8.80±0.50 | 9.06±0.35 |
| *Citrus sinensis* | 8 | 2.23±0.12 | 3.00±0.05 |
| | 4 | 3.76±0.33 | 4.23±0.08 |
| | 2 | 4.86±0.12 | 5.00±0.11 |
| | 0 | 8.76±0.31 | 7.63±0.24 |
| *Citrus paradisi* | 8 | 2.09±0.17 | 2.80±0.11 |
| | 4 | 3.23±0.16 | 3.40±.11 |
| | 2 | 4.80±0.17 | 4.23±0.20 |
| | 0 | 8.66±0.61 | 8.63±0.26 |
| *Citrus aurantium* | 8 | 2.05±0.18 | 2.36±0.14 |
| | 4 | 3.40±0.30 | 3.96±0.34 |
| | 2 | 4.03±0.31 | 4.19±0.17 |
| | 0 | 8.53±0.17 | 8.40±0.25 |

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