# Insecticidal Activities Of Two Citrus Oils Against *Tribolium Castaneum* (HERBST)

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**ABSTARCT:** The effect of volatile compounds of *Citrus reticulata* and *Citrus sinensis* oils were studied on the stored grain pest *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) the oils were extracted from the fruit peels and seeds using hydrodistillation. Result indicated that the essential oil of *Citrus reticulata* showed more toxic effects than that of *Citrus sinensis* against larvae and adult of *Tribolium castaneum*. The $LC_{50}$ of *Citrus reticulata* against larvae of *Tribolium castaneum* was 20.70 µl and 14.82 µl at 48 and 72 h exposure. Similarly the $LC_{50}$ of *Citrus sinensis* was 41 .58 µl and 40.28 µl at 48 and 72 h exposure. The $LC_{50}$ of *Citrus reticulata* and *Citrus sinensis* against adult of *T. castaneum* was 53.00 µl, 43.81 µl, 53.28 µl, and 44.55 µl at 48 and 72 h exposure respectively.
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## INTRODUCATION

Higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods for insect control (Arnason *et al*., 1989).Insecticidal activity of many plants against several insect pests has been demonstrated (Jilani and Su, 1983; Isman, 2000; Carlini and Grossi-de-Sá, 2002). The deleterious effects of plant extracts or pure compounds on insects can be manifested in several manners including toxicity, mortality, antifeedant growth inhibitor, suppression of reproductive behaviour and reduction of fecundity and fertility. (Yang and Tang 1988) reviewed the plants used for pest insect control and found that there is a strong connection between medicinal and pesticidal plants.

*Tribolium castaneum* (Herbst) is considered as a major pest of stored grains (Howe, 1965). Annual post-harvest losses resulting from insect damages, microbial deterioration and others factors are estimated to be 10- 25% of worldwide production (Matthews, 1993). Control of these insects relies heavily on the use of synthetic insecticides and fumigants. However, their widespread use has led to some serious problems including development of insect strains resistant to insecticides (Zettler and Cuperus, 1990; White, 1995; Ribeiro et al., 2003), toxic residues on stored grain, toxicity to consumers and increasing costs of application. However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly. Considerable efforts have been focused on plant-derived materials, potentially useful as commercial insecticides. *Citrus reticulate* and *Citrus sinensis* are common plants in Pakistan. Simpson (1995) had reported that Essential oils are believed to act as allelopathic agents or as irritants that protect plants from predation by insects and infestation by parasites.

The aim of our study was to evaluate the insecticidal activity of the methanol extracts from *citrus reticulate* and *Citrus sinensis* against larvae and adults of *Tribolium castaneum*. We assessed the effect of different extracts on (1) Mortality rate of adult (2) mortality rate of larvae.

## MATERIAL AND METHOD

The studies were conducted in the Grain Research, Training, and Storage Management Cell of the Department of Agri Entomology, University of Agriculture, Faisalabad, during the year 2008-2010.

## COLLECTION OF INSECTS

Heterogeneous samples of *T. castaneum* were collected for rearing in the laboratory, from various godowns of Punjab Food Department and Grain Markets located in Faisalabad and Lodhran District.

## REARING OF INSECTS

The insect culture was maintained in jars placed in the incubator at 30±2°C and 60% ± 5 R.H to get the homogenous population. The culture medium was the whole meal wheat and wheat flour sterilized at 60°C for 60-90 minutes. Thirty beetles from the heterogeneous population (20F+10M) were liberated in 250gm of wheat flour placed in different jars. The mouths of jars were covered with muslin cloth, tied with rubber bands to avoid the escape of beetles. Beetles were allowed to remain in the culture medium for 3 days for egg laying and then removed from jars with the help of sieves and fine camel hair brushes for

continuation of culture. The flour containing eggs was placed again in the same jars. The population received from these jars after a month that was considered as a uniform age for experimentation.

**EXTRACTION OF OIL**

The seeds of citrus cultivars were washed with tap water to remove the pulp and then dried in the oven for 48 hours at 60°C and later were grounded in an electric grinder (Anex Germany). While other plant materials like fruit peels, leaves and rhizomes were also dried. The grounded material was put in Soxhlet apparatus for the extraction of oil by steam distillation method (Vogel, 1978)

**LARVAL MORTALITY**

Larvicidal property of Citrus reticulate and *Citrus sinensis* essential oils were tested against newly molted 4th instars of *Triboluim castaneum* larvae by contraction. whatman no 1 filter papers were cut according to shape of petri dish and treated with soloution of differient concentration, 2, 4, 6, 8 and 10 µl oils in 100 µl acetone using micropipette. The treated filter papers were dried to evaporate the solvent completely. The treated filter papers were placed at the bottom in glass petri dish (90 mm). Twenty larvae of *Triboluim castaneum* taken from the laboratory culture were placed with 1 gram of wheat flour in petri dish. The flour was spread uniformly along the whole surface of the petri dish. All closed petri dishes were kept in dark and three replicates were set for each concentration. After 24, 48 and 72 h larval mortality was recorded.

**ADULT MORTALITY**

The toxic effect of *Citrus reticulate and Citrus sinensis* essential oils were tested against adult of *Triboluim castaneum* by contraction. The methodology used was the same as the used in determining the toxic effect of larval mortality in *Triboluim castaneum*

**STATISTICAL ANALYSES**

The $LC_{50}$ was calculated by POLO programme (Roberson *et al.* 2007). Correlation and linear regression analysis were conducted to define all dose response relationships (Sokal *et al.*1973). Analysis of variance was performed to test the equality of regression coefficient (Sokal *et al.*1973).

**RESULTS**

The essential oils of fruit peels and seeds of *Citrus reticulata* and *Citrus sinensis* killed the larvae and adults of *Triboluim castaneum* by contact action. The $LC_{50}$ of *Citrus reticulata* oil was found 30.62 µl, 20.70 µl and 14.82 µl at 24, 48, and 72 h against larvae of *T. castaneum*. (Table 1). The adult toxicity of *Citrus reticulata* against *Triboluim castaneum* was 58.31 µl, 53.00 and 43.81 µl at 24, 48, and 72 h exposure. Whereas larval toxicity of *Citrus sinensis* was found 42.48 µl, 41.58 µl and 40.28 µl at 24, 48 and 72 h exposure. The adult toxicity of *Citrus sinensis* was 45.46 µl, 53.28 µl and 44.55 µl at 24, 48, and 72 h exposure. (Table 1).

The regression analysis showed a concentration dependent significant correlation of *Citrus reticulata* with larval mortality ($F=46.728$, $P<0.01$) at 24 h, ($F=46.234$, $P<0.01$) at 48 h and ($F=45.546$, $P<0.01$) at 72 h exposure. While the concentration dependent significant correlation of *Citrus reticulata* with adult mortality ($F=17.051$, $P<0.01$) at 24 h, ($F=22.991$, $P<0.01$) at 48 h and ($F=29.980$, $P<0.01$) at 72 h exposure. (Table 2).

The concentration dependent significant correlation of *Citrus sinensis* with larval mortality ($F=27.110$, $P<0.01$) at 24 h, ($F=56.001$, $P<0.01$) at 48 h and ($F=31.675$, $P<0.01$) at 72 h exposure whereas the adult mortality ($F=22.112$, $P<0.01$) at 24 h, ($F=27.324$, $P<0.01$) at 48 h and ($F=57.88$, $P<0.01$) at 72 h exposure respectively (Table 2).

Table 1 The toxicity assay of Citrus reticulata and Citrus sinensis essential oils against larvae and adult of *Triboluim castaneum*

| Essential oils | stage | exposure time(h) | $LC_{50}$ [a] | LCL-UCL b | g-value c | t- ratio c | Heterogeneity c |
|---|---|---|---|---|---|---|---|
| Citrus reticulata | Larvae | 24 | 30.62 | 16.77-358.88 | 0.299 | 5.12 | 0.13 |
| | | 48 | 20.70 | 13.55-63.89 | 0.234 | 4.34 | 0.18 |
| | | 72 | 14.82 | 9.43 -68.43 | 0.209 | 4.11 | 0.10 |
| | Adult | 24 | 58.31 | 21.69-16591 | 0.332 | 3.04 | 0.29 |
| | | 48 | 53.00 | 21.05-12449 | 0.314 | 4.31 | 0.31 |
| | | 72 | 43.81 | 18.11-6199.3 | 0.281 | 4.42 | 0.27 |
| Citrus sinensis | Larvae | 24 | 42.48 | 18.67 -846.09 | 0.196 | 3.05 | 0.14 |
| | | 48 | 41.58 | 18.82-433.01 | 0.145 | 4.56 | 0.19 |
| | | 72 | 40.28 | 16.72- 341.0 | 0.111 | 3.01 | 0.23 |
| | Adult | 24 | 45.46 | 19.69-12412.1 | 0.414 | 4.26 | 0.34 |
| | | 48 | 53.28 | 20.64-12040.7 | 0.360 | 4.13 | 0.38 |
| | | 72 | 44.55 | 15.54-641.4 | 0.319 | 3.99 | 0.30 |

[a]$LC_{50}$ represent the median lethal concentration. UCL and LCL represent upper confidence limit and lower confidence limit. g value, t- ratio and heterogeneity were significant at all probability levels (90%, 95%, 995)

Table 2 regression parameters of insecticidal effects of Citrus reticulata and Citrus sinensis essential oils against larvae and adult of *Triboluim castaneum*

| Essential oils | stage | exposure time(h) | intercept | slope | regression coefficient | F-value* (df=3.20) P<0.01 |
|---|---|---|---|---|---|---|
| Citrus reticulata | Larvae | 24 | -0.97 | 0.221 | 0.998 | 46.728 |
| | | 48 | -0.111 | 0.253 | 0.981 | 46.234 |
| | | 72 | -0.126 | 0.267 | 0.987 | 45.546 |
| | Adult | 24 | -0.816 | 0.41 | 0.977 | 17.051 |
| | | 48 | -0.601 | 0.233 | 0.991 | 22.991 |
| | | 72 | -0.672 | 0.247 | 0.998 | 29.980 |
| Citrus sinensis | Larvae | 24 | -0.81 | 0.223 | 0.932 | 27.110 |
| | | 48 | -0.156 | 0.239 | 0.991 | 56.001 |
| | | 72 | -0.201 | 0.253 | 0.971 | 31.675 |
| | Adult | 24 | -0.978 | 0.260 | 0.999 | 22.112 |
| | | 48 | -0.583 | 0.269 | 0.997 | 27.324 |
| | | 72 | -0.019 | 0.241 | 0.976 | 57.88 |

Regression analysis was performed between differient concentartions of essential oils and response of pest * significant at 99% probability level.

**DISCUSSION**

Different essential oils and their constitutes have been studied to possess potential as alternative compound and gaining tremendous importance particularly for the management of stored product ecologically safe and biodegradable (Sokal *et al*.1973, Nerio *et al* 2009). Rutaceae is the large family containing 130 genera in seven subfamilies with many important fruits and essential oils product. Lemon essential oils has highest value of all essential oils and is widely used as flavouring agent in different bakery, as a fragrance in perfumery and also for pharmaceutical applications.(Weiss 1997).

In this study, the result showed that insecticidal effect of essentials oils from peel and seeds of citrus fruit on *Triboluim castaneum* after 24, 48, and 72 h exposure. This study showed that this essential oils has significant ($p<0.01$) and good toxicity against *Triboluim castaneum*. Previously for the management of economic loss caused by *Triboluim castaneum* .several essential oils of botanical origin have been reported for their insecticidal activites. The essential oil of *Citrus sinensis* showed contact toxicity against *Zabrotes subfasciatus* L. (Zewde *et al*, 2010). Essential oils derived from orange peel and seeds is known to have toxic, feeding deterrent and poor development effect on lesser grain borer, *Rhyzopertha dominica* (F), rice weevils, *Sitophilus oryze* (L) and red flour beetle, *Triboluim castaneum* (Herbst) (Tripathi *et al,* 2003). The gas chromatographic analysis of citrus peels and seeds oils and components of these essential oils have been tested against *Callosobruchus maculatus.* Several compounds including the major component of all citrus peel and seeds oils has been found to be bioactive, which have a strong vapor insecticidal activity. A combined study has established that in artificial mixture several pure components of citrus peel oils potentiate their individual fumigant action in a constitute manner with a preservative model against *Callosobruchus maculatus.*(Don-Pendo, 1996).

The peel oil was also reported to have toxicity towards *Culex pipiens* .(Mwaiko *et al*, 1992) and cow pea weevils, *Callosobruchus maculatus* (F).(EL- Syed *et al*, 1991). Furthermore the peel and seed oil has fumigant action against fleas.(Weinzier and Henn, 1992) and house hold insects *Blatella germanica* (L) and *Musca domestica* (L) and stored products *Sitophilus oryze*.(Karr and Coats, 1988). The essential oils from differient part of plants , fruits of *Schyzygum aromaticum*, leaves of *Aegle marmelos*, seeds of *Corriandrum sativum*, and peeil of *Citrus reticulate* fruits extracted by a water distillation method showed strong repellency against *Triboluim castaneum* at low concentrations but its repellency was more marked towards *Sitophilus oryze*,(Mishra and Tripathi, 2011). The essential oils extracted from citrus genus have monocyclic monoterpenoide and its major components is d-limonene (ɖ-mentha-1,8-dene) and they have insecticidal activity against insects pests (Karr and Coats, 1988). Similarly, in the present study the essential oils of *Citrus reticulate* showed more toxicity at 48 and 72 h exposure against larvae and adult of *Triboluim castaneum* than that of *Citrus sinensis*, which show less toxicity against adult, and larvae of *Triboluim castaneum.* Varying activity by differient essential oils indicated that the pest controlling and repellent factors were not uniformly present in every aromatic plant. Therefore, essential oils from fruit peel and seeds of citrus reticulate and citrus sinensis may be recommended as a cheap, easily available at farmer level, eco-friendly with low mammalian toxicity and good alternative to synthetic insecticide. It could further reduce the use of synthetic insecticide.

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