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# Chronic Activity of Plant Volatiles Essential Oils in Management of Rice Weevil *Sitophilus oryzae* (Coleoptera: Curculionidae)

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## ABSTRACT

Stored food faces severe damage due to infestation by insects. The essential oils extracted from leaves of *Aegle marmelos*, *Mentha arvensis*, peels of *Citrus reticulata* and clove of *Syzygium aromaticum* by hydrodistillation method were screened as fumigant for chronic activity against rice weevil *Sitophilus oryzae* (Coleoptera: Curculionidae) in laboratory assay. All four essential oils showed chronic activity in a dose dependent manner. Fumigation with sub-lethal concentration of essential oils significantly ($p<0.01$) reduced oviposition capsity and exhibited ovicidal activity. Highest reduction in oviposition (35.66%) and feeding deterrence index (74.52%) observed in *A. marmelos* essential oils against *S. oryzae* in comparison to control groups. These studies showed strong insecticidal activity of all four essential oils and its potential role as a fumigant against *S. oryzae*. From this study it is concluded that these essential oils have potential for application in Insect Pest Management programs for stored-grain insect pests because of its fumigant action.

**Key words:** Chronic activity, *Sitophilus oryzae*, essential oils, oviposition, antifeedent activity

## INTRODUCTION

*Sitophilus oryzae* (Coleoptera: Curculionidae) is a major pest of stored-grain products which occurs worldwide (Park *et al.*, 2003; Athanassiou *et al.*, 2008; Derbalah *et al.*, 2012). Its local name is “Ghun” and commonly found in humid climatic conditions in the sub-tropical regions. The progeny production rate of *S. oryzae* is so high. The fourth instars larvae are highly active in rainy season and cause very high infestation.

The damage to stored-grains causes more economic loss of total world-wide production annually (Via, 1999; Weston and Rattlingourd, 2000). According to an estimate, the overall damage caused by stored-grain insect pests was 10-40% loss in temperate regions and tropical regions (Matthews, 1993). For control of *S. oryzae* many chemical fumigants and contact synthetic insecticides are commonly used to prevent the loss of stored products throughout the world. These chemical pesticides are still most effective against insect infestation but their repeated use has disrupted biological control by natural enemies and led to outbreaks of other insect species and sometimes resulted in the development of pest resistance (Ignatowicz, 1999; Zeng, 1999). Besides this chemical pesticides also play negative role in ozone depletion, environmental pollution and toxicity to non-target organisms (Zhang and Van Epenhuijsen, 2004).

To overcome this adverse impact on environment and human beings alternatives of chemical pesticides are being searched. Therefore, it is urgent need to develop certain insecticides which should be ecologically safe, biodegradable and cause no toxicity to non-target animals. Botanical pesticides have high insecticidal activity against insect pests that check its infestation stage (Isman, 2000; Sahaf and Moharamipour, 2009; Ikbal *et al.*, 2007). The insecticidal activity of essential oils against different stored-product pests has been evaluated and is environmentally compatible (Shaaya *et al.*, 1991; Sarac and Tunc, 1995; Aslan *et al.*, 2005; Ayvaz *et al.*, 2007; Oparaeke and Kuhiep, 2006; Asmanizar *et al.*, 2012). In present time, botanical insecticides in the form of essential oils presently constitute 1% of the world insecticide market (Rozman *et al.*, 2007).

The essential oils of *Aegle marmelos* (Rutaceae), *Citrus reticulata* (Rutaceae), *Mentha arvensis* (Laminaceae) and *Syzygium aromaticum* (Myrtaceae) are known to exhibit antifungal, antibacterial, antimicrobial properties (Jeeva *et al.*, 2007; Koochak *et al.*, 2010; Kingston *et al.*, 2009). Their repellent and toxic effect against *S. oryzae* has already been determined and finds their lethal concentration values (Mishra and Tripathi, 2011; Mishra *et al.*, 2011, 2012, 2013). This study was undertaken to evaluate the bioefficacy of the essential oils from *A. marmelos, M. arvensis, C. reticulata* and *S. aromaticum* against *S. oryzae* in chronic exposure at sub-lethal concentration.

## MATERIALS AND METHODS

**Extraction of essential oils:** For plant collection and extraction of essential oils, leaves of *A. marmelos, M. arvensis*, peels of *C. reticulata* were collected and cloves of *S. aromaticum* was purchase from the local area of Gorakhpur district of Uttar Pradesh, India. The specimens were identified and authenticated by Department of Botany, D. D. U. Gorakhpur University, Gorakhpur. The buds and leaves (1 kg of each) were dried in the absence of sun light at room temperature (30±5°C) and grounded using a domestic mixer. The essential oils were extracted with distilled water by hydro-distillation using a modified Clevenger apparatus. Distillation was done continuously for 5 h to yield essential oils (1-1.5 mL approximately). Anhydrous sodium sulphate was used to remove water after extraction. The superior phase was collected from the condenser in glass containers and stored in appendorff tube at 5°C until their use for further experiments.

**Insect rearing:** Rice weevils *S. oryzae* were used to examine the activity of essential oils. For experiments adults of insects were taken from laboratory stock cultures at 28±2°C, 75±5% RH and at a photoperiod of 10:14 (L:D). The adults were reared on grains and flours of rice (*Oryza sativa*) and wheat (*Triticum aestivum*) at 12-13% moisture content. The newly born 10 days old unsexed adult weevils were used to determine the insecticidal property of essential oils.

**Effect of essential oils on oviposition capability and hatchability of eggs:** The effect of *A. marmelos, C. reticulata, M. arvensis and S. aromaticum* essential oils on oviposition capability and hatchability of eggs was tested against *S. oryzae* by fumigation. Ten adults taken from the laboratory culture (1-2 week old) were placed in 1 g of wheat flour and grain in glass petri dish (height 15 mm×radius 45 mm). Flour and grain was spread uniformly along the whole surface of the petri dish. A paper strip (2 $cm^2$) treated with 40, 60 and 80% sub-lethal concentration of 24 h $LC_{50}$ of essential oils in acetone was pasted on the inner surface of the cover of each petri dish. Another paper strip (2 $cm^2$) was treated with absolute acetone only used as control. All the closed petri dishes were kept in dark and six replicates were set for each concentration. After 72 h of fumigation, the treated adults of *S. oryzae* were transferred to fresh petri dish having fresh wheat flour and grain. After 7 days of treatment, the adults of both stored-grain pests were removed and discarded. The number of the larvae hatched was counted for the treated as well as for control groups.

Hatchability was calculated as percentage:

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

C = No. of larvae in control
T = No. of larvae in test

**Determination of chronic toxicity of essential oils:** The chronic toxic activity of *A. marmelos, C. reticulata, M. arvensis and S. aromaticum* essential oils was tested against adults of *S. oryzae* by contraction method with two sub-lethal concentrations. Whatman No. 1 filter papers were cut according to the shape and size of petri dishes and treated with sub-lethal concentrations of essential oils prepared in acetone (30 and 60% of 24 h $LC_{50}$) by using micropipette. The treated filter papers were dried to evaporate the solvent completely. The treated filter paper placed at the bottom in glass petri dish (height 15 mm×radius 45 mm). Ten adults taken from the laboratory culture (1-2 week old) were placed with 1 g of wheat flour and grain in petri dish. Flour and grains were spread uniformly along the whole surface of the petri dish. All the closed petri dishes were kept in dark and six replicates were set for each concentration. After 30 days per cent grains damage by stored-grain insect pests were recorded. The adults surviving after treatment were used for experiments.

**Data analysis:** Correlation and linear regression analysis were conducted to define all dose-response relationships (Sokal and Rohlf, 1973). Analysis of variance was performed to test the equality of regression coefficient (Sokal and Rohlf, 1973).

## RESULTS

The essential oils significantly ($p<0.01$) inhibited oviposition capacity of *S. oryzae* at three sub-lethal concentrations compared to control. The maximum oviposition inhibitory activity of *A. marmelos, C. reticulata, M. arvensis and S. aromaticum* essential oils against adults of *S. oryzae* was 35.66, 53.31, 44.90 and 43.31% at 80% sub-lethal concentration of 24 h $LC_{50}$, respectively (Table 1).

The hatchability percentage of *A. marmelos, C. reticulata, M. arvensis and S. aromaticum* essential oils was 47.41, 30.45, 38.05 and 39.55% reduced in compared to control at 80% of 24 h $LC_{50}$ against *S. oryzae*, respectively (Table 1).

The essential oils of *A. marmelos, C. reticulata, M. arvensis* and *S. aromaticum* caused less damage to the grains by adults of stored-grain insect pest *S. oryzae* by fumigation action. The percent grains infection was reduced by *A. marmelos, C. reticulata, M. arvensis* and *S. aromaticum* essential oils against *S. oryzae* was 74.520±0.82, 66.23±0.56, 57.30±0.61 and 59.56±0.48 at 60% sub-lethal concentration of 24 h $LC_{50}$ (Table 2).

The decrease in oviposition potential of *S. oryzae* ($F = 222.440$; 102.425; 260.782 and 202.163) ($df = 3.20$; $p<0.01$) was significant when fumigated with *A. marmelos, C. reticulata, M. arvensis and S. aromaticum* essential oils, respectively (Table 3).

The regression analysis indicated that per cent damage grain reduction of adults of *S. oryzae* by essential oils showed a significant negative correlation ($df = 2.15$; $p<0.01$) with concentration when fumigated with *A. marmelos* ($F = 606.31$), *C. reticulata* ($F = 517.54$), *M. arvensis* ($F = 408.75$)

Table 1: Effect of fumigation of *Aegle marmelos, Citrus reticulata, Mentha arvensis* and *Syzygium aromaticum* essential oils on oviposition of stored-grain insect pest *Sitophilus oryzae*

| Essential oils | 24 h $LC_{50}$ (µL) | Treatment 24 h $LC_{50}$ (%) | No. of eggs/larvae produced per 10 insects (Mean±SE) | Eggs/larvae produced per 10 insects (%) | Hatchability (%)[a] |
|---|---|---|---|---|---|
| | | Control | 261.66±3.06 | 100 | 100 |
| *Aegle marmelos* | 18.488 | 40 | 165.00±4.281 | 63.05 | 22.65 |
| | | 60 | 135.00±5.163 | 51.59 | 31.93 |
| | | 80 | 93.33±6.146 | 35.66 | 47.41 |
| *Citrus reticulata* | 24.471 | 40 | 200.83±5.83 | 76.75 | 13.15 |
| | | 60 | 176.66±5.57 | 67.51 | 19.39 |
| | | 80 | 139.50±5.31 | 53.31 | 30.45 |
| *Mentha arvensis* | 17.326 | 40 | 183.33±3.33 | 70.06 | 17.60 |
| | | 60 | 133.33±4.94 | 50.95 | 32.48 |
| | | 80 | 117.50±4.42 | 44.90 | 38.05 |
| *Syzygium aromaticum* | 18.326 | 40 | 178.33±4.77 | 68.15 | 18.93 |
| | | 60 | 148.33±4.77 | 56.68 | 27.64 |
| | | 80 | 113.33±4.94 | 43.31 | 39.55 |

[a]Hatchability percentage was calculated as 100(C-T)/(C+T), where C and T represent the No. of eggs/larvae produced in the control and in the test, respectively

Table 2: Effect of fumigation of *Aegle marmelos, Citrus reticulata, Mentha arvensis and Syzygium aromaticum* essential oils on damage caused by stored-grain insect pest *Sitophilus oryzae*

| Essential oils | 24 h $LC_{50}$ (µL) | Treatment 24 h $LC_{50}$ (%) | Grain damage reducinon (Mean±SE) (%) |
|---|---|---|---|
| *Aegle marmelos* | 18.488 | 30 | 48.53±0.35 |
| | | 60 | 74.52±0.82 |
| *Citrus reticulata* | 24.471 | 30 | 39.81±0.62 |
| | | 60 | 66.23±0.56 |
| *Mentha arvensis* | 17.326 | 30 | 33.87±0.38 |
| | | 60 | 57.30±0.61 |
| *Syzygium aromaticum* | 18.326 | 30 | 47.23±0.60 |
| | | 60 | 59.56±0.48 |

Table 3: Regression parameters of sub lethal and chronic activity of *A. marmelos, C. reticulata, M. arvensis* and *S. aromaticum* essential oils on stored-grain insect pest *S. oryzae* by fumigation method

| Treatment | Parameters (%) | Intercept | Slope | Regression equation | Regression coefficient | *F-value (p<0.01) |
|---|---|---|---|---|---|---|
| *Aegle marmelos* | Oviposition | -1.54 | 1.24 | Y = -1.54+1.24X | 0.996 | 222.440* |
| | FDI | -1.69 | 0.79 | Y = -1.69+0.79X | 0.991 | 606.31** |
| *Citrus reticulata* | Oviposition | -1.40 | 1.40 | Y = -1.40+1.40X | 0.997 | 102.425* |
| | FDI | -1.59 | 0.89 | Y = -1.59+0.89X | 0.993 | 517.59** |
| *Mentha arvensis* | Oviposition | 0.49 | 1.73 | Y = 0.49+1.73X | 0.998 | 260.782* |
| | FDI | -1.47 | 1.03 | Y = -1.47+1.03X | 0.994 | 408.75** |
| *Syzygium aromaticum* | Oviposition | -0.73 | 1.36 | Y = -0.73+1.36X | 0.989 | 202.163* |
| | FDI | -2.17 | 0.90 | Y = -2.17+0.90X | 0.947 | 488.36** |

FDI (%): Feeding deterrence index. *F values were significant at all probability levels (90, 95 and 99%), *df = 3,20; **df = 2, 15

and *S. aromaticum* (F = 488.36) essential oil (Table 3). Analysis of variance revealed that *A. marmelos* essential oil had strong chronic activity with sub-lethal concentration against rice weevil in comparison to other essential oils (Table 3).

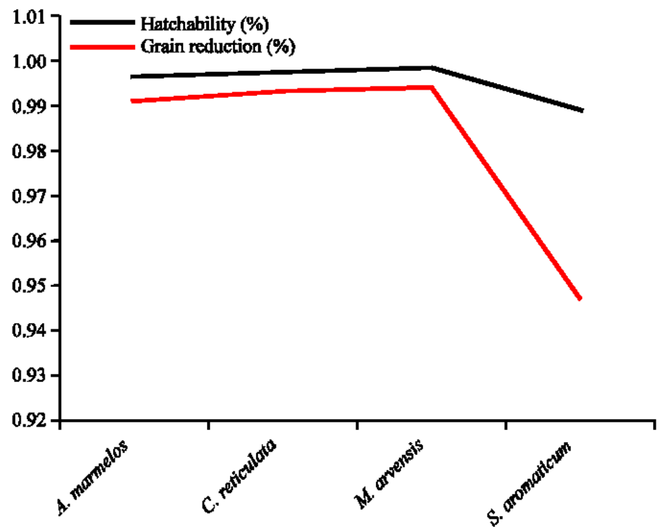


Fig. 1: Percent hatchability and percent grain reduction assay when *Sitophilus oryzae* adults were fumigated with essential oils by chronic exposure

## DISCUSSION

This insecticidal activity in essential oils are may be due to presence of volatile chemicals present in essential oils having different functional groups which persist for longer time if used against stored grain insects in closed chambers. On an average each essential oils extract has shown 60-75% oviposition and damaging food reduction. This high oviposition inhibition and feeding deterrence in all four essential oils was due to volatile components present in essential oils available in leaves, peels and buds.

In GC-MS analysis, the essential oils of *A. marmelos*, *C. reticulata*, *M. arvensis* and *S. aromaticum* show different chemical components. The leaf essential oil of *A. marmelos* contain 15 compounds, in all of this limonene was the main constituent (Kaur *et al*., 2006). The *Citrus* genus extracts are monocyclic monoterpenoides and the main constituents are d-limonene show insecticidal activity (Karr and Coats, 1988). The major constituents reported from essential oils of *M. arvensis* are L-menthone (29.41%), menthol (21.33%), isomenthone (10.80%), eucalyptol (6.91%), neo-menthol (4.70%), cis-piperitone oxide (3.62%), linalool (2.20%), thymol (1.60%), di-Limonene (1.47%) and a-Phellandrene (3.20%) (Mishra *et al*., 2012). In clove essential oil of *S. aromaticum*, eugenol, caryophyllene, eugenol acetate and alpha-humelene are present and in all of these, eugenol is the main constituent (Viuda-Martos *et al*., 2007).

When adult weevils were exposed to sub-lethal dose of essential oils; these have shown significantly oviposition inhibition in female insects and block the emergence of F1 individuals from exposed eggs. From the result it was found that oviposition inhibition in *S. oryzae* was dose and time dependent because when the concentration of essential oils increased oviposition inhibition in adults was also increased.

It is clear from the results section that all the essential oils showed a significant ($p<0.01$) reduction in oviposition and chronic toxic activity against adults of *S. oryzae* compared to control. Maximum oviposition inhibition and chronic toxic activity was observed by *A. marmelos* against adults of *S. oryzae* (Table 1, 2).

The present findings are similar to the observations of Kumar *et al.* (2008) who tested insecticidal activity of *A. marmelos* essential oil against four stored-grain insect pests and found significant reduction in oviposition and chronic activity against four stored-grain insect pests. The monoterpenoids are present in *A. marmelos* essential oil and they inhibit reproduction of stored insects at several steps of their life cycles. Sur *et al.* (1999) reported anti spermatogenic acitivity of ethanolic extract of *A. marmelos* leaves in rats. Similarly, Remya *et al.* (2009) studied the effect of ethanol extracts of leaves of *A. marmelos* for their *in-vitro* effect on sperm motility and suggested that extracts had a considerable effect on the motility of sperms.

In the present investigation, *C. reticulata* essential oil also showed the reduction in adult emergence which could either is due to the egg mortality or larval mortality or even reduction in hatching of the eggs. Similarly, Zewde and Jembere (2010) evaluated the orange peel *C. sinensis* as a source of protectant against *Zabrotes subfasciatus* (Coleoptera: Bruchidae) and found significant reduction in progeny emergence of *Z. subfasciatus*. The current findings are supported with to the results of Tripathi *et al.* (2003) who has also reported oviposition reduction (94.5%) effects of orange peel oil against *T. castaneum*.

Mentha oil is lipophilic in nature and accumulates with lipids, it had a strong morphogenic effect acting by fumigation and disrupting adult development. The reduction in oviposition and adult emergance may be due to suffocation and inhibition of various biosynthesis processes of the insects at developmental stages. Mesbah *et al.* (2006) reported that all the efficiency tested essential oils acted principally as insect growth inhibitors causing disruption of insect development and abnormal adults that were lead finally to death.

Bhat and Kempraj (2009) found that the hatchability of the eggs was affected by the presence of leaf and buds of clove oils. The present investigation was also supported to the result of Sharma and Meshram (2006) that evaluated insecticidal activity of essential oils of *Acorus calamus* (Acoraceae) and *S. aromaticum* (Myrtaceae) against *S. oryzae* as seed protector. The use of both essential oils exhibited inhibition of F1 progeny from 61.08-91.52%.

Recently, Jeyasankar *et al.* (2010) tested the antifeedent and growth inhibitory activities of *Syzygium lineare* leaves against fourth instars larvae of *Spodoptera litura* and found that high pupal, adult deformities and decreased adult emergence of *S. litura*. Ho *et al.* (1994) investigated the biological activity of *S. aromaticum* oil against stored-products pests. They found that this oil suppress progeny development of *T. castaneum* with isoeugenol being particularly active.

## CONCLUSION

The present study indicates that after fumigation of the essential oils obtained from *A. marmelos, C. reticulata, M. arvensis* and *S. aromaticum* reduce the oviposition of *S. oryzae*. Observation further reveal that per cent infestation rate of *S. oryzae* is also drastically affected. Therefore, the essential oils obtained from the aforesaid plants products can be used to protect stored-grains from the infestation of rice weevil.

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