Repellent effect of leaves essential oils from *Eucalyptus globulus* (Mirtaceae) *and Ocimum basilicum* (Lamiaceae) against two major stored grain insect pests of Coleopterons

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Abstract: The essential oils were extracted by water distillation method from two medicinal plants *Eucalyptus globulus* (Mirtaceae) and *Ocimum basilicum* (Lamiaceae) leaves and its repellent activity was investigated against two major economic important stored-grain insect pests red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) and rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionide) adults. The results indicated that the repellency of *E. globulus* and *O. basilicum* was 9.16±0.30 and 8.50±0.22 for *T. castaneum* and 8.66±0.33 and 8.16±0.30 for *S. oryzae.* The repellency of both insect pests increased with concentration from 0.05% to 0.40% at exposure time of 4 h. These observations confirmed that the essential oils which were extracted from these two medicinal plants leaves have more repellency against both insect pests.

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Introduction

Rice weevil *Sitophilus oryzae* L. (Coleoptera: Curculionide) and red flour beetle *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae) are very common pest infesting on many flours, cereals, meal, grains etc. They have world-wide distribution and are among the most economically important stored product pests (Aitken, 1975; Weston and Rattlingourd, 2000; Pugazhvendon et al., 2009). These stored grain insect pests have been damaging our economy by infesting agricultural stored products. According to an estimate these are responsible for worldwide loss of 10-40% in the stored-grain annually (Matthews, 1993).

For the control of these insect pests many synthetic insecticides and fumigants are used, but their widespread use led to some serious problems including development of insect resistance to insecticides (Zettler and Cuperus, 1990; Ribeiro et al., 2003), toxic residues in food, toxicity to consumers and increasing coast of application (Sighamony et al., 1990). The uncontrolled use of these synthetic pesticides caused great hazards for environment and consumers due to residual property (White, 1995). However, there is an urgent need to develop safe alternatives that are of low cost, convenient to use and environmentally friendly (Hassanali et al., 1990; Jember et al., 1995). The plants volatile essential oils extracted from aromatic plants have insecticidal properties could be considered as alternative insecticides (Shaaya et al., 1991; Shukla et al., 2008). Many plant volatile essential oils and their constituents have been studied to posses potential as alternative compound and gaining tremendous importance for the management of stored products and these are ecologically safe and biodegradable (Rajendran and Sriranjini, 2008; Batish et al., 2008; Cosmi et al., 2009). The essential oils are volatile with high insecticidal efficiency and very low persistence. The active compounds present in essential oils are specific to particular insect groups (Huang et al., 1997) and not to mammals (Isman, 2000), many of them are not dangerous to human.

Eucalyptus globulus (Mirtaceae) and *Ocimum basilicum* (Lamiaceae) are two important medicinal plants. The essential oils of different species of both plants leaves and their constituents have pesticidal properties against insect pests (Nagssoum et al., 2007). Therefore, the purpose of present work was to investigate the repellent properties of leaves essential oils of *Eucalyptus globulus* and *Ocimum basilicum* against stored grain insect pests.

Material and Methods

Isolation of essential oils

Essential oils were extracted from leaves of *Euculyptus globulus* and *Ocimum basilicum.* The leaves were collected from the local area of Gorakhpur, district of Uttar Pradesh, India during March to June 2011. The leaves were dried in absence of sunlight at room temperature (30±5^{0}C)

and grounded by domestic mixer. The dried powdered material was hydro-distilled in Clevenger apparatus continuously for five hours to yield essential oils. The oils were collected in glass containers and kept in appendorff tubes at 5^0C till their use.

Insect rearing

Red flour beetle *T. castaneum* and rice weevil *S. oryzae* were used to determine the repellent activity of essential oils. The insects were reared on wheat flour and grain in laboratory at 30±2^{0}C, 75±5% RH and at photoperiod of 10:14 (L: D) hours.

Repellency

The repellency test used was adopted after (McDonald et al., 1970; Talukder and Howse, 1993, 1994). Four solutions of 0.05%, 0.10%, 0.20% and 0.40% of essential oils were prepared by dissolving essential oils in acetone. Whatman no. 1 filter papers ware cut into two equal halves one half of each dish was treated with essential oil solution as uniform as possible by using micro pipette. The other half of the filter paper was treated with acetone only. The essential oil treated and acetone treated filter papers halves were dried to evaporate the solvent completely. Essential oil treated and acetone treated half-dishes were then attached lengthwise, edge-to-edge with adhesive tape and placed at the bottom in glass petri dish (height 15 mm × radius 45 mm). Ten adults of insects were released at the centre of the petri dishes and then petri dishes were covered and kept in dark. Six replicates were set for each concentration of essential oils. Number of the insects on both treated and untreated halves were recorded after four hours in mild light.

Data analysis

All the values obtained during observation were represented as mean ± SE. Chi-square test was applied to establish the repellent activity of the essential oils tested (Sokal and Rohlf, 1973).

Result

The repellent action of these two essential oils was studied against *T. castaneum* and *S. oryzae*. Data in (Table-1) showed that at highest concentration 0.40%, the essential oil of *Eucalyptus globulus* had strong repellent action (9.16±0.30) and (8.66±0.33) against *T. castaneum* and *S. oryzae*. Similarly, at highest concentration 0.40% the *Ocimum basilicum* essential oil showed (8.50±0.22) and (8.16±0.30) against *T. castaneum* and *S. oryzae*, respectively (Table-2). The repellent activity of *E. globulus* and *O. basilicum* progressively increase with increase in concentration against both stored-grain insect pests.

The chi-square test showed a significant ($P<0.05$) repellency of the both plants leaves against both stored grain insect pests but *E. globulus* had strong repellency in comparison to *O. basilicum* against *T. castaneum* and *S. oryzae.*

Discussion

Plant products especially in the form of essential oils having considerable potential as insecticidal compounds are gaining tremendous importance in recent years. In our present study the leaves essential oil of *Eucalyptus globulus* and *Ocimum basilicum* was significantly ($P<0.05$) repellent at very low concentration against the flour beetle and rice weevil and join a series of some other essential oils which have similar insecticidal effects on the management of pests and provides a scientific relation for their use in control of stored-grain insect pests (Hassanali et al., 1990; Bekele et al., 1996; Bouda et al., 2001). The repellent effect of volatile essential oils may have an important implication in traditional post harvest storage system and have potential activity for their local availability make it attractive candidate in management of stored-grain insect pests.

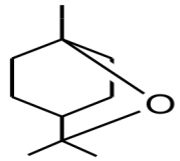
Simillar observation on the other plants extracts effect on several insect pests have been reported that the essential oils of *Schyzygium aromaticum, Aegle marmelos, Corriandrum sativum* and *Citrus reticulata* extracted by a water distillation method showed strongly repellency against *S. oryzae* and *T. castaneum* even at low concentration but its repellency was more marked towards *S. oryzae* (Mishra and Tripathi, 2011). The essential oil of *Piper nigrum* (L.) repelled the adults of *T. castaneum* at low concentration (Upadhyay and Jaiswal, 2007). The plant volatile essential oils isolated from *Trachyspermum ammi*, *Anethum graveolens*, *Nigella sativa* show insecticidal activity against *T. castaneum* (Chaubey, 2007)**,** while the leaf essential oil of *Melaleuca cajuputi* had different insecticidal activity against *Sitophilus zeamais* and *T. castaneum* but 100% repellency was only occurred in *T. castaneum* (Ko et al., 2009). The essential oil derived from Citrus peels possesses pesticidal activity against both insect pests *T. castaneum* and *S. oryzae* (Mishra et al., 2011). The effect of essential oil of *Ocimum grattissimum* leaves on *S. zeamais* was assessed for repellency and toxicity. The oil was found to be moderately repellent to the *S. zeamais* (Aswalam et al., 2008). Similarlly, the ethyl acetate extract of *Citrullus colocynthis* showed highest repellent activity in lower concentration against *Callosobruchus maculates* (Prabhu Seenivasan et al., 2004**).**

Essential oils and their constituents of different *Eucalyptus species* (Mirtaceae) and *Ocimum species*

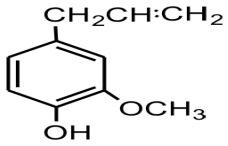
(Lamiaceae) show many insecticidal activity against stored-grain insect pests (Keita et al., 2001; Jirovetz et al., 2005; Brito et al., 2006; Ngassoum et al., 2007). The main component present in essential oils of *Eucalyptus species* are 1-8 cineole and in *Ocimum basilicum* are eugenol which showed different insecticidal properties against insect pests (Lee et al., 1997; Aswalam et al., 2008).

All the above findings clearly support the result of the present study. The mode of action of both essential oils is yet to be confirmed but the repellency of adults stored-grain insect pests may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism (Don-Perdo, 1989).

Therefore, essential oils from leaves of *Eucalyptus globulus* and *Ocimum basilicum* may be recommended as cheap, easily available at farmer level, eco-friendly with low mammalian toxicity and good alternative to synthetic insecticides. It could further reduce the application of the synthetic chemicals.



1,8 Cineole



Eugenol

Table 1: Repellency caused by *Eucalyptus globulus* against adults of *Tribolium castaneum* and *Sitophilus oryzae* after four hours in filter paper test

Insect pests	Con. (%) vol: vol	Mean number of insects untreated±SE	Mean number of insects treated ±SE	χ2 value P<0.05 (df=5)
Tribolium castaneum	0.05%	5.66±0.21	4.33±0.21	0.072 [NS]
	0.10%	7.33±0.33	2.66±0.33	1.058 [S]
	0.20%	8.00±0.25	2.00±0.25	1.800 [S]
	0.40%	9.16±0.30	0.83±0.30	3.461 [S]
Sitophilus oryzae	0.05%	5.16±0.30	4.83±0.30	0.005 [NS]
	0.10%	6.83±0.30	3.16±0.30	0.669 [S]
	0.20%	7.66±0.33	2.33±0.33	1.415 [S]
	0.40%	8.66±0.33	1.33±0.33	2.679 [S]

Adults of *T. castaneum* and *S. oryzae* were used in filter paper repellency assay. For each concentration of essential oils six replicate were carried out and 10 adults were used per replicate. Mean of untreated and treated halves in filterpaper repellency assay.

a. Not significant (NS) as the calculated values of χ2 were less than the table values at probability levels (99%). Significant (S) at probability levels (99%).

Table 2: Repellency caused by *Ocimum basilicum* against adults of *Tribolium castaneum* and *Sitophilos oryzae* after four hours in filter paper test

Insect pests	Con. (%) vol: vol	Mean number of insects untreated ±SE	Mean number of insects treated ±SE	χ2 value P<0.05 (df=5)
Tribolium castaneum	0.05%	5.33±0.21	4.66±0.21	0.021 [NS]
	0.10%	7.00±0.25	3.00±0.25	0.800 [S]
	0.20%	7.50±0.22	2.50±0.22	1.250 [S]
	0.40%	8.50±0.22	1.50±0.22	2.450 [S]
Sitophilus oryzae	0.05%	5.16±0.30	4.83±0.30	0.005 [NS]
	0.10%	6.83±0.30	3.16±0.30	0.669 [S]
	0.20%	7.16±0.40	2.83±0.40	0.933 [S]
	0.40%	8.16±0.30	1.83±0.30	1.997 [S]

Adults of *S. oryzae* and *T. castaneum* were used in filter paper repellency assay. For each concentration of essential oils six replicate were carried out and 10 adults were used per replicate. Mean of untreated and treated halves in filterpaper repellency assay.

a. Not significant (NS) as the calculated values of $\chi 2$ were less than the table values at probability levels (99%). Significant (S) at probability levels (99%).

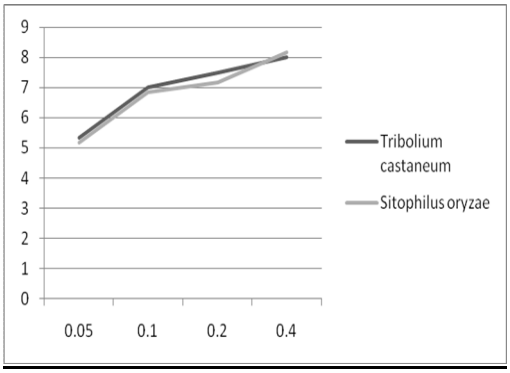


Figure 1. Repellent activity of the essential oil from *Eucalyptus globulus* against 2 major stored-grain insect pests.

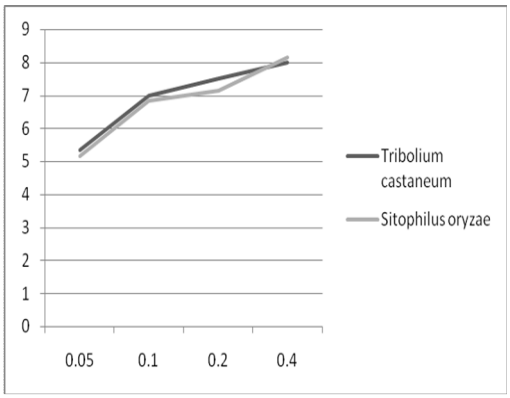


Figure 2. Repellent activity of the essential oil from *Ocimum basilicum* against 2 major stored-grain insect pests.

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