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# Biological activities of terpenes against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae)

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**ABSTRACT**

*Two terpenes, α-pinene and β-caryophyllene were investigated for repellent, insecticidal, ovipositional and egg hatching inhibition its activities against pulse beetle, Callosobruchus chinensis (Coleoptera:Bruchidae). α-Pinene and β-caryophyllene repelled bruchid adults in choice oviposition assay .α-Pinene and β-caryophyllene caused both fumigant and contact toxicity in C. chinensis adults in a concentration dependent manner. These two terpenes reduced oviposition in C. chinensis adults significantly when treated with sublethal concentrations by fumigation and contact method both. Reduction in oviposition was more when terpene compounds were applied by contact method than fumigation method. Both terpenes significantly reduced $F_1$ progeny emergence, damage and weight loss in grains in chronic toxicity assay. Reduction in $F_1$ progeny emergence, damage and weight loss in grains was more pronounced in case of β-caryophyllene than α-pinene. Application of these two terpenes showed no effect on germination of seeds. Present study suggests that α-pinene and β-caryophyllene can be useful as promising agent in insect pest management programme.*

**Key words:** α-Pinene, β-Caryophyllene, oviposition deterrence index, hatching inhibition rate

## INTRODUCTION

Since the beginning of agricultural practices, storage of food grains as a safeguard against poor harvests and famine started and since then insect pests are damaging stored grains both quantitatively and qualitatively. This damage amounts to10-40% in countries depending on traditional storage technologies. In India, this approaches10% of total production at farm level [1,2]. This creates a major problem in storing food grains leading to its wastage. Among important stored-product insect pests, pulse beetle *Callosobruchus chinensis* (Order: Coleoptera, Family: Bruchidae) is a serious pest infesting gram, cowpea, beans, lentil and other pulses. Under storage condition, *C. chinensis* causes 32-64% loss especially during April to October [3]. Only grubs are infective stages. These make holes in grains and consume inner part leaving empty kernel, and damaged grains become unpalatable for human, incapable of producing sprout and lose its market value.

Application of different synthetic fumigants, sprays and dusts are in practice for the insect pest management programmes, but some of them have been proved unsuccessful because of high migration rate of insect pests during transportation from field to godowns [4]. Excessive and continuous uses of chemicals have developed resistance in them causing loss of several billion dollars each year [5-7]. Besides these, synthetic insecticides

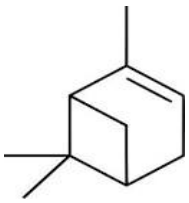
increase chances of ozone depletion, neurotoxicity, carcinogenicity, teratogenicity and mutagenic effects in non-target animals and cross- and multi-resistant in targets [8-11]. These problems have diverted scientific interest regarding human and environment safety and plant products come into existence in stored-grain insect pest management programme since last two to three decades. Different workers have used different plant based insecticidal agents especially essential oils [12-15]. Essential oils being secondary metabolites produced in different plant parts have strong odour, volatility and lower density [16]. Due to their volatility, essential oils are non persistent environmentally and 'generally recognized as safe' by United States Food and Drug Administration [17]. Essential oils are produced in different members of families like Apiaceae, Asteraceae, Cupressaceae, Myrtaceae, Lamiaceae, Lauraceae, Piperaceae, Poaceae, Rutaceae and Zinziberaceae. The chemical constituents and their biological activities of essential oils vary with plant parts used for extraction, extraction method, plant phenology, harvesting season, plant age, soil and environmental conditions of habitat [18,19]. The biological activities of essential oils depend on the major constituents present. In the present study, two terpenes viz. α-pinene and β-caryophyllene have been evaluated for their repellent insecticidal, antiovipositional and egg hatching inhibitory activities against pulse beetle, *C. chinensis.*

α-Pinene is a monoterpene containing a reactive four-membered ring. It is found as a important constituent in essential oils of *Nepta racemosa*, *Ferulago* spp., *Syzygium aromaticum*, *Biden pilosa*, *Zingiber officinale*, *Eucalyptus* spp., *Citrus* spp. and *Vicia dadianorum* [20-29]. β-Caryophyllene, a bicyclic sesquiterpene having cyclobutane ring, has been reported in *Piper cubeba*, *Scutellaria pinnat*, *Ferulago* spp., *Syzygium aromaticum*, *Biden pilosa*, *Eucalyptus* spp., *Citrus* spp. and *Pistacia lentiscus* essential oils [21,22,24-26,29-32].

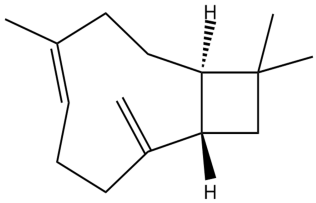
## MATERIALS AND METHODS

***Terpenes***

Two pure terpenecompunds viz. monocyclic monoterpene, α-pinene **(**(±)-2-Pinene, 2, 6, 6 Trimethylbicyclo [3.1.1] hept-2-ene**)** and bicyclic sesquiterpene, β-caryophyllene (4,11,11-trimethyl-8-methylene-bicyclo [7.2.0] undec-4-ene) were purchased from Sigma Chemicals, USA.



**(±)-2-Pinene,2,6,6 Trimethylbicyclo[3.1.1]hept-2-ene**




**4,11,11-trimethyl-8-methylene-bicyclo[7.2.0]undec-4-ene**

***Insect***

Pulse beetle, C. *chinensis* were used to investigate the biological activities of pure terpene compounds. The insects were reared on cow pea seeds in laboratory at30±2°C,75±5%RH and a photoperiod of 12:12(L:D)h.

***Repellency assay***

In a plastic box (10 cm diameter and 13 cm height), two transparent glass vials ( 3 cm diameter and 10 cm height) with screw cap interconnected horizontally by a plastic tube (2 cm long and 1.5 cm diameter) 2 cm above the base were taken. One vial of the pair was supposed as treated while other as untreated. A filter paper disc (2.5 cm diameter) treated with 0.5 ml aliquot of solution (prepared by dissolving 5, 10, 15 and 20 µl of pure terpene compound in acetone) was pasted under the cover of vial (treated). In the untreated vial of the box, filter paper treated with acetone only was applied as in the treated. Solvent was allowed to evaporate from filter paper disc for 5 minutes. Neck of the vial was blocked by a piece of plastic mesh. In each vial, 20 cowpea seeds were taken and introduced 0-24 h old 10 adults of mixed sex into it. After 96h, the number of eggs laid on cow pea seeds was counted in treated and untreated vial of the box. For each concentration of compound, six replicates were set. Percent Repellency (PR) was measured by comparing the number of eggs laid on cowpea seeds in treated vial against the number of eggs laid on cow pea seeds in untreated using the

formula:

$$PR = \frac{N_{UT} - N_T}{N_{UT} + N_T} \times 100$$

$N_{UT}$ = Number of eggs in untreated vial, and $N_T$ = Number of eggs in treated vial

***Toxicity assays***

**Fumigant Toxicity assay:** Fumigant toxicity of α-pinene and β-caryophyllene was determined against 2-4 days old bruchid adults using glass vials (3 cm diameter and 10 cm height) with screw cap. Test solutions of different concentrations were prepared by diluting terpene compounds with acetone. For fumigation, filter paper strip (2.5 cm diameter) impregnated with 100µl aliquot of test solution was pasted on the inner side of cap and solvent was allowed to evaporate for 5 min. Neck of vial was blocked with a piece of plastic mesh to avoid the contact effect of test solution. Twenty cowpea seeds were taken in each vial and into it ten adults were introduced. Open end of vial was closed by screw cap so that compound treated filter paper remained inside vial. All the vials were kept in conditions maintained for insect culture. Mortality in adults was recorded after 24h, 48h, 72h and 96 h of treatment. In control, filter paper impregnated with solvent only was used. For each compound, four different concentrations and for each concentration of terpene compound and control six replicates were set.

**Contact toxicity assay:** Contact toxicity of α-pinene and β-caryophyllene was determined against 2-4 days old bruchid adults using glass vials (3 cm diameter and 10 cm height) with screw cap. Test solutions of different concentrations were prepared by diluting pure compounds with acetone. A 2 ml aliquot of test solution was applied on whole inner surface of vials and under surface of screw cap by rolling it. The treated vial was kept open for 5 min to evaporate solvent. Twenty cowpea seeds were taken in each vial and into it ten adults were introduced. Vials were closed and kept in conditions maintained for insect culture. Mortality in adults was recorded after 24h, 48h, 72h and 96h of treatment. In control, filter paper impregnated with solvent only was used. For each compound, four different concentrations and for each concentration compound and control six replicates were set.

***Oviposition inhibition assay***

**By fumigation method:** In this assay, ten 0-24h old bruchid adults were fumigated with two sublethal concentrations (40% of 96h-$LC_{50}$ and 40% of 96h-$LC_{50}$ determined in fumigation toxicity assay) of terpene compound solutions as was done in fumigation toxicity assay. After 96h of fumigation, number of eggs laid over the cowpea seeds was counted. For each concentration of terpene compound as well as control group, six replicates were set. In control group only solvent was used.

**By contact method:** In this assay, ten 0-24h old bruchid adults were treated with two sublethal concentrations (40 and 80% of 96h-$LC_{50}$ determined in contact toxicity assay) of terpene compound solutions as was done in contact toxicity assay. After 96h of treatment, number of eggs laid over the cowpea seeds was counted. For each concentration of terpene compound as well as control group, six replicates were set. In control group only solvent was used.

Percent Oviposition Deterrence Index (%ODI) was calculated as:

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

C = Number of eggs in control, and T = Number of eggs in test

***Ovicidal assay***

In ovicidal assay, 25 eggs were fumigated with test solutions prepared by diluting terpene compounds with acetone. A 100 µl aliquot of test solution was applied on filter paper strip (2.5 cm diameter) and solvent was allowed to

evaporate for 5 min. The filter paper was pasted to under surface of screw cap of vial (3 cm diameter and 10 cm height). Cap of vial was screwed and incubated for 96 h in conditions maintained for insect culture. After fumigation, eggs were allowed to hatch and number of egg hatched was recorded after 14 days of treatment. Four different concentrations of each terpene were used and for each concentration and control six replicates were set. Percent Hatching Inhibition Rate (% HIR) was calculated as:

$$\%HIR = \frac{Cn-Tn}{Cn} \times 100$$

Cn= Number of adults in control, and Tn= Number of adults in test

**Chronic toxicity assay:** In chronic toxicity assay, 100 gm of cowpea seeds was taken into a plastic box (7 cm diameter and 11 cm height), and mixed well with 2 ml aliquot of test solution prepared by diluting pure terpene compounds in acetone. Twenty 0-24 h old bruchid adults were introduced into the box. Number of $F_1$ progeny emerged was counted after 24 days of initiation of the experiment and removed.The counting and removal of $F_1$ progeny emerged was continued for 5 days more. The potency of the terpene compounds was estimated as percent protection (PP) using formula:

$$PP = \frac{N_{UT} - N_T}{N_T} \times 100$$

$N_{UT}$ = Number of $F_1$ progeny in untreated, and $N_T$ = Number of $F_1$ progeny in treated

After 90 days, weight loss in the seeds was estimated and represented as percent weight loss. The damaged and undamaged cowpea seeds were counted and represented as percent grain damage.

***Seed germination***

Hundred cowpea seeds used in the chronic were taken at the end of the chronic toxicity experiment and placed on the moistened filter paper and allowed to germinate. The number of cowpea germinated seeds was recorded for ten days. For each concentration of terpene compound and control, sex replication was made.

***Data analysis***

Median lethal concentration ($LC_{50}$) was calculated by POLO programme [33] and one way analysis of variance (ANOVA) was performed to test significancy of data [34].

## RESULTS

***Repellency assay***

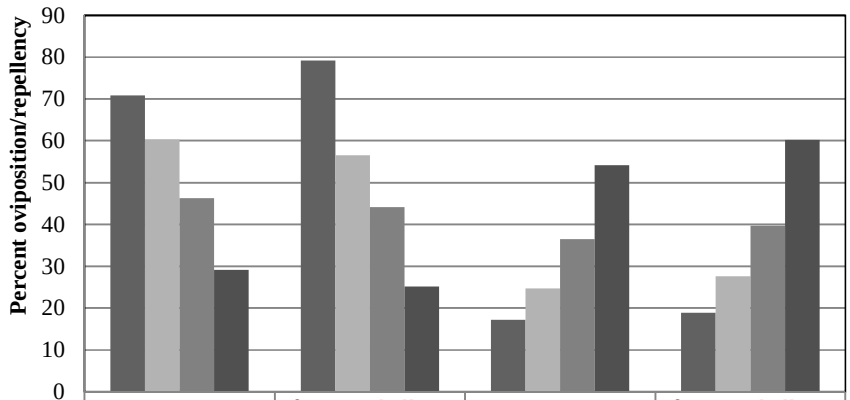
α-Pinene and β-caryophyllene inhibit oviposition in repellency assay in concentration dependent manner. Oviposition was reduced to 70.85, 60.25, 46.27 and 29.15%; and 79.15, 56.56, 44.18 and 25.18% at 0.071, 0.142, 0.213 and 0.284 µlcm$^{-3}$ concentration of α-pinene and β-caryophyllene respectively (Figure 1). This reduction in oviposition was estimated in terms of percent repellency (PR). PR was found 17.19, 24.70, 36.47 and 54.17; and 18.93, 27.62, 39.71 and 60.21 at 0.071, 0.142, 0.213 and 0.284 µlcm$^{-3}$ concentration of α-pinene and β-caryophyllene respectively (Figure 1).

***Toxicity assay***

α-Pinene and β-caryophyllene caused both fumigant and contact toxicity in *C. chinensis* adults. In fumigation toxicity assay, median lethal concentrations ($LC_{50}$) were determined 0.688, 0.459, 0.427 and 0.316 µl cm$^{-3}$air; and 0.885, 0.629, 0.587 and 0.505 µl cm$^{-3}$ air for α-pinene and β-caryophyllene after 24, 48, 72 and 96 hours respectively (Figure 2). In contact toxicity assay, median lethal concentrations ($LC_{50}$) were determined 2.73, 2.38, 1.94 and 1.29; and 0.184, 0.138, 0.124 and 0.081 µl cm$^{-2}$ for α-pinene and β-caryophyllene after 24, 48, 72 and 96 hours respectively (Figure 3).In fumigant toxicity assay, α-pinene showed higher toxicity than that of β-caryophyllene. On other hand, in contact toxicity assay, β-caryophyllene showed higher toxicity than that of α-pinene. Regression analysis showed concentration dependent correlation of terpene compounds and mortality in bruchid adults (Table 1).

**Table 1. Regression parameters of toxicity assays to study the effects of α-pinene and β-caryophyllene against *C. chinensis* adults**

| Compound | Toxicity | Exposure period | Intercept | Slope | Regression equation | Regression coefficient |
|---|---|---|---|---|---|---|
| α-Pinene | Fumigant | 24h | - 7.79 | 1.28 | Y = - 7.79+ 1.28X | 0.979 |
| | | 48h | - 5.72 | 1.96 | Y = - 5.72+ 1.96X | 0.981 |
| | | 72h | - 10.0 | 2.18 | Y = - 10.0+ 2.18X | 0.953 |
| | | 96h | - 9.59 | 2.99 | Y = - 9.59+ 2.99X | 0.959 |
| | Contact | 24h | - 8.90 | 1.68 | Y = - 8.90+ 1.68X | 0.955 |
| | | 48h | - 8.24 | 1.89 | Y = - 8.24+ 1.89X | 0.975 |
| | | 72h | - 9.55 | 2.33 | Y = - 9.55+ 2.33X | 0.960 |
| | | 96h | - 10.82 | 3.69 | Y = - 10.82+3.69 X | 0.934 |
| β-Caryophyllene | Fumigant | 24h | - 7.03 | 0.97 | Y = - 7.03+ 0.97X | 0.969 |
| | | 48h | - 7.08 | 1.24 | Y = - 7.08+ 1.24X | 0.976 |
| | | 72h | - 9.84 | 1.51 | Y = - 9.84+ 1.51X | 0.947 |
| | | 96h | - 10.76 | 1.89 | Y = - 10.76+ 1.89X | 0.960 |
| | Contact | 24h | - 10.09 | 24.29 | Y = - 10.09+ 24.29X | 0.968 |
| | | 48h | - 7.12 | 31.42 | Y = - 7.12+ 31.42X | 0.979 |
| | | 72h | - 11.86 | 40.29 | Y = - 11.86+ 40.29X | 0.946 |
| | | 96h | - 11.69 | 59.87 | Y = - 11.69+ 59.87X | 0.898 |

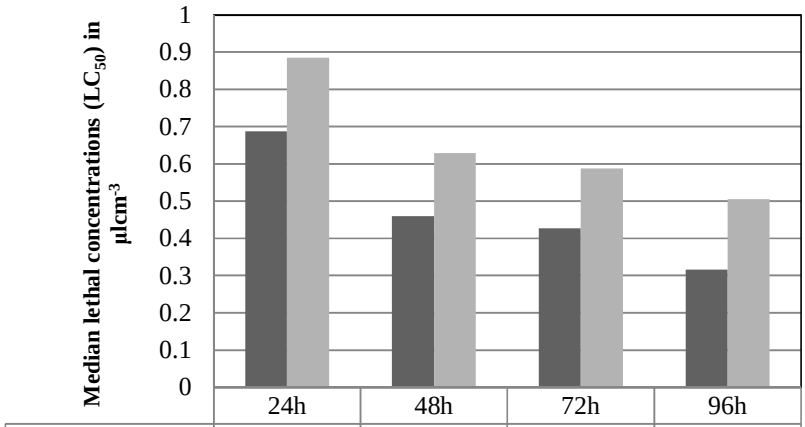



| | α-Pinene | β-Caryophyllene | α-Pinene | β-Caryophyllene |
|---|---|---|---|---|
| | Percent oviposition | | Percent repellency | |
| 0.071 | 70.85 | 79.15 | 17.19 | 18.93 |
| 0.142 | 60.25 | 56.56 | 24.7 | 27.62 |
| 0.213 | 46.27 | 44.18 | 36.47 | 39.71 |
| 0.284 | 29.15 | 25.18 | 54.17 | 60.21 |

**Figure 1. Effect of α-Pinene and β-Caryophyllene at different concentration of compound (0.071, 0.142, 0.213 and 0.284 µl $cm^{-3}$) on percent oviposition and percent repellency of *C. chinensis* adults in repellency assay**

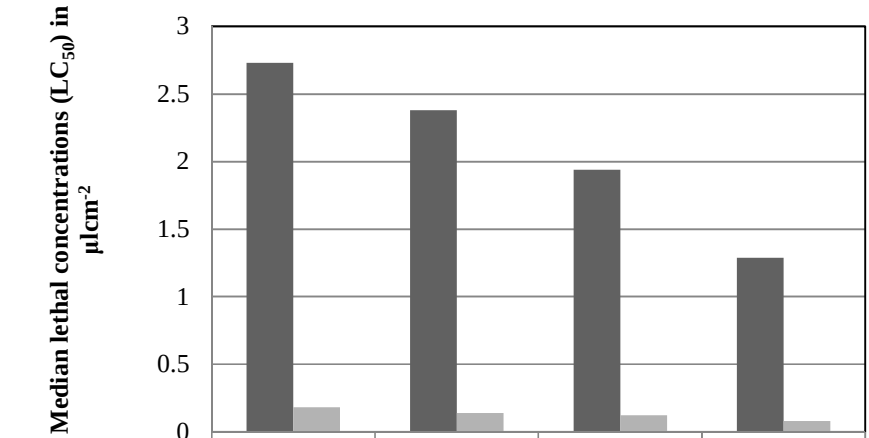
***Oviposition inhibition assay***

α-Pinene and β-caryophyllene significantly ($P<0.05$) reduced oviposition potency of bruchid adults when exposed to α-pinene and β-caryophyllene. In fumigation oviposition inhibition assay, mean numbers of eggs laid per insect was 16.23 and 10.83; and 15.83 and 11.68 when bruchid adults were fumigated with 40 and 80% of 96h-$LC_{50}$ of α-pinene and β-caryophyllene respectively as compared to 19.88 eggs laid per insect in control (Figure 4). In contact oviposition inhibition assay, mean numbers of eggs laid per insect was 12.55 and 8.17; and 11.96 and 7.28 when bruchid adults were fumigated with 40 and 80% of 96h-$LC_{50}$ of α-pinene and β-caryophyllene respectively as compared to 19.88 eggs laid per insect in control (Figure 4). Similarly, %ODI was calculated 10.10 and 29.59; and 11.34 and 25.98 when adults were fumigated with 40 and 80% of 96h-$LC_{50}$ α-pinene and β-caryophyllene respectively (Figure 5). The %ODI was calculated 22.60 and 45.31; and 29.16 and 46.56 when adults were treated with 40 and 80% of 96h-$LC_{50}$ of α-pinene and β-caryophyllene respectively (Figure 5).




| | 24h | 48h | 72h | 96h |
|---|---|---|---|---|
| α-Pinene | 0.688 | 0.459 | 0.427 | 0.316 |
| β-Caryophyllene | 0.885 | 0.629 | 0.587 | 0.505 |

**Figure 2. Median lethal concentrations ($LC_{50}$) of α-Pinene and β-Caryophyllene against *C. chinensis* adults at different exposure periods (24, 48, 72 and 96 h) in fumigant toxicity assay**




| | 24h | 48h | 72h | 96h |
|---|---|---|---|---|
| α-Pinene | 2.73 | 2.38 | 1.94 | 1.29 |
| β-Caryophyllene | 0.184 | 0.138 | 0.124 | 0.081 |

**Figure 3. Median lethal concentrations ($LC_{50}$) of α-Pinene and β-Caryophyllene against *C. chinensis* adults at different exposure periods (24, 48, 72 and 96 h) in contact toxicity assay**

***Ovicidal assay***

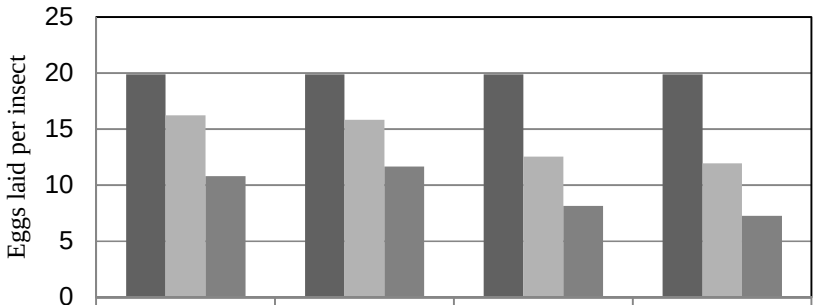
α-Pinene and β-caryophyllene significantly (P<0.01) reduced hatching rate in *C. chinensis* eggs when fumigated. Mean number of eggs hatched per 25 eggs was reduced to 20.16, 17.83, 12.83 and 9.5; and 19.33, 15.66, 11.5 and 8.0 when fumigated with 0.28, 0.42, 0.56 and 0.70 µlcm$^{-3}$ air of α-pinene and β-caryophyllene respectively as compared to 22.16 eggs hatched in control (Figure 6). Increase in %HIR was 9.72, 20.15, 42.54 and 57.45; and 13.43, 29.87, 48.59 and 64.17 when fumigated with 0.28, 0.42, 0.56 and 0.70 µlcm$^{-3}$ air of α-pinene and β-caryophyllene respectively (Figure 6).

***Chronic toxicity assay***

α-Pinene and β-caryophyllene significantly (P<0.01) reduced grain damage, weight loss and $F_1$ progeny production during chronic exposure of *C. chinensis* adults in comparison to untreated group. When *C. chinensis* adults were exposed to α-pinene and β-caryophyllene at concentration of 0.1, 0.2, 0.4 and 0.6 µl gm$^{-1}$in chronic toxicity assay, grain damage was reduced to 14.13%, 10.83%, 8.98% and 6.23%; and 11.72%, 9.81%, 7.03% and 4.0% respectively in comparison to the untreated where grain damage was reported 18.46% (Figure 7). In the chronic toxicity assay, weight loss in the treated cowpea seeds was recorded 6.72%, 5.86%, 4.52% and 3.43%; and 5.4%, 2.97%, 1.57%

and 0.46% when *C. chinensis* adults were exposed to α-pinene and β-caryophyllene at concentration of 0.1, 0.2, 0.4 and 0.6 µl $gm^{-1}$(Figure 7). This grain damage and weight loss in the cow pea seeds were due to the reduction in the $F_1$ progeny. The $F_1$ progeny represented in terms of percent protection was reduced to 14.85%, 26.09%, 33.55% and 52.2%; and 27.75%, 58.05%, 87.51% and 95.99% when *C. chinensis* adults were exposed to α-pinene and β-caryophyllene at concentration of 0.1, 0.2, 0.4 and 0.6 µl $gm^{-1}$ (Figure 8).

For α-Pinene: F = 24.56 (Fumigation method), 60.49 (Contact method)
For β-Caryophyllene: F = 21.01 (Fumigation method), 65.74 (Contact method)

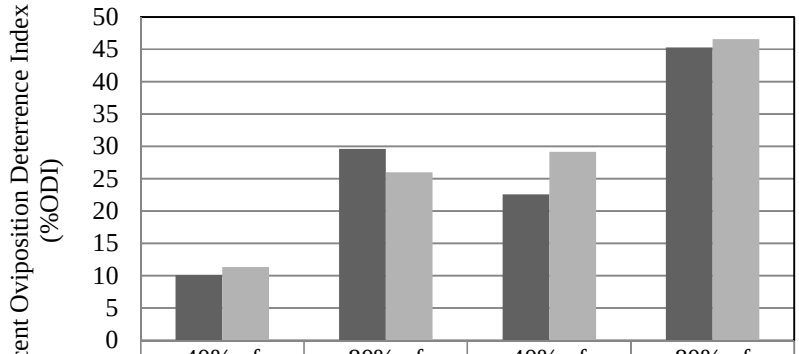



| | Fumigation method | | Contact method | |
|---|---|---|---|---|
| | α-Pinene | β-Caryophyllene | α-Pinene | β-Caryophyllene |
| Control | 19.88 | 19.88 | 19.88 | 19.88 |
| 40% of 96h-LC50 | 16.23 | 15.83 | 12.55 | 11.96 |
| 80% of 96h-LC50 | 10.8 | 11.68 | 8.17 | 7.28 |

**Figure 4. Effect of treatment with 40% and 80% of 96h-$LC_{50}$ of α-Pinene and β-Caryophyllene for 96 h by fumigation and contact method on eggs laid per insect when *C. chinensis*adults**

***Seed germination***

No adverse effect was observed on the seed germination when cowpea seeds were treated with α-pinene and β-caryophyllene in chronic toxicity assay. Percent seed germination was observed 99.5%, 99.66%, 98.66% and 98.5%; and 98.66%, 99.33%, 98.66% and 99% at concentration 0.1, 0.2, 0.4 and 0.6 µl $gm^{-1}$of α-pinene and β-caryophyllene respectively in comparison to 99.5% in the untreated cowpea seeds (Figure 9).




| | Fumigation method | | Contact method | |
|---|---|---|---|---|
| | 40% of 96h-LC50 | 80% of 96h-LC50 | 40% of 96h-LC50 | 80% of 96h-LC50 |
| α-Pinene | 10.1 | 29.59 | 22.6 | 45.31 |
| β-Caryophyllene | 11.34 | 25.98 | 29.16 | 46.56 |

**Figure 5. Effect of α-Pinene and β-Caryophyllene on oviposition deterrence index (%ODI) when *C. chinensis* adults**

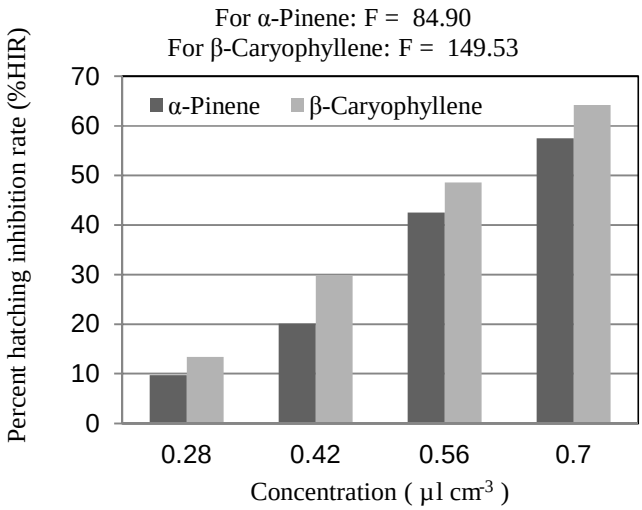
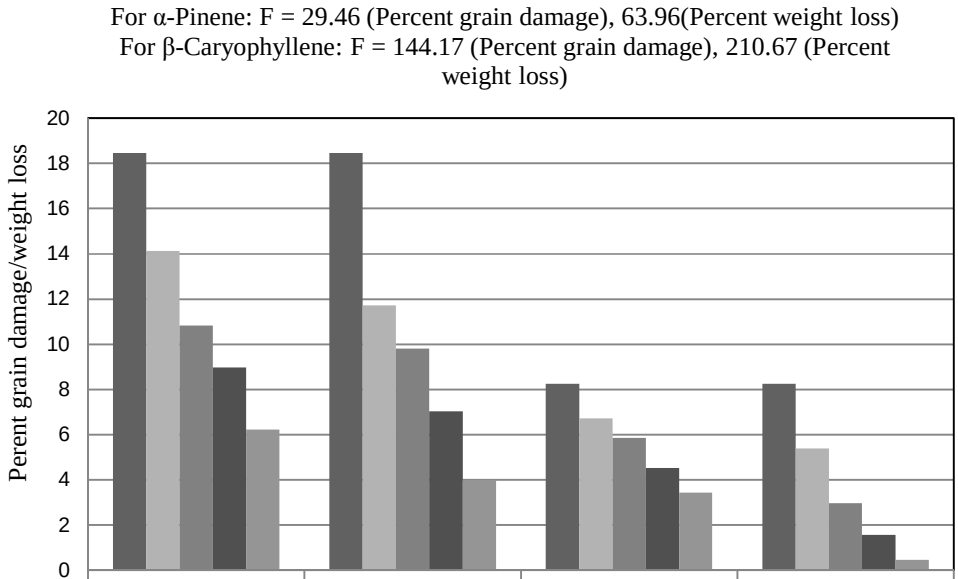



**Figure 6. Effect of α-Pinene and β-Caryophyllene on hatching rate of *C. chinensis* egg**




| | Percent grain damage | | Percent weight loss | |
|---|---|---|---|---|
| | α-Pinene | β-Caryophyllene | α-Pinene | β-Caryophyllene |
| Control | 18.46 | 18.46 | 8.25 | 8.25 |
| 0.1 | 14.13 | 11.72 | 6.72 | 5.4 |
| 0.2 | 10.83 | 9.81 | 5.86 | 2.97 |
| 0.4 | 8.98 | 7.03 | 4.52 | 1.57 |
| 0.6 | 6.23 | 4 | 3.43 | 0.46 |

**Figure 7. Effect of α-Pinene and β-Caryophyllene on grain damage and weight loss in cowpea during chronic exposure of *C. chinensis* adults at concentrations 0.1, 0.2, 0.4 and 0.6 µl gm$^{-1}$**

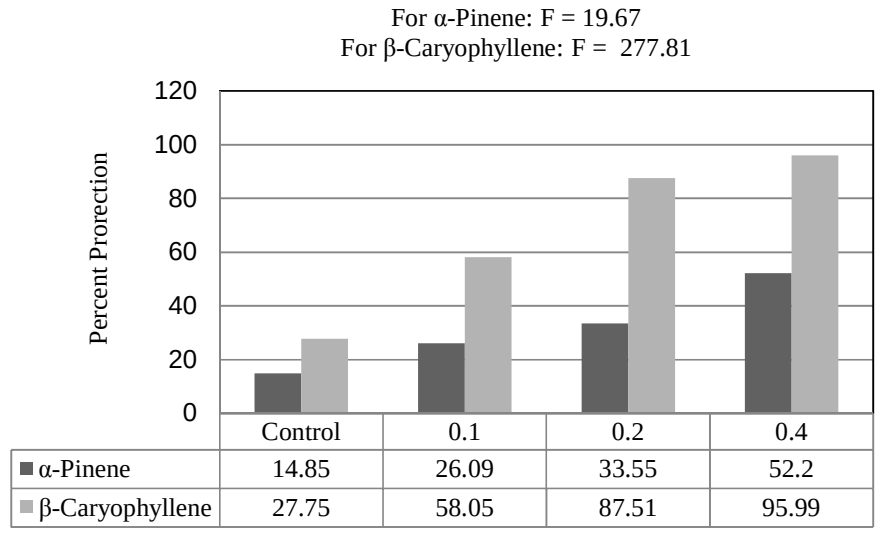



**Figure 8. Effect of α-Pinene and β-Caryophyllene on percent protection during chronic exposure of *C. chinensis* adults at concentrations 0.1, 0.2, 0.4 and 0.6 µl gm$^{-1}$**

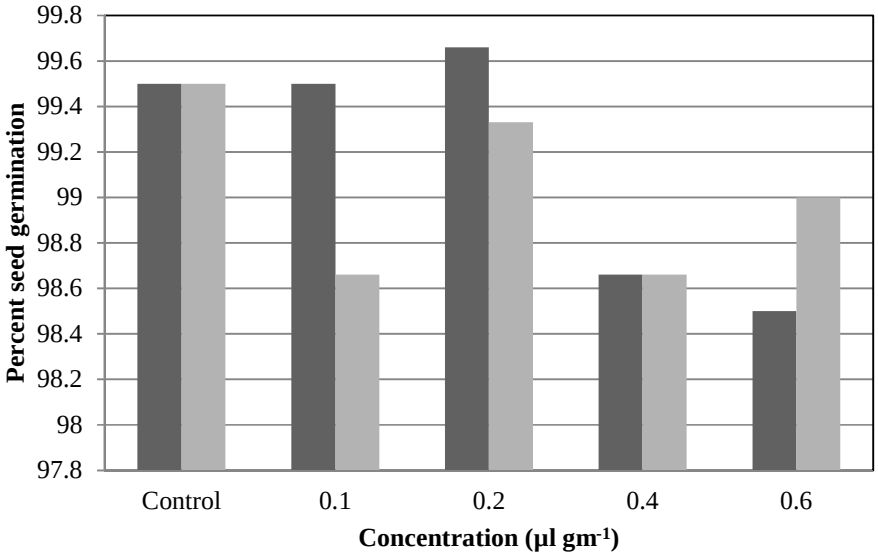



**Figure 9. Effect of α-Pinene and β-Caryophyllene on germination of cowpea seed treated during chronic toxicity assay**

## DISCUSSION

Use of natural products especially plant volatiles and its various components as pesticides is gaining importance in integrated pest management programmes because synthetic insecticides have created major environmental and health hazard [13,14,35-41]. The volatile components of essential oils can be classified into four main groups viz. terpenes, benzene derivatives, hydrocarbons and other miscellaneous compounds [42]. Terpenes and terpenoids are the most representative molecules constituting 90% of the essential oils and allow a great variety of structures with diverse functions. Many of the volatile components of various chemical groups have also been evaluated for their role in insect pest management programme. Don-Perdo (1996) has studied effect of citrus peel oils and its components against *C. maculatus* [43]. Several compounds including the major component of citrus peel oils, limonene has been found to be insecticidal [43]. A combined study has established that in artificial mixtures, several pure components of citrus peel oil potentiate their individual fumigant activity [43]. Linalool has been demonstrated

to act on the nervous system affecting ion transport and the release of acetylcholinesterase in insects [44]. Carvone and menthol are effective as fumigant while 1,8-cineole exhibits both contact and fumigant toxicity against *Tribolium castaneum* and *C. maculatus* [45]. *l*-Carvone has been reported to cause more fumigant toxicity than its contact toxicity to *Rhizopertha domenica* [36]. *Trans*-anethole, thymol, 1,8-cineole, carvacrol, terpineol and linalool have been evaluated as fumigants against *T. castaneum* but only compound to show significant effect against this insect species is trans-anethole [46]. A comparative study has been conducted to assess contact and fumigant toxicities of monoterpenes viz. camphene, camphor, carvone, 1-8-cineole, cuminaldehyde, fenchone, geraniol, limonene, linalool, menthol and myrcene on *Sitophilus oryzae* and *T. castaneum*. In fumigant toxicity assays, 1-8-cineole has found most effective against *S. oryzae* and *T. castaneum*. Structure-toxicity investigations reveal that carvone has the highest contact toxicity. *In vitro* inhibition studies of acetylcholine esterase from adults of *S.oryzae* show that cuminaldehyde inhibits enzyme activity most effectively followed by 1-8-cineole, limonene, and fenchone. 1-8-Cineole is the most potent inhibitor of acetylcholinesterase activity from *T. castaneum* larvae followed by carvone and limonene [47].

Earlier attempts to explore the toxicity of essential oils against *C. chinensis* have been made by several scientific groups. Essential oils can affect insects by antifeedant activity, repellent activity, oviposition inhibitory activities, ovicidal activities, by inhibiting $F_1$ progeny production and by disrupting metabolic pathways [19,48-52]. In the present study, α-pinene and β-Caryophyllene significantly repelled the bruchid adults at very low concentration as the oviposition capacity decreased in choice oviposition assay. Both these terpenes caused fumigant and contact toxicity in bruchid adults in a concentration dependent manner. In fumigant toxicity assay, α-pinene showed higher toxicity than that of β-caryophyllene. On other hand, in contact toxicity assay, β-caryophyllene showed higher toxicity than that of α-pinene. α-Pinene and β-Caryophyllene reduced egg laying capacity in *C. chinensis* adults in oviposition inhibition assay performed either by fumigation or contact method. Antiovipositional activities of both terpenes were more pronounced when bruchid adults were treated by contact method. α-pinene and β-Caryophyllene reduced hatching rate in *C. chinensis* eggs when fumigated. Elhag (2000) have shown oviposition inhibition activity of several essential oils against *C. maculatus*[53]. The essential oil of *Artemisia annua* has been shown for toxic, repellent, and ovicidal towards *C. maculatus* and *T. castaneum* [54]. Vapour of tridecanone affects the number of eggs laid, egg hatching and adult emergence [55]. The exposure of the cowpea seeds to the vapour of tridecanone is very effective to control their infestation by *C. maculates* since adult emergence was reduced as compared to untreated seeds [55]. The number of eggs laid and fecundity of *C. maculates* on seeds of chickpea has been reduced when fumigated with garlic essential oils [56].

In chronic toxicity assay, numbers of $F_1$ progenies emerged was reduced from seeds when treated with α-pinene and β-caryophyllene. Reduction in progeny production in beetle was inhibited maximally in chickpea seeds when treated with β-Caryophyllene. In general, higher the concentration of terpene compounds, the lower the progeny emergence and the higher the reduction in adult emergence in the chronic toxicity assay. The reduction in adult emergence could either be due to the reduction in egg hatching rate or death of larva. Larvae hatched must penetrate the seeds to ensure survival. However, the larvae are unable to do so unless the eggs are firmly attached to the seeds [57]. The amount of seed damage and grain weight losses caused by *C. chinensis* was reduced in chronic toxicity assay when exposed to α-pinene and β-caryophyllene as compared to the untreated group. The minimum seed damage was observed with β-caryophyllene. This grain damage and weight loss in the cow pea seeds were due to the reduction in the $F_1$ progeny. Similarly, tridecanone exhibits fumigant toxicity and its efficacy in protecting the cowpea seeds against *C. maculatus* which is mainly due to its ovicidal activity. Since adult emergence is based on the proportion of hatched eggs that develop into adults inside the seeds, the results suggest that tridecanone vapour can cross the seed coat and therefore, interfere with the larvae development [56]. There were no significant differences among treatments in seed germination. Similarly, treatment of chickpea seeds with plant products did not show any adverse effect on germination of seeds [58-60].

The mode of action of essential oil constituents has not yet been known but it may be due to suffocation and inhibition of various biosynthetic processes of the insect [43]. The toxicity of menthol, methonene, limonene, α-pipene, β-pipene and linalool against *S. oryzae* is proved to its exert on acetylcholinesterase enzyme activity [61]. It must be kept in mind that essential oils/constituents should be toxic to target insects and but not toxic to non-target organisms such as other beneficial insects and other animals such as fish, birds and humans. There are several other factors that must be considered during the evaluation of insecticides like risk associated to users, mode of exposure, degradation in the environment and chronic toxicity to be used effective for control of stored-product insect populations.

## CONCLUSION

Present study indicates that essential oil constituents can be considered as an alternative in the eco-friendly management of stored-grain insects.

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