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# Insecticidal Efficacy of Essential Oil from *Cinnamomum Zeylanicum* Blume and Its Two Major Constituents against *Callosobruchus maculatus* (F.) and *Sitophilus oryzae* (L.)

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**Abstract** Essential oil isolated from *Cinnamomum zeylanicum* Blume was analyzed by mass spectroscopy (MS) and two of its main constituents were identified. Contact and fumigant activity of *C. zeylanicum* essential oil and its two constituents viz., cinnamaldehyde and linalool were examined against two stored product pests, *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) and *Sitophilus oryzae* L. (Coleoptera: Curculionidae). Responses varied with respect to doses of compounds, insect species and exposure time. In contact activity assay, the cinnamon oil at 1.2 mg/cm$^2$ caused 98 and 80% mortality against *C. maculatus* and *S. oryzae* adults respectively, after 24 hours of treatment. 0.3 mg/cm$^2$ of cinnamaldehyde caused 100 % mortality in *C. maculatus* whereas, similar dose of linalool caused 89% mortality in *S. oryzae* after an exposure period of 12 hrs. The fumigant action as compared to contact toxicity indicated high percentage of mortality for adult insects even at low doses of compounds and less duration of exposure. The essential oil of cinnamon along with its major constituents demonstrates higher contact and fumigant toxicity to *C. maculatus* than *S. oryzae* adults. Further, cinnamaldehyde showed higher toxicity than linalool and it may be attributed to its chemical structure.

**Keywords:** Essential oil, cinnamaldehyde, linalool, contact toxicity, fumigant toxicity.

## Introduction

Stored insect pests are a major problem throughout the world as they significantly reduce the quantity and quality of grain. The post-harvest grain losses due to insect pests and other bio-agents ranged from 10 to 40% (Raja *et al.*, 2001; Papachristos and Stamopoulos, 2002). It is evident from literature that the cowpea weevil, *Callosobruchus maculatus* (F.) and the rice weevil, *Sitophilus oryzae* (L.) are considered as the most widespread and destructive

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primary insect pests of stored legumes and cereals (Park *et al.*, 2003). The main method of grain protection and to avoid or control insect infestations is the use of chemical agents since, it is the simplest and most cost effective means of dealing with stored product pests (Hidalgo *et al.*, 1998). But the excessive use of traditional chemical insecticides leads to a number of serious problems, like persistence in the atmosphere, resistance to chemical insecticides, pests resurgence, exclusion of economically beneficial insects, toxicity to humans and environment and higher cost of crop production (Khan and Selman, 1989). So, there is an urgent need to develop safe alternatives to conventional fumigants and insecticides to control the insect infestation in stored grain products. Continued screening for such systems of grain protection that target the pest species more accurately is required (Cox 2004). Plant derived insecticides as compared to synthetic ones are safer for the environment, generally cost effective, easy to handle and used by small industries and farmers (Belmain *et al.,* 2001). Botanical pesticides are often active against a number of species, are often biodegradable, nontoxic and appropriate for use in integrated pest management (Kim *et al.,* 2003). In this context, plant extracts including essential oils can play an important role in protecting stored products against insect infestations. Essential oils derived from plants are volatile in nature (Guenther, 1948) and their constituents have revealed adequate activity as botanical pesticides (Singh and Upadhyah, 1993). Spices are considered as rich sources of essential oils and are known to be effective against various insect pests including stored product insect pests (Jacobson, 1989). Clove oil was found toxic to *S. oryzae* and castor oil to *C. maculatus* and *C. phaseoli* in stored conditions (Pacheco *et al.,* 1995). Fumigant toxicity of essential oils from several spices like anise (*Pimpinella anisum* L.) and peppermint (*Mentha piperita* L.) was found against four major stored product pests including, *Ryzopertha dominica* (F.), *Tribolium castaneum* (Herbst), *S. oryzae* and *Orzyaephilus surinamensis* (L.) (Shaaya *et al.*, 1991).

In this work, we have examined the insecticidal activity of essential oil from *Cinnamomum zeylanicum* and its two major components cinnamaldehyde and linalool against *C. maculatus* and *S. oryzae* adults. This insecticidal study will put emphasis on the practice of essential oils and their components as botanical insecticides against variety of insect pests.

Objectives: • To reduce the negative impacts on human health and environment natural products are the best alternative to synthetic pesticides.

• In the present study cinnamon oil and its two major constituents were shown great efficiency against the two stored products insect pests (*C. maculatus* and *S. oryzae*), responsible for post-harvest losses.

- Essential oils are target specific, biodegradable, less expensive, promising grain protectants and relatively less harmful to environment and mammalian health.

## Materials and methods

### *Chemicals*

Cinnamaldehyde (>99% purity) and linalool (>99% purity) were purchased from Sigma Aldrich and tested by bioassay. Methanol was purchased from SD fine-chem Ltd, Mumbai, India. The chemical structure of compounds has been shown in figure 1.

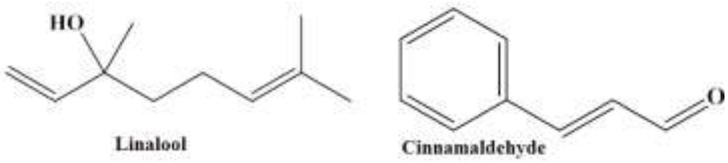

**Figure 1.** Chemical structure of linalool and cinnamaldehyde.

### *Insect culture*

Two species of insects *S. oryzae* and *C. maculatus* were reared in the culture. Cultures of *S. oryzae* and *C. maculatus* were maintained in the laboratory without exposure to any insecticide on rice grain and cowpea respectively, in glass containers at 25 ±1°C and 50 - 60% rh.

### *Extraction of essential oil from cinnamon*

Cinnamon barks (5g) was grinded into fine powder and then transferred into a 100 ml round-bottomed flask (RBF). 20 ml of ethyl acetate along with few pieces of anti-bumping granules were added to the round-bottomed flask and then a water-cooled condenser was attached to the flask followed by refluxing of the mixture for 15-20 minutes with constant heating at 70 °C. The mixture was cooled to room temperature and filtered into a clean conical flask. After that the mixture was passed through anhydrous sodium sulphate to remove the moisture. Transfer the Filtrate was transferred into the round-bottomed flask (RBF) and the solvent from mixture removed under reduced pressure in a rotary flash evaporator at 45 °C and oily residue left behind is the essential oil.

### *Mass spectroscopy*

Mass spectra of cinnamon oil was recorded on Bruker micrOTOF Q II Mass spectrometer. The prime components of essential oil were identified by mass spectroscopy and mass spectrum of essential oil isolated from cinnamon has been shown in figure 2.

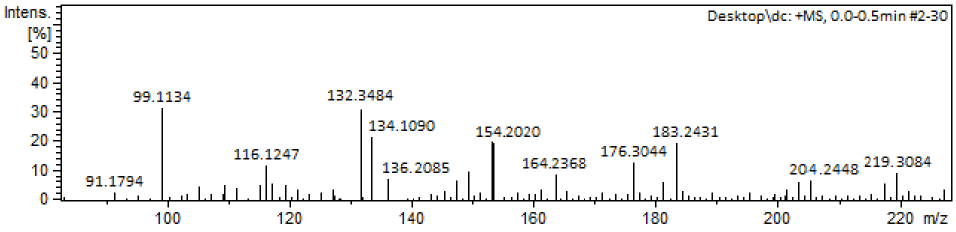

**Figure 2.** Mass spectrum of cinnamon oil.

### *Bioassay*

#### *a) Contact toxicity*

Insecticidal activities of cinnamon oil and its two constituents against adults of *C. maculatus* and *S. oryzae* have been determined by direct contact application as cited in literature (Kim *et al.* 2003; Usha Rani 2010). Cinnamon oil and its constituents were prepared in methanol at concentrations of (0.6, 0.8 & 1.2 mg/cm$^2$) and (0.1, 0.2 & 0.3 mg/cm$^2$) respectively. Aliquots of 1 ml of each solution were applied on filter papers (Whatman No. 1). Solvent was allowed to evaporate for 10 - 15 minutes before the introduction of insects. Then each dried paper was placed at the bottom of a Petri dish (5.5 cm × 1.2 cm) and 10 adults each of *C maculatus* and *S. oyzae* were placed in each Petri dish and covered with a lid. Controls received only 1000 µl methanol alone. Each set of treatment was repeated three times and number of dead insects in each petri-dish was counted at an interval of 3, 6, 8, 12, 24 48 hour respectively. Percentage mortality was calculated by using the Abott formula.

#### *b) Fumigant toxicity*

Vapour toxicity of essential oil and its constituents against the adult insects were determined via impregnated paper assay following the method of Park *et al.* (2002) with some modifications. Plastic jars of 250 ml capacity with screw lids were used as exposure chambers. Different doses of 30 µl, 20 µl and 10 µl of cinnamaldehyde and linalool dissolved in methanol were applied to a circular filter paper (Whatman No. 1, 3 cm diameter). The treated filter paper discs were then introduced into the plastic jars (250 ml capacity) to achieve final concentrations of 0.04, 0.08, 0.12, 1.2, 2.4 and 3.6 µl ml$^{-1}$, respectively, with respect to volume of the jars. After allowing the solvent to evaporate for 10 - 15 minutes, the filter paper was attached to the inner surface of the screw lid of the jar using adhesive tape. Ten adults of each insect species (2 to 4 days old) were released into each jar containing 50 seeds. Insect mortalities were determined and calculated after 3, 5, 7, 12 and 24 hours from exposure. Three replicates were set up for each dose and control.

### *Statistical analysis*

All the data concerning mortality were corrected by using Abbott's formula (Abbott 1925). Tests for contact and fumigant toxicity were performed in triplicate and data presented are mean ± SE. The mean values were compared by one-way ANOVA and Tukey's multiple comparison tests using software SPSS, version 11.5.

## Results

### *Components of cinnamon essential oil*

The mass spectroscopy has been used to elucidate the components of the cinnamon oil. The essential oil from *C. zeylanicum* contains different component have been summarized in table 1.

**Table 1.** Chemical constituents of the essential oil (*C. zeylanicum*) and parent ion peak (m/Z) values of components.

| **Components** | **m/Z** | |
|---|---|---|
| | **calculated** | **Observed** |
| **alpha-pinene** | 136.23 | 136.20 |
| **Myrcene** | 154.24 | 154.20 |
| **Linalool** | 154.13 | 154.20 |
| **1,8-cineole** | 154.24 | 154.20 |
| **Cinnamaldehyde** | 132.05 | 132.34 |
| **Eugenol** | 164.08 | 164.23 |
| **beta-caryophyllene** | 204.36 | 204.24 |
| **cinnamyl acetate** | 176.21 | 176.30 |
| **para-cymene** | 134.21 | 134.10 |
| **alpha-terpineol** | 154.25 | 154.20 |

The parent ion peak (m/Z) for the different components of cinnamon oil has been observed in mass spectrum at m/z 132.34, 134.10, 136.20, 154.20, 164.23, 176.30 and 204.244 and these signals account for cinnamaldehyde (calculated m/z = 132.05), para-cymene (calculated m/z = 134.21), alpha-pinene (calculated m/z = 136.23), myrcene (calculated m/z = 154.24 ), linalool (calculated m/z = 154.13), 1,8-cineole (calculated m/z = 154.24), alpha-terpineol (calculated m/z = 154.25), eugenol (calculated m/z = 164.08), cinnamyl acetate (calculated m/z = 176.21) and beta-caryophyllene (calculated m/z = 204.36). The mass spectroscopy revealed that the *C. zeylanicum* oil composed of a mixture of components mentioned above. A detailed survey of literature regarding the insecticidal activity of essential oils suggests that the phenolic/aromatic compounds affected the insecticidal activity positively as compared to non-aromatic compounds. Therefore in the present work one aromatic (cinnamaldehyde) and one non-aromatic (linalool) component of essential oil were chosen to explore the insecticidal activities.

### *Contact activity with treated filter paper*

The contact activity method has been widely used to investigate the toxicity of insecticides. The findings of contact toxicity unveiled that the mode of action of essential oil and its components against adults of *C. maculatus* and *S. oryzae* was dosage and exposure time dependent. It has been noticed that the cinnamon essential oil showed 52 and 65% mortality against adult *C. maculatus* at a dose of 0.6 and 0.8 mg/cm$^2$ respectively. Further increase the concentration of oil to 1.2 mg/cm$^2$, yielded higher mortality of 98% against adult *C. maculatus*. A similar trend of mortality versus concentration has been shown by essential oil against *S. oryzae* (table 2) but mortality rate was slightly lower in case of *S. oryzae*. But no mortality was obtained in the control within the same time period.

**Table 2.** Contact activities of essential oil of cinnamon against adults of *C. maculatus* and *S. oryzae.*

| | **Dose (mg/cm$^2$)** | **Mortality%** | |
|---|---|---|---|
| | | ***C. maculatus***<br>(24 hour*) | ***S. oryzae***<br>(48 hour*) |
| **Essential oil** | 0.6 | 52.6 ±3a | 45.4 ±5a |
| | 0.8 | 65.3 ±2b | 56.5 ±4b |
| | 1.2 | 98.2 ±1c | 95.3 ±2c |

*Exposure time and % values are mean (*n* = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

Results in figure 3 displayed the components of essential oil cinnamaldehyde and linalool showed strong toxicity against both the insect species than the cinnamon oil. In a test with *C. maculatus* adults, cinnamaldehyde at a dose of 0.3 mg/cm$^2$, caused 88 and 100% mortality at the time interval of 6 and 8 hour treatment respectively. But in case of *S. oryzae* maximum mortality of 95% was achieved by cinnamaldehyde after 12 hour of treatment at a dose of 0.3 mg/cm$^2$.

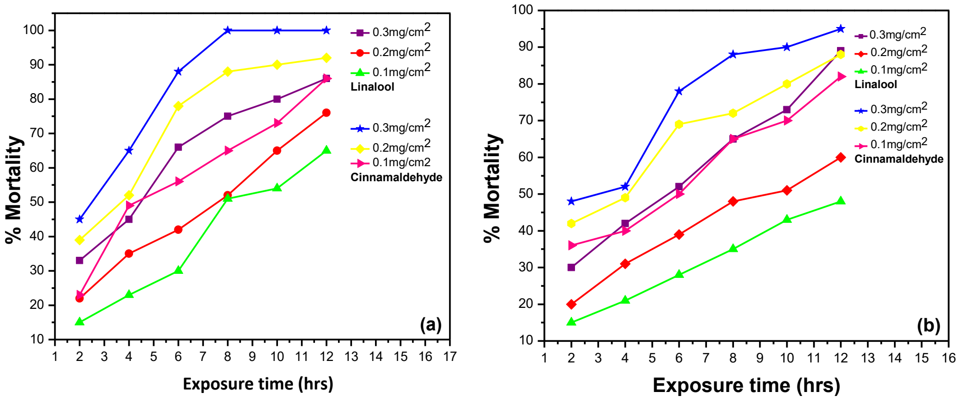

**Figure 3.** Represents the percentage mortality of a) *C. maculatus* and b) *S. oryzae* respectively, exposed to cinnamaldehyde and linalool by contact activity assay.

However, in linalool a dose of 0.3 mg/cm$^2$ caused 66, 75 and 86% mortality in *C. maculatus* within 6, 8 and 12 hour of treatment respectively and in *S. oryzae* 52, 65 and 89% mortality within same exposure time (table 3 and 4).

**Table 3.** Contact activities of constituents of essential oil from cinnamon against *C. maculatus.*

| | **Dose (mg/cm$^2$)** | **Mortality%** | | |
|---|---|---|---|---|
| | | 6 hour* | 8 hour* | 12 hour* |
| **Linalool** | 0.3 | 66.2 ±2cd | 75.6 ±3de | 86.3 ±1a |
| | 0.2 | 42.5 ±6bc | 52.4 ±5a | 76.4 ±3de |
| | 0.1 | 30.8 ±4e | 51.6 ±4a | 65.5 ±3cd |
| **Cinnamaldehyde** | 0.3 | 88.2 ±2cde | 100 ±0b | 100 ±0b |
| | 0.2 | 78.3 ±3ef | 88.4 ±4d | 92.4 ±1ef |
| | 0.1 | 56.2 ±5d | 65.6 ±2fg | 86.8 ±2a |

*Exposure time and % values are mean (*n* = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests

Furthermore, the minimum mortality achieved by cinnamaldehyde against *C. maculatus* and *S. oryzae* was 56 and 50% respectively, at a concentration of 0.1 mg/cm$^2$ and 6 hour of exposure. Similarly, linalool also showed mortality of 30 and 28% at lowest dose of 0.1mg/cm$^2$ against *C. maculatus* and *S. oryzae* respectively, 6 hour after treatment. The study revealed that the mortality was greatly affected by exposure time as well as concentration (table 3 and 4). The results also shows that the toxicity was more pronounced in cinnamaldehyde than linalool and this may be accredited to aromatic nature of cinnamaldehyde.

**Table 4.** Contact activities of constituents of essential oil from cinnamon against *S. oryzae.*

|  | **Dose (mg/cm$^2$)** | **Mortality%** |  |  |
|---|---|---|---|---|
|  |  | 6 hour* | 8 hour* | 12 hour* |
| **Linalool** | 0.3 | 52.4 ±1bc | 65.4 ±3a | 89.1 ±1de |
|  | 0.2 | 39.5 ±1cd | 48.2 ±1e | 60.5 ±2cd |
|  | 0.1 | 28.2 ±4de | 35.8 ±2fg | 48.9 ±1cde |
| **Cinnamaldehyde** | 0.3 | 78.3 ±2e | 88.6 ±2de | 95.2 ±5fg |
|  | 0.2 | 69.2 ±1f | 72.6 ±4b | 88.6 ±3e |
|  | 0.1 | 50.7 ±1g | 65.7 ±1a | 82.4 ±2c |

*Exposure time and % values are mean (*n* = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

### *Fumigant toxicity*

The efficacy of investigated compounds (essential oil, cinnamaldehyde and linalool) has further been evaluated by fumigation toxicity. The cinnamon oil at a dose of 1.2 µlml$^{-1}$ caused 36 and 32% mortality of *C. maculatus* and *S. oryzae* respectively, after 7 hour of treatment whereas, at higher concentration (3.6 µlml$^{-1}$) the essential oil caused 100 and 95% mortality against both the species of insects at same exposure of time (figure 4). *C. maculatus* was found to be more susceptible to essential oil than *S. oryzae.*

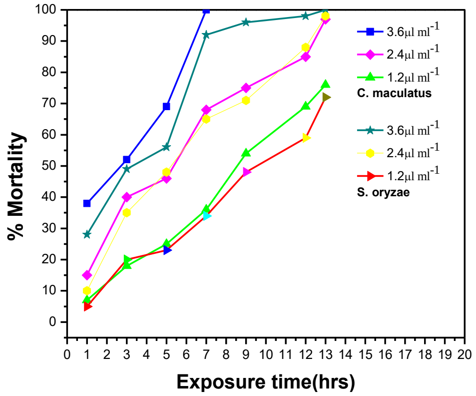

**Figure 4.** Percentage mortality of *C. maculatus* and *S. oryzae* exposed to fumigant action of cinnamon essential oil.

At a dose of 0.12 µlml$^{-1}$ both the components of essential oil caused 100% mortality in both insect species after 7 hour of treatment (figure 5). Similarly, at a concentration of 0.04 µlml$^{-1}$, cinnamaldehyde caused 66 and 82% mortality of *C. maculatus* 3 and 5 hour after exposure whereas, 63 and 79% mortality was achieved in *S. oryzae* at the same duration of exposure respectively (table 5).

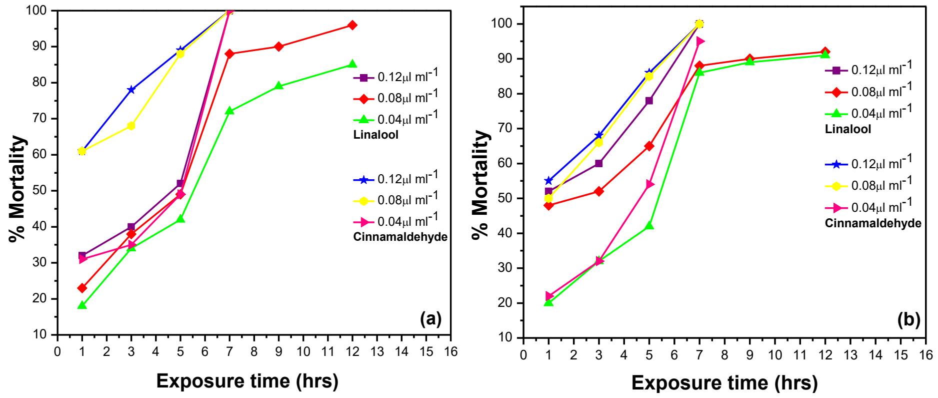

**Figure 5.** Represents the percentage mortality of a) *C. maculatus* and b) *S. oryzae* respectively, exposed to cinnamaldehyde and linalool by fumigant action.

**Table 5.** Fumigant toxicity of essential oil/ constituents against adults of *C. maculatus.*

| | **Doses (µlml$^{-1}$)** | **Mortality %** | | |
|---|---|---|---|---|
| | | 3 hour* | 5 hour* | 7 hour* |
| **Cinnamon oil** | 3.6 | 52.2 ±1b | 69.4 ±2cd | 100 ±0a |
| | 2.4 | 40.5 ±3bc | 46.7 ±3b | 68.5 ±5fg |
| | 1.2 | 28.6 ±2c | 25.8 ±2efg | 36.4 ±2gh |
| **Linalool** | 0.12 | 60.8 ±3a | 67.3 ±5cd | 100 ±0a |
| | 0.08 | 52.3 ±1b | 54.4 ±ef | 88.6 ±1def |
| | 0.04 | 34.4 ±2de | 46.2 ±3b | 72.5 ±3ghi |
| **Cinnamaldehyde** | 0.12 | 78.4 ±4cd | 89.1 ±2a | 100 ±0a |
| | 0.08 | 68.1 ±2cde | 88.5 ±2a | 100 ±0a |
| | 0.04 | 66.5 ±5cde | 82.2 ±4cde | 100 ±0a |

*Exposure time and % values are mean (*n* = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

However linalool at dose of 0.04 µlml$^{-1}$ gave 46 and 34% mortality of *C. maculatus* and *S. oryzae* respectively, 5 hour after treatment. Again no mortality was observed under controls. The increased exposure time and concentrations increased the mortality of insects. The fumigant activity of cinnamaldehyde against *C. maculatus* increased by 1 - 2.2 folds as concentration increases by 3 times at any exposure of time whereas, the activity of linalool increases by 1.3 - 1.7 folds against the same species of insect. Further, the fumigant action of the essential oil pronounced by 1.8 - 2.9 folds against *C. maculatus* with 3 times increase in dose concentration at any exposure of time. The vapour toxicity of cinnamaldehyde was found more than the cinnamon essential oil and linalool. Also the mortality rate was significantly affected by concentration in case of essential oil than other investigated compounds (cinnamaldehyde and linalool). *C. maculatus* was found to be more susceptible to essential oil and its constituents.

**Table 6.** Fumigant toxicity of essential oil/ constituents against adults of *S. oryzae*.

| | **Doses (µlml$^{-1}$)** | **Mortality %** | | |
|---|---|---|---|---|
| | | 3 hour* | 5 hour* | 7 hour* |
| **Cinnamon oil** | 3.6 | 49.7 ±1ab | 56.5 ±2cd | 95.2±2b |
| | 2.4 | 35.7 ±3bc | 48.4±5de | 65.2 ±5ef |
| | 1.2 | 20.8 ±2bcd | 23.8 ±2bcd | 32.8 ±2efg |
| **Linalool** | 0.12 | 56.3 ±1cd | 62.5 ±5ef | 100 ±0a |
| | 0.08 | 42.3 ±4cde | 48.6 ±4de | 82.4 ±1e |
| | 0.04 | 32.4 ±2a | 34.3 ±3bc | 69.9 ±3gh |
| **Cinnamaldehyde** | 0.12 | 68.3 ±4b | 86.2 ±2d | 100 ±0a |
| | 0.08 | 66.8 ±2b | 85.1 ±1d | 100 ±0a |
| | 0.04 | 63.5 ±5b | 79.4 ±4efg | 96.1 ±3b |

*Exposure time and % values are mean (*n* = 3) ± SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests.

## Discussion

The present study unveiled that the essential oil of *C. zeylanicum* and its two components exhibits contact and fumigant toxicity against the adults of both insect species. The toxic effects of cinnamon essential oil are attributed to its major constituent monoterpenes which are highly volatile and possess high fumigant toxicity. Many plant derived materials such as monoterpenoids have fumigant action against a variety of insect pests attributed to their high volatility (Coats *et al.,* 1991; Shaaya *et al.,* 1997; Ahn *et al.,* 1998). Monoterpenoids (limonene, linalool, terpineol, carvacrol and myrcene) are the main insecticidal constituents of many essential oils effective against stored product insects (Regnault-Roger *et al.*, 1995). Based on previous investigation (Regnault-Roger and Hamroui, 1995) two monoterpenes cinnamaldehyde and linalool were selected from different constituents of cinnamon essential oil for comparative study of their contact and fumigant action with essential oil of cinnamon against two stored product pests. The essential oil of cinnamon along with its major constituents revealed higher toxicity to *C. maculatus* than adults of *S. oryzae*. El- Nahal *et al.* (1984) studied the toxic effect of *Acoras calamus* essential oil on five stored product insect species and found the declining order of susceptibility as *C. chinensis*, *S. granaries*, *S. oryzae*, *T. confusum* and *R.*

*dominica*. Both the prime components showed better insecticidal activity than the essential oil. In the present study contact toxicity assay demonstrated highest mortality within 12 hours of treatment with cinnamaldehyde and linalool while complete mortality by fumigation was reached within 7 hours of treatment in both the insect species. However in contact paper assay cinnamon oil caused 98 and 95% mortality at a high dose of 1.2 mg/cm$^2$ against *C. maculatus* and *S. oryzae* adults respectively in a time period of 24 and 48 hours of treatment. The results indicated that the fumigant activity of cinnamon oil, linalool and cinnamaldehyde was more as compared to their contact toxicity. The insecticidal activity of mustard oil, horse radish and foeniculum fruit extract against *Lasioderma serricorne* adults was recorded more in closed cups than in open ones and also insecticidal mode of action of these materials was largely attributed to their fumigant action. (Kim and Ahn *et al.,* 2003). Ahn *et al*. (1998) reported that the monoterpene carvacrol has a wide range of insecticidal activity against various agricultural, stored product and medical insect pests, and possess fumigant activity. The phenylpropenes (E)-anethole, estragole and monoterpene fenchone exhibits fumigant activity against adults of *S. oryzae, C. chinensis* and *L. serricorne* (Kim and Ahn, 2001). The adulticidal activity of cinnamon essential oil and its constituents was found to be both dose and exposure time dependent. Cinnamaldehyde showed higher toxicity than linalool may be due to its aromatic structure. Fumigant activity and reproductive inhibition induced by monoterpenes with chemical skeleton having a non-substituted phenolic moiety was found more against *Acanthoscelides obtectus* (Regnault–Roger and Hamraoui, 1995). Present study demonstrates that essential oil and its constituent monoterpenes can be used for managing stored product insects in enclosed spaces such as storage bins, glasshouses and buildings etc. because of their fumigant action. But Karr and Coats (1992) have reported that when monoterpenoids are used as potential insecticides, direct toxicity as well as adverse effects on biotic potential must be considered in the evaluation of overall insecticidal efficacy.

It is possible that essential oils from plants and their derived constituents have sufficient potential to replace the more problematic fumigants and insecticides. Therefore they might be considered as better consolidates in the avenue of botanical insecticides for pest management. But further investigations are required to increase knowledge horizon for the effective and widespread use of these technologies especially on commercial bases.

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