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Insecticidal Efficacy of Essential Oils from *Cinnamomum zeylanicum*, *Thymus vulgaris*, *Ferula assafoetida* L on *Callosobruchus maculatus* F

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Authors' contributions

This work was carried out in collaboration among all authors. Author PE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AND and RK managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: The plants have well-developed defense mechanisms against pests and are excellent sources of new insecticidal substances.
Materials and methods: An experiment was conducted on Hamedan Agricultural Research Center, as factorial based on completely randomized blocks in 3 replications during 2009. Experimental treatments included 10 levels concentration with *Cinnamomum zeylanicum* (0, 5, 10, 15, 20, 25, 75, 100, 200, 300) µl, 7 levels concentration with *Thymus vulgaris* (0, 0.25, 0.50, 0.75, 10, 20, 30) µl, 6 levels concentration with *Ferula assafoetida* L (0, 30, 60, 90, 120, 150) µl as the time at 3 levels (8, 24, 48) hours as the second factor. 1 ml of solutiones were applied on filter papers with Whatman No. 1. Then each dried paper was placed and 10 C. maculatus adults was placed. Each set of treatment was repeated 3 times and number of dead insects in was counted at an interval of 8, 24, 48 hours respectively.

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Conclusion: Percentage mortality was calculated. It has been results that the *C. zeylanicum*, *T. vulgaris*, *F. assafoetida* L essential oils showed 13%, 56%, 10% mortality adult of *C. maculatus* at dose of 5 µl of *C. zeylanicum*, 0.25 µl of *T. vulgaris*, 30 µl of *F. assafoetida* L respectively. Further concentration increase to 300 µl of *C. zeylanicum* oil, 30 µl of *T. vulgaris*, 150 µl *F. assafoetida* L yielded mortality of 100% against adult C. maculatus respectively.

Keywords: Callosobruchus maculatus; Cinnamomum zeylanicum; Ferula assafoetida L; Thymus vulgaris.

1. INTRODUCTION

Presence of volatile monoterpenes or essential oils in the plants provides an important defense strategy to the plants.

monoterpenes or essential oils act as signaling molecules and depict evolutionary relationship with their functional roles. The interest in essential oils has regained momentum during the last decade, primarily due to their contact insecticidal activities and the less stringent regulatory approval mechanisms for their exploration due to long history of use [1]. It is primarily because essential oils are easily extractable [1,2]. Role extractable pattern important in plant protection against pests [1]. These properties of essential oils permit their use even in sensitive areas such as schools, restaurants, hospitals and homes. These compounds can act like fumigants offering the prospect for use in stored-product protection [3,4]. Essential oils may have numerous types of effect: Fumigant activity [5]; Inside the insect body as contact insecticides [6,7]. Antifeedants [8]. Several studies [9,10,11,12,13] were reported on the toxicity of essential oils against various insect species in Iran.

Essential oils from different families have been studied for insecticidal toxicity. Essential oils from *Achillea millefolium* L., *Achillea wilhelmsii* C. Koch, *Agastache foeniculum* Kuntze, *Allium sativum* L., *Anethum graveolens* L., *Artemisia aucheri* Boiss, *Artemisia dracunculus* L., *Artemisia haussknechtii* Boiss., *Artemisia scoparia* Waldst et Kit, *Artemisia sieberi* Besser, *Artemisia unnua* L., *Azilia eryngioides Hedge et Lamond*, *Bunium persicum* Boiss, *Carthamus tinctorius* L., *Carum copticum* C. B. Clarke, *Carum carvi* L., *Cinnamomum zelanicum* Blume, *Cinnamomum camphora* (L.), Citrus aurantium Risso, *Citrus limon* (L.), Citrus paradisi Macf, *Citrus sinensis* (L.), *Cominum cyminum* L., *Coriandrum sativum* L., *Cupressus arizonica* E.L. Greene, *Cymbopogon olivieri* bar, *Elletaria cardamomum Maton., Eucalyptus camaldulensis* Denhardt, *Eucalyptus globulus* Labill, *Ferula gummosa* Boiss., *Foeniculum vulgare* Mill, *Heracleum persicum* Desf., *Helianthus annuus* L., *Juniperus Sabina L., Laurus nobilis L., Lavandula angustifolia Mill., Lavandula stoechas L., Lippia citrodora Kunth, Melissa officinalis L., Mentha longifolia (L.), Mentha piperita L., Mentha pulegium L., Mentha spicata L., Nepeta cataria L.,Perovskia atriplicifolia (Benth), Prangos acaulis Bornm, Pulicaria gnaphalodes Boiss, Rosmarinus officinalis L., Salvia bracteata Banks and Soland, Salvia multicaulis Vahl, Salvia sclarea L., Satureja hortensis L., Sesamum indicum L., Syzygium aromaticum (L.), Tagetes minuta L., Thymus daenensis Celak, Thymus persicus (Roniger ex Reach F.), Thymus vulgaris L., Verbascum cheiranthifolium Bioss, Verbascum speciosum Schard, Vitex pseudo-negundo (Hausskn), Zataria multiflora Boiss, Zingiber officinale Rosci and Zhumeria majdae Rech F.* and *Wendelbo* were introduced as insecticides [9]. Different phenological stages as well as environmental , as shown in the studies of *Thymus vulgaris* [10]. *Thymus vulgaris* L. is a perennial plant belonging to the Lamiaceae family [11]. Thyme is an aromatic medicinal plant of increasing economic importance [12].

The pharmacological properties of the plant and of its different extracts,in particular the essential oils,has been thoroughly studied the many industrial mainly as food and cosmetic additive and medical applications [12].The yield of herb of thyme can be influenced by environmental factors, by agricultural practice [13,14,15]. The genus Ferula, the third largest genus of the Apiaceae (alt .Umbelliferae) family, is composed of ca. 180 species [16], 15 of which are endemic to Iran [17], nine species to Turkey, seven to China [18] and one species to Italy, and the rest are indigenous entities of several other countries.The majority of the Ferula plants have a pungent odor and can beused for different purposes. In the literature, numerous reports have described various biolo-gical and medicinal activities for different essential oils and ex-tracts of the Ferula plants.These include anticancer [19,20]. Anti helmintic [20], anti-epileptic [21,22], aphicidal, anti-oxidant [18,23,24,25,26], antimicrobial [18,27,28,29], antihypertensive [30], antifungal [20,29], antidepressant [31], phytotoxic [18,29], antiproliferative [32,33] acetylcholinesterase inhibitory and muscarinic receptors inhibitory, antiprotozoal activity [34,35], anti-hemolytic, antimycobacterial antiulcer [36], antitumor [27,37], anticoagulant [38], antifertility, antispasmodic [20,39], anticonvulsant [21,29], relaxant, antinociceptive [40], hypnotic [41], hypotensive [20], muscle relaxant [20], memory enhancing [20], enhancing digestive enzyme [20], antiviral [20,42,43], anxiolytics [20], antihyperlipidemic [24,44], antigenotoxic [45], anti-in-flammatory [18,37], cytotoxic, anti-hyperglycemic [24,44,46], acaricidal [47], antidiabetic [48], hepatoprotective [20] and antibiotic modulation [18] activities. Cinnamon is the genus *Cinnamomum* in the family *Lauraceae* [49,50]. This plant is also found in West tropical Africa where it has multiple uses its consumption as food (bark), sauces, condiments, spices, flavorings , and drink, the bark infusion is used as a remedy for pain, arthritis, rheumatism, nasopharyngeal affections and stomach troubles whereas leaf, bark, and roots are used to heal diarrhea and dysentery [51]. *C. cassia* Blume is native to China and exotic to southern and eastern Asia and to Africa [52] and South Africa [53]. Traditionally, *C. cassia* is used as an spice [54]. There are several reports related to its pharmacological effects [54]. *C. burmannii* is native to Indonesia [53]. To this order Insecticidal Efficacy of Essential Oils from *Cinnamomum zeylanicum, Thymus vulgaris*, *Ferula assafoetida* L on *Callosobruchus maculatus* F. an experiment was conducted on Hamedan Agricultural Research Center, in 2009.

2. MATERIALS AND METHODS

2.1 Stock Culture

Callosobruchus maculatus (fabricus) was used in the present investigation. A small population of beetles was reared and bred under laboratory conditions on the seeds of cowpea (*Vigna unguiculata*) inside a growth chamber at 30±20°C, 12:12 L:D and 70% RH.

2.2 Plant Material

Samples of *C. zeylanicum, T. vulgaris, F. assafoetida* L were collected in May, 2009.The dried aerial parts were submitted to Hydro distillation for 3 h using Clevenger type apparatus, according to the European Pharmacopoeia (European Pharmacopoeia, 1996). The essential oil was collected, dried over anhydrous sodium sulphate and stored at 4°C until used.

2.3 Mass Spectrometry Analysis

The oil was analysed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 6890 mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph. The MS operating parameters were as follows: ionisation potential, 70 eV; ionisation current, 2 A; ion source temperature, 200°C, resolution, 1000. Mass unit were monitored from 30 to 450 m/z .Identification of components in the oil was based on retention indices relatives to n-alkanes and computer matching with the WILLEY275.L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the lite rapture [55].

2.4 Contact Toxicity

Experimental treatments included 10 levels concentration with *C. zeylanicum* (0, 5, 10, 15, 20, 25, 75, 100, 200, 300) µl, 7 levels concentration with *T. vulgaris* (0, 0.25, 0.50, 0.75, 10, 20, 30) µl, 6 levels concentration with *F. assafoetida* L (0,30, 60, 90, 120, 150) µl a with 3 levels of time (8, 24, 48) hours as the second factor. 1 ml of solutiones was applied on filter papers with Whatman No. 1. Then each dried paper was placed at the bottom of a Petri dish (5.5 cm × 1.2 cm) and 10 adults of *C. maculatus* was placed and covered with a lid. Controls received only with water. Each set of treatment was repeated 3 times and number of dead insects in was counted at an interval of 8, 24, 48 hours respectively.

2.5 Statistics Analysis

Statistical analysis was carried out using SAS software version 9.1 and Comparing averages were carried out by one-way ANOVA using Duncan test.

3. RESULTS

Chemical compositiones of *Cinnamomum zeylanicum*, *Ferula assafoetida* L., *Thymus vulgaris*. Essential oil of *T. vulgaris* components were identified. Among those, The major constituents of the oil were eugenol (73.1%), β-caryophylene (7.7%), α-pinene (3.4%), phellandrene(3.6%) (Fig. 1).

Essential oil of *T. vulgaris* components were identified. Among those, The major constituents of the oil were Carvacrol (34.01%), thymol

(21.07%), Ƴb-bisabolene (11.90%), g Terpinene (4.35%), Caryophyllene (2.69%) and Carvacryl acetate (2.59%). Other components were 23.12% (Fig. 2).

Chemical composition of *Thymus vulgaris* Essential oil of *T. vulgaris* components were identified. Among those, isospathulenol (21.68%), α-Humulene epoxide II (21.04%), Caryophellene oxide (20.45%), Spathulenol (20.42%), Viridiflorene (18.34%) were the major oil components (Fig.3).

The results of comparing the means with indicated that there was a significant difference between different levels of essential oils of *Cinnamomum zeylanicum, Ferula assafoetida* L., *Thymus vulgaris* with control (Fig.1). The results of the analysis of variance showed that treatment of essential oil of *C. zeylanicum, F. assafoetida L., T. vulgaris* with control (Fig. 1). The results of the analysis of variance showed that treatment of essential oil of *T. vulgaris C. zeylanicum, F. assafoetida L.,* had significant effect on death rate of *C. maculatus* at probability level of 1%. About essencial oils, the experiment indicated that the rate of losses of *C. maculatus* had the lowest amount in all experiments in control treatment (no use of essential oils). The *C. zeylanicum, T. vulgaris, F. assafoetida* L

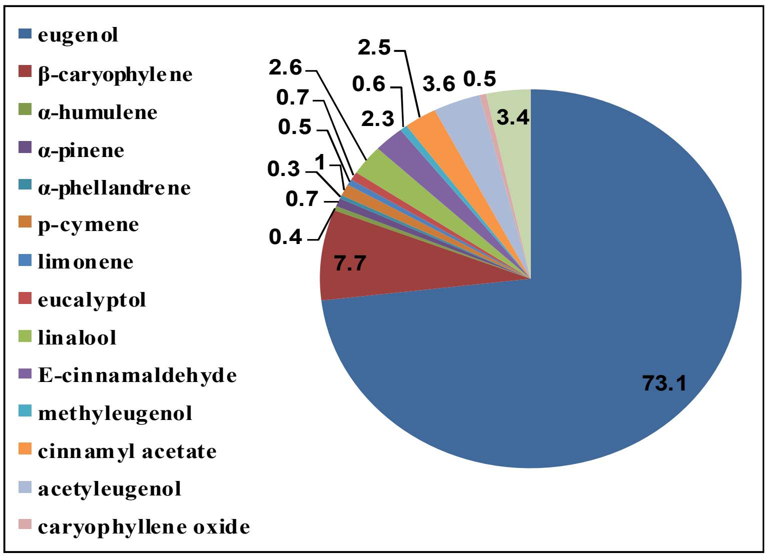

Fig. 1. Chemical composition of *Cinnamomum zeylanicum*

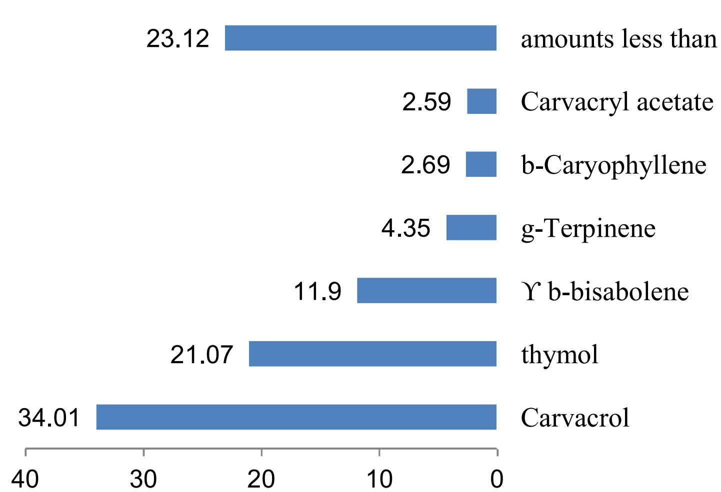

Fig. 2. Chemical composition of *Ferula assafoetida* L

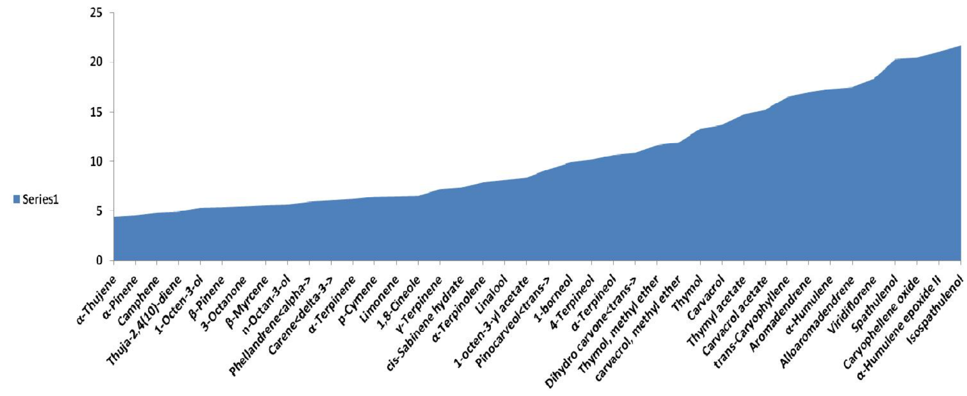

Fig. 3. Chemical composition of *Thymus vulgaris*

essential oils showed 13%, 56%, 10% mortality adult *C. maculatus* at dose 5 μl *C. zeylanicum* (Fig. 4), 0.25 μl *T. vulgaris* (Fig.6), 30 μl *F. assafoetida* L (Fig. 5) respectively. Further concentration increase to 300 μl of *C. zeylanicum* oil, 30 μl *of T. vulgaris* oil, 150 μl *of F. assafoetida L* oil yielded mortality of 100% against adult *C. maculatus* respectively.

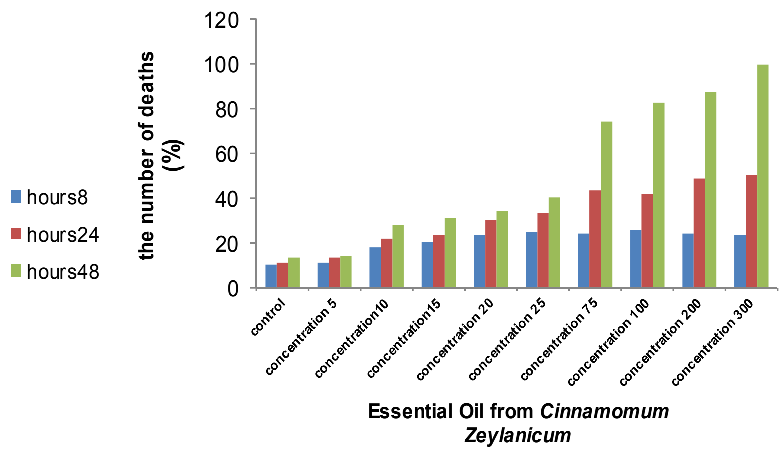

Fig. 4. Effect of *Cinnamomum zeylanicum* essential oil on *Callosobruchus maculatus*

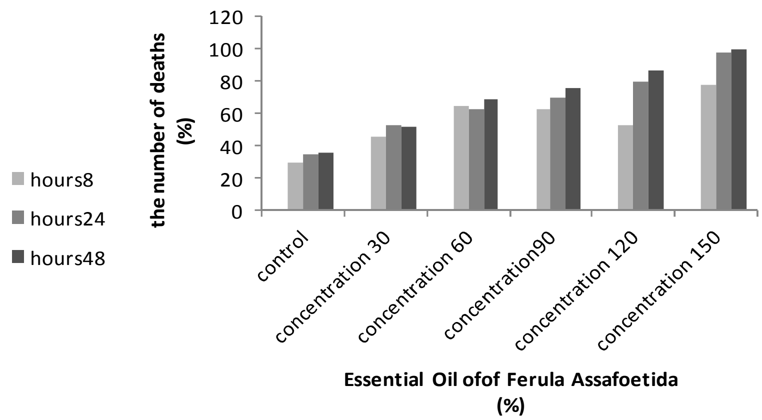

Fig. 5. Effect of *Ferula assafoetida* L essential oil on *Callosobruchus maculatus*

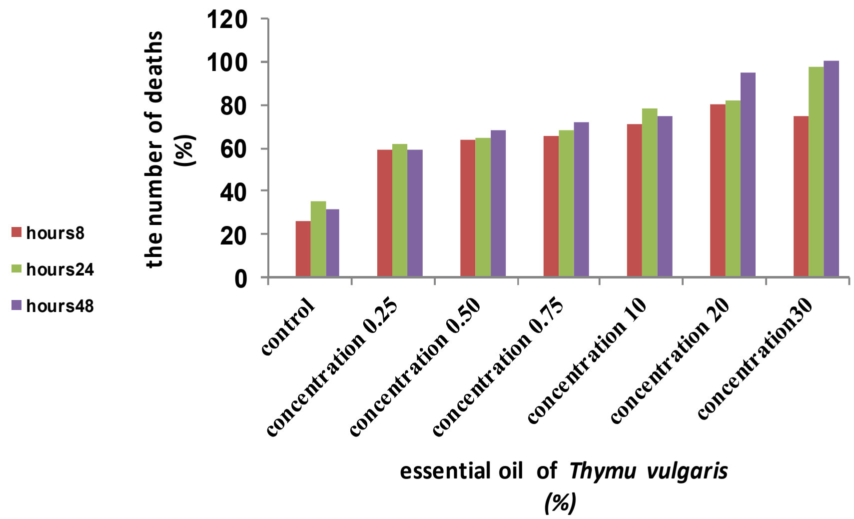

Fig. 6. Effect of *Thymus vulgaris* essential oil on *Callosobruchus maculatus*

4. DISCUSSION

Essential oil are natural products that as monoterpenes, sesquiterpenes and aliphatic compounds that provide characteristic odors [56]. Essential oil components and quality vary with geographical distribution, harvesting time, growing conditions [27]. The monoterpenoids have drawn the greatest attention for insecticidal activity against stored-product insects. Many essential oils isolated from various plant contain relatively high of monoterpenes [8]. Monoterpenes are responsible for the characteristic odours of many plants [57]. These are easily degradable [2]. Studies have shown that the toxicity of essential oils obtained from aromatic plants against storage pests is related to the oil's main components [58,59] such as 1.8 Cineole, Carvacrol, Thymol, Eugenol, Terpinene, Limonene, α-Pinene, among others. The essential oil of a plant may contain hundreds of different constituents but certain components will be present in larger quantities. 1,8-cineole was predominant in the essential oils of *Achillea millefolium* (22%), *Artemisia aucheri* (22.8%), *Eucalyptus camaldulensis* (69.46%), *Eucalyptus globulus* (31.42%), *Lavandula stoechas* (48.5%), *Laurus nobilis* (4.02) and *Perovskia atriplicifolia* (20.74). In recent years, several studies were reported on the toxicity of some essential oil constituents against various insect species. Obeng-Ofori et al. [60] found 1,8-cineole to be highly repellent and toxic to *Sitophilus granarius L., S. zeamais, Tribolium confusum du Val and Prostephanus truncatus* (Horn). Antifeedant activity of 1,8-Cineole has been demonstrated against *T. castaneum* [61]. Application of 1, 8-Cineole reduced oviposition rate by 30-50% at concentration of 1.0%, as compared to controls [62]. Lee et al. [63] reported that 1,8-cineole was the most fumigant constituent against the adults of *Tribolium castaneum* Herbst. Plant essential oils are among options for cheaper, safer and eco-friendly replacements for synthetic insecticides [64,65,66]. Applications of cinnamon essential oil adequately controlled *C. maculatus* on stored this research demonstrated the Essential oil, such as cinnamon oil, are very complex natural mixtures and can contain various compounds at different concentrations with two or three major components that will determine the biological properties of the essential oil [67]. Synergistic effects between the components of essential oils have been frequently reported in studies [68,69]. Chemical analyses of cinnamon essential oil revealed that their primarily components were eugenol (>70.0%), the sesquiterpene β-caryophyllene (between 7.0 and 12%). These results are in concordance with previous studies that reported [18,67,70]. Cinnamon essential oil, despite its major components, also contained a range of other compounds, including acetyleugenol, benzyl benzoate, linalool, cinnamyl acetate and cinnamaldehyde (between 2 and 4%) [67,71]. Several studies have reported the insecticidal toxicity of clove and cinnamon essential oils and their primary compounds that control stored product pests [70,72,73,74,75]. Other insects [67]. These investigations have attributed essential oil insecticidal activities to their major constituents (i.e., eugenol and β-caryophyllene)

[76,77]. Negative effects on developmental traits, such as rates of growth and progeny emergence of bruchid insects such as *C. maculatus*, have been reported for various essential oils [8,72,75,78,79,80]. The toxic effects of cinnamon essential oil to its major constituent monoterpenes which are highly volatile and possess high fumigant toxicity. Many plants derived materials monoterpenoids have fumigant action against a variety of insect pests attributed to their high volatility [3,81,82]. Monoterpenoids (limonene, linalool, terpineol, carvacrol and myrcene) are the main insecticidal constituents of effective against stored product insects [83]. Based on investigation [83]. Two monoterpenes cinnamaldehyde and linalool were selected 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 revealed higher toxicity to *C. maculatus* than adults of *S. oryzae*. The insecticidal activity of mustard oil, horse radish and foeniculum fruit extract against *Lasioderma serricorne* adults was recorded more and also insecticidal mode of action of these materials was largely attributed to their fumigant action [84]. Ahn et al. [84] reported that the monoterpene carvacrol has a wide range of insecticidal activity against various agricultural, stored product, and possess fumigant activity. The adulticidal activity of cinnamon essential oil was found to be both dose and exposure time dependent [85]. The phenylpropenes (E)-anethole and monoterpene fenchone exhibits fumigant activity against adults of *S. oryzae, C. chinensis and L. serricorne* [86]. Investigations demonstrates that essential oil and its constituent monoterpenes can be used for managing stored product insects in enclosed spaces such as glasshouses and buildings etc. because of their fumigant action. But Karr and Coats [86] have reported that when monoterpenoids are used as potential insecticides, as well as adverse effects on biotic potential must be considered in the evaluation of overall insecticidal efficacy. Essential oils from plants and their derived constituents have sufficient potential to replace the more problematic fumigants and insecticides. Therefore 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. In pest management strategies, aromatic plants with long lasting insecticidal efficiency considerations must take into account the pest species or the type of stored products. certain compounds in the oils exhibit much stronger activity . Plant should be sought that produce these compounds in larger quantities, or synthetic production methods should be explored. Essential oils possess a wide spectrum of biological activity against insects and provides a simple and environment friendly alternative pest control. Essential oils have strong toxicity in the vapour form against a wide range of insects, they could be commercially exploited as a fumigant thus preventing the insect infestation.

5. CONCLUSION

Percentage mortality was calculated. It has been results that the *C. zeylanicum, T. vulgaris*, *F. assafoetida* L essential oils showed 13%, 56%, 10% mortality adult of *C. maculatus* at dose of 5 µl of *C. zeylanicum,* 0.25 µl of *T. vulgaris*, 30 µl of *F. assafoetida L* respectively. Further concentration increase to 300 µl of *C. zeylanicum* oil*,* 30 µl *of T. vulgaris*, 150 µl *F. assafoetida* L yielded mortality of 100% against adult *C. maculatus* respectively.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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