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Fumigant toxicity study of different essential oils against stored grain pest *Callosobruchus chinensis*

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Abstract

Essential oils were extracted from five aromatic plant species namely Cinnamon essential oil (*Cinnamomum zeylanicum,* C. verum), Clove essential oil (*Syzygium aromaticum*), Rosemary essential oil (*Rosmarinus officinalis*), Bergamot essential oil (*Citrus Bergamia*) and Japanese Mint essential oil (*Mentha arvensis*) by steam distillation. Bioassays, i.e. fumigation with pure essential oils were carried out on adults. Observations were taken after 24 h, 48h, 72 h and 96 h respectively. The LC_{50} values obtained for 24 h, 48 h, 72 h and 96 h was 0.712(0.597-0.859)%, 0.345(.069-0.474)%, 0.479(0.332-0.602)% and 0.397(0.234-0.511)% respectively. The LC_{50} values obtained for 24 h, 48 h and 72 h for rosemary essential oil are 3.282, 4.261 and 1.509 respectively while the LC_{50} values obtained for clove oil for 24 h, 48 h and 72 h were 0.334, 0.341 and 0.059 respectively. The present study demonstrated fumigant toxicity against the stored grain pest *Callosobruchus chinensis*. The essential oil showed potential to be developed as possible natural fumigants or repellents for control of the pulse beetle.

Keywords: Essential oil, aromatic, stored grain pest

Introduction

Stored grain infestation is a very serious problem as various life stages of insects cause economic damage and deteriorates the quality of food grains and food products. There are number of stored grain insect pests that infest food grains in farmer stores and public warehouses and massively surge due to un-controlled environmental conditions and poor ware housing technology used. Mungbean (*Vigna radi*ata) is extensively grown in India, where it is stocked jute bags and bins or in cemented dark storerooms from season to season. Although there are many pests of mung bean, the pulse beetle, *Callosobruchus chinensis* L. (*Bruchidae: Coleoptera*), causes substantial losses to the pulses in the storage (Righi Assia et al., 2010). Initial infestation begins in the field, where female insect lays eggs on the green pods, grubs feed on the pod cover and remain concealed inside the developing seeds and when such seeds are harvested and stored, the pest population increases rapidly and results in total destruction within a short span of 3-4 months. *C chinensis* accounts for about 8.5% loss in pulses during post harvest handling and storage in India. (Rahman and Talukder, 2006).The pulse beetle is reported to cause 10-95 per cent loss in the seed weight and 45.5-66.3 per cent loss in protein content of the seeds under normal conditions and the intensity of damage increases with the duration of storage (Gujar and Yadav, 1978). The germination rate of the pulses seed is also reduced to a great extent due to infestation (Yadav, 1985). The degree of damage depends on types of legumes, duration of exposure time, storage facilities and other factors associated with the seeds. Losses reported during storage by *Callosobruchus chinensis* L. of blackgram, mungbean, chickpea and pea as 56.26%, 46.70%, 44.08% and 30.26%, respectively (Rustamani et al. 1985). The bruchids cause weight loss in seeds, they decrease the germination potential and reduce the commercial value of the seed (Booker, 1967 & Okunola, 2003). It is a serious pest of peas, mung beans, cowpeas and lentil and has also been reported attacking cotton seed, sorghum and maize (Ahmed et al., 2003).

Botanicals have been used since time immemorial for protection of stored products against common pests. Traditionally, plant essential oils have been used to prevent insect infestation (Isman, 2006) [6] and are being deliberated as an alternative to conventional pesticides because of their low toxicity. Many spices and herbs, and their extracts, are known to possess insecticidal properties that are frequently present in the essential oil fraction (Brattsten 1983; Shaaya et al. 1991) [19]. Literature shows that some chemical constituents of these oils interfere with the nervous system in insects. Due to the recent surge in use of green pesticides, the use of plant based pesticides is gaining a lot of impetus.

Cinnamon (*Cinnamomum zeylanicum*) a spice collected from the bark of several trees from the genus *Cinnamomum* and *Lauracea* family. The oil from cinnamon bark oil is rich in cinnamaldehyde. It is also rich in β-caryophyllene, linalool and other terpenes are present (Paranagama 1991). Kong et al 2007 [9] reported nematicidal activity due to cinnamon oils of which the main components reported are ethyl cinnamate, α-methyl-(E)-cinnamaldehyde, methyl cinnamate, cinnamoyl chloride and allyl cinnamate. Kim et al.(2003) [7, 8] reported that cinnamon oil produced 100% mortality within 1 day after treatment against adults of *Sitophilus oryzae* (L.) and *Callosobruchus chinensis* (L.), using the filter paper diffusion method, exposed to 0.7 mg/cm2. The paper also reported 100% mortality over a 24 hr period for *Sitophilus oryzae* (L.) and *Callosobruchus chinensis* (L.) using the direct contact in closed container and vapour in closed container method. *Rosmarinus officinalis* L. (Family Lamiaceae) popularly named rosemary, is a common household plant grown around the world. Lee *et al*. (2001) reported that rosemary essential oil had potent fumigant activities against the rice weevil having a sLD"30.5 l/l air). Literature has reported that the aromatic vapor of rosemary oil exhibited ovicidal and larvicidal effects toward several stored product pests (Tunc, I. *et al*., 2000 & Papachristos and Stamopolus 2002). Rosemary is a strong aromatic plant, which is commonly used in conventional medicine, food and the medicine industry because of their antioxidant and antimicrobial properties (Biljana *et al*. 2007). Giordani et al. (2004) reported that the main antimicrobial property of rosemary essential oil is attributed to the presence of α-pinene, 1,8-cineole, camphene, limonene, camphor, verbenone and borneol. Bergamont essential Oil is steam distilled from leaves of the Mentha citrata plant. Melliou et al. (2009) reported larvicidal activity due to bergamot oil while Ansari et al., 2000 reported larvicidal activities of peppermint oil.

Although toxicities of various essential oils and extracts on stored grain pest *Callosobruchus chinensis.* has been studied by several researchers there is very less literature available against toxicities with essential oils like Bergamot essential oil (*Citrus Bergamia*) and Japanese Mint essential oil (*Mentha arvensis*). In comparison to this, commonly used essential oils used for pest repellancy like Cinnamon essential oil (*Cinnamomum zeylanicum*, *C. verum*), Clove essential oil (*Syzygium aromaticum*), Rosemary essential oil (Rosmarinus officinalis) were also studied to compare the results.

Materials and Methods

This study was carried out during September-December, 2016 at ICAR-Directorate of Medicinal and Aromatic Plants Research, Anand. Essential oils used in the study were procured from Fragrance and Flavour Development Centre, Kannauj, India.

Rearing of insects

The pulse beetle, *C. chinensis*, was reared on fresh mung bean seeds in the Entomology laboratory at the Directorate of Medicinal and Aromatic Plants Research, Anand, India. The cultures were raised on healthy seeds using a single pair of freshly emerged adults and maintained under controlled conditions of temperature 29±1˚C and relative humidity 60±5% in the B.O.D. Incubator. The insects were raised for about 6-8 generations before starting the experiments.

Fumigant Toxicity Assay

Exposure time in all the tests was kept the same that is 24 hrs. In order to test the toxicity of essential oil vapours to the adults of *C.chinensis* gas tight glass bottles of 300ml volume with plastic screw caps were used as exposure chambers. A small piece (6x8) cms filter paper strips were kept inside the glass bottle to serve as an oil diffuser after the appropriate amount of pure essential oil has been applied on it. Doses were calculated based on nominal concentrations and assumed 100% volatilization of the oils in the exposure vessels / glass bottles. In each bottle 5 insects/replication were used and kept inside plastic vials fitted with 40 copper wire net on both the ends. This arrangement with the insects was suspended into the 300 ml glass bottle and then sealed with its cap. This whole set was considered as one replication. 3 such replications for each concentration of oil was taken. After 24 hrs of exposure to essential oil vapours the dead insects were counted.

Contact Toxicity assay

The insecticidal activity of various essential oils against the adults of *C.chinensis* was evaluated by direct contact application assay. (Qui and Burkholder 1981, Broussali's et al 1999). 20, 40, 60 80 and 100 µl /ml (2,4,6 and 8% solutions) in acetone were prepared). Males and females of *C.chinensis* were transferred into Petri dishes and chilled for 2-5 min to reduce their mobility. One µl of the test solution was applied to the dorsal surface of the insect insects with the micropipette. Ten insects were treated /conc of the test solution and this was termed as one replication. Ten such replications for each dose were done. After treatment, insects were transferred into empty 12 cms diameter glass Petri dishes. Insects were examined after 24 hrs of treatment.

Statistical analysis

Percent mortality was calculated by using the ratio of dead insects to the total number of insects after 24 hours. Insects were considered to be dead if no movement was observed by touching with a probe under a magnifying glass for several seconds. Probit Analysis® was used to calculate LC_{50} (concentration causing 50% mortality compared with the control) values and their confidence intervals.

Result and Discussion

The lethal concentration LC_{50} of cinnamon oil, clove oil, bergamont oil, Japanese mint oil and rosemary oil, against *C. chinensis* is reported in Table 1.

The LC_{50} values of cinnamon oil obtained for 24 h, 48 h, 72 h and 96 h was 0.712(0.597-0.859)%, 0.628(0.506-0.764)%, 0.479(0.332-0.602)% and 0.397(0.234-0.511)% respectively. Mondal, M. (2006) [14] reported that in fumigation bioassay cinnamon oil provided the highest toxicity to adult and 10, 14, and 18-day old larvae, with LD_{50} values of 0.03, 0.05, 0.088 and 0.09 mg cm-3 respectively. Tarigan *et al*. (2016) reported that cinnamon oil has the highest efficacy against egg, larva, and adult of *C. maculatus* with an LC_{50} of 0.01%, 0.132%, and 0.186%, respectively.

Papachristos *et al*. (2004) [17] and Huang, Y., & Ho, S. H. (1998) [4] studied the contact, fumigant and antifeedant effects of cinnamaldehyde against *T. castaneum* adults and larvae and *S. zeamais* adults. They reported that *T. castaneum* and *S. zeamais* adults showed similar susceptibility to the contact toxicity of cinnamaldehyde, both having an LC_{50} of 0.7 mg cm-' and an LC_{95} of 0.9 mg cm^{-2}. However, cinnamaldehyde had a higher level of fumigant toxicity to *T. castaneum* than to *S. zeamais*, with LC_{50} values of 0.28 and 0.54 mg cm^{-2}, respectively, and LC_{95} values of 0.32 and 1.78 mg cm^{-2}, respectively. *T. castaneum* adults were more susceptible than larvae to the contact and fumigant actions of cinnamaldehyde. Cinnamon oil vapour exhibited 100 % mortality against *S.*

oryzae adults when given at a dosage of 0.7 mg/cm2 for 24 h. (Kim *et al*., 2003) [7, 8]

The LC_{50} values obtained for 24 h, 48 h 72 h and 96 h for bergamot essential oil was 2.073,1.527,1.153 and 1.153 respectively. Cosimi, et al., A., (2009) [1] studied that the Bergamot oil showed the highest repellent activity at a concentration of 0.1% after 24 h; a repellent effect of this oil on beetles was observed also at the concentration of 0.01% after 3 and 24 h.

In the case of rosemary essential oil, the LC_{50} values obtained for 24 h, 48 h and 72 h for are 3.282, 4.261, and 1.509 respectively. Literature studies demonstrate the efficacy of rosemary oil as a repellent (Koschier & Sedy, 2003) [10] along with fumigant and contact insecticide properties against a broad range of insect species, particularly with respect to stored product pests (Shaaya et al., 1991) [19].

The LC_{50} values obtained for clove oil for 24 h, 48 h and 72 h were 0.334, 0.341, and 0.059 respectively. The main efficacy of clove oil is due to the its main constituents of the essential oil are phenylpropanoids such as carvacrol, thymol, eugenol and cinnamaldehyde. Its biological activity has been investigated on several microorganisms and parasites, including pathogenic bacteria.

In the present study the LC_{50} values of *Mentha arvensis* oil l for 24 h, 48 h, 72 h and 96 h were 2.1666,1.575,1.036, and 0.447 respectively. Raja *et al*. (2001) [18] observed seed damage of cowpea by C. maculatus by taking 100 seeds in airtight plastic containers fumigated with, *Mentha arvensis, Mentha spicata and Mentha piperata* EO prevented seed damage at least upto 2 months. Results of these studies indicate that due to their fumigant action, EO and their compounds might be useful for managing *Callosobruchus* spp. In enclosed spaces such as storage bins, glasshouse and buildings.

Conclusion

Botanicals have been used for hundreds of years to provide food safety by controlling bruchid population but were replaced from the market by synthetic insecticides. Some newer plant-derived products and their application technologies deserve proper attention for use in control of infestations of food commodities infested by different species of *Callosobruchus.* Most of the botanical formulations would be farmer friendly as these can often be easily available from the local flora. Hence, the use of botanical insecticides is more beneficial in developing countries where farmers are unable to afford synthetic insecticides. Cinnamon essential oil (*Cinnamomum zeylanicum*, C. verum), Clove essential oil (*Syzygium aromaticum*), Rosemary essential oil (*Rosmarinus officinalis*), Bergamot essential oil (*Citrus Bergamia*) and Japanese Mint essential oil (*Mentha arvensis*) demonstrated fumigant toxicity and contact toxicity against the stored grain pest *Callosobruchus chinensis*. They showed potential to be developed as possible natural fumigants or repellents for control of the pulse beetle but needs to be further evaluated for safety in humans and to enhance its activity.

Table 1: Lethal concentration (LC_{50}) of on time (24 - 96 h) for *C.chinensis*

Essential oil	Concentration (mg l^{-1}), (95 % confidence limits)			
	24 h	**48 h**	**72 h**	**96 h**
Cinnamon oil	0.712(.597-.859)	0.628(0.506-0.764)	0.479(0.3320.602)	0.397(0.234-0.511)
Bergamont oil	2.073(1.704-2.545)	1.527(1.078-1.920)	1.153(0.467-1.559)	1.153(0.467-1.559)
Clove oil	0.334(-0.013-0.483)	0.341(0.171-0.450)	0.059	
Japanese mint oil	2.166(1.551-3.471)	1.575(0.387-2.364)	1.036(-0.190-1.539)	0.447(-3.000-1.103)
Rosemary oil	3.282	4.261	1.509	1.509

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