Fumigant and contact toxic potential of essential oils from plant extracts against stored product pests

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ABSTRACT

Essential oils isolated from pine (*Pinus longifolia* L.), Eucalyptus (*Eucalyptus obliqua* L'Her) and coriander (*Coriandrum sativum* L.) were screened for contact and fumigant activities against rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), adzuki bean weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) and rice moth *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) in laboratory assays. Responses varied with test material, insect species, and exposure time. In fumigation assay, coriander and eucalyptus oils at 130µg/cm^2, caused 100% toxicity to all the species within 24 hrs of treatment, whereas pine oil revealed, 90% mortality at same concentration after 72 hrs of treatment. In contact assay, the test oils were effective against adults of *S. oryzae*, *C. chinensis* and *C. cephalonica* producing about 90% toxicity only after 72 hrs of treatment. Against *C. chinensis* adults, all test materials revealed potent insecticidal activities than other two insects in both fumigation and contact assays even at lower concentrations. These studies showed the strong insecticidal activity of coriander, eucalyptus and pine oils and its potential role as a fumigant for *S. oryzae*, *C. chinensis* and *C. cephalonica*. From this study we conclude that these essential oils have potential for applications in IPM programs for stored-grain pests because of its high volatility and fumigant activity.

Key words: *Callosobruchus chinensis*, *Corcyra cephalonica*, essential oils, fumigation, *Sitophilus oryzae*

INTRODUCTION

The rice weevil, *Sitophilus oryzae* L. (Coleoptera: Curculionidae), adzuki bean weevil, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) and rice moth, *Corcyra cephalonica* S. (Lepidoptera: Pyralidae) are the most widespread and destructive insect pests of stored grains as well as stored products (Kim *et al.*, 2003; Athanassiou *et al.*, 2008; Usha Rani and Rajasekharreddy, 2010). Control of these pests is primarily dependent upon repeated application of synthetic insecticides (Hasan and Reichmuth, 2004). Methyl bromide and phosphine fumigants have been used for decades to control stored pests (Islam *et al.*, 2009) and belong to the most effective treatments to protect stored food, feedstuffs, and other agricultural commodities. Growers are moving away from using methyl bromide as post-harvest fumigant because of its ozone-depleting nature (Zhang and Van Epenhuijsen, 2004) and phosphine, due to repeated use as it disrupts biological system leading to the development of pest resistance (Ignatowicz, 1999; Zeng, 1999). In order to control this kind of species without disturbing the environment, natural products have been screened for their insecticidal activity (Sukumar *et al.*, 1991). Botanical insecticide composed of essential oils may be a sound alternative to the more persistent synthetic pesticides for managing the major pests of stored product insects (Sahaf and Moharramipour, 2008).

Plant essential oils have traditionally been used to kill or repel insects (Isman, 2006) being considered as an alternative to stored-grain conventional pesticides because of their low toxicity to warm-blooded mammals and their high volatility (Shaaya *et al.*, 1997; Li and Zou, 2001). The toxicity of essential oils to stored-product insects is influenced by the chemical composition of the oil, which in turn depends on the source, season and ecological conditions, method of extraction, time of extraction and plant part used (Don-Pedro, 1996; Lee *et al.*, 2001). For example, while Rahman and Schmidt (1999) were working with the essential oil of

Acorus calamus against adults and larvae of *Callosobruchus phaseoli* (Gyllenhal), showed that the essential oil of Indian origin (containing 66% b-asarone) was more toxic than that of Yugoslavian origin (6% b-asarone content) or Russian origin (7% b-asarone). Among the essential oil components, the monoterpenoids have drawn the greatest attention for fumigant activity against stored-product insects (Rajendran and Sriranjini, 2008; Ahn *et al.*, 1998). Several reports indicate that monoterpenoids cause insect mortality by inhibiting acetylcholinesterase enzyme (AChE) activity (Houghton *et al*., 2006).The monocyclic monoterpene 1, 8-Cineole (eucalyptol) is the major component of different species of *Eucalyptus* having fumigant action against *Tribolium castaneum* (Rajendran and Sriranjini, 2008). Lee *et al.* (2004) observed that phosphine-resistant strains of *T. castaneum* did not show any cross-resistance to 1, 8-cineole. In earlier studies, toxic effects of few essential oils were assessed to determine possible fumigant, contact and ingestion activity against *R. dominica, S. oryzae* and *T. castaneum* (Lee *et al*., 2001).

The leaves of *Pinus longifolia* L. (Pinaceae) commonly known as Pine, yield oil which is traditionally used for the protection from mosquito bites (Ansari *et al*., 2005). It is also used as an herbal medicine in some rural areas in India (Ansari *et al.*, 2005). Repellent properties of pine oil to forest insects had been demonstrated earlier (Nijholt *et al.*, 1981) and also it was reported to be a feeding deterrent for the pine weevil, *Pissodes strobi* (Alfro *et al.*, 1984). Essential oil from *Coriandrum sativum* L. (Apiaceae) contains several compounds including linalool, camphor, g-terpinene, limonene, geraniol and carvone (López *et al*., 2008). The essential oil of *C. sativum* exhibited volatile toxicity to stored product insects (Pascual-Villalobos and Ballesta-Acosta, 2003; López *et al*., 2008). In earlier reports, essential oil from *C. sativum* exhibited good fumigant, repellent and toxic properties against larvae and adult of *T. castaneum* (Islam *et al*., 2009; Farhana *et al.*, 2006). In the present study, the insecticidal activity of three essential oils like pine, Eucalyptus and coriander were assayed against three major stored product insects- *Sitophilus oryzae* L., *Callasobruchus chinensis* L. and *Corcyra cephalonica* S. under laboratory conditions.

MATERIALS AND METHODS

Insects

The insect species were obtained from laboratory cultures in whole wheat (*Triticum aestivum* L.) for *S. oryzae*; green gram (*Phaseolus mungo* L.), for *C. chinensis* and jowar (*Sorghum vulgare* L.) for *C. cephalonica.* Insect rearing were maintained in the laboratory of Indian Institute of Chemical Technology (IICT), Hyderabad, India and the cultures maintained at 28 ± 2° C and 65 ± 5% relative humidity. Initially, 50 pairs of 1-2 day-old adults were placed in a jar containing their respective food grains (1kg). The jars remained sealed for a maximum period of 7 day to allow mating and oviposition. Then parental stocks were removed and the remaining of the content (diet and layed eggs) of each jar was used to infest to fresh seeds or respective, of each species, diet with pieces of cloth fastened with rubber bands to prevent the insects from escaping. The subsequent progenies of the beetles were used for all experiments.

Test materials

Industrially extracted oils of eucalyptus, pine and coriander oil were obtained from Central Institute of Medicinal and Aromatic Plants (CIMAP), Hyderabad. Only 95% pure oils were used throughout the experiments. The pure essential oils were diluted with acetone to obtain the required doses 1, 2, 3, 4 and 5% concentrations for the evaluation.

Bioassays

Contact toxicity of the tested plant oils

The insecticidal activity of eucalyptus, pine and coriander oils against adults of three stored product insects was evaluated by direct contact application assay (Kim *et al*., 2003; Usha Rani and Rajasekharareddy, 2010). Each essential oil prepared in acetone (100µL) at different concentrations (30, 60, 100 and 130µg/cm^2) were applied on filter papers (Whatman No. 1, cut into 15cm^2 pieces). Solvent was allowed to evaporate for 10-15 min prior to introduction of insects. Then each paper (dried) was placed at the bottom of a Petri dish (5.5cm diameter x 1.2cm), and 10 adults each of *S. oryzae* (7–10 day old), *C. chinensis* (3–5 day old), and *C. cephalonica* (1–2 day old) were placed in each petri dish and covered with a lid. The inner side of the lid was coated with Vaseline to prevent insect staying on lid. Controls received 100 µL acetone alone. There were a total of 15 replicates per treatment, and the treatments were done on different days. Mortality percentages were measured after exposure for 24, 48, and 72 hrs of treatment.

Vapour toxicity of the tested plant oils

The vapour toxicity of the eucalyptus, pine and coriander oils was evaluated according to a method described by Kim and Ahn, 2001; Usha Rani and Rajasekharreddy, 2010. In brief, small diet cups (3.6cm diameter x 4cm) were used as fumigation chambers and groups of 10 adults were placed in diet cups and covered with a nylon 60 mesh cloth. Each filter paper (Whatman No. 1, cut into $10cm^2$ pieces) treated with each test oils (10, 30, 60 and $100/cm^2$) previously dissolved in acetone (100 µL), it was placed in the bottom of a polyethylene cup (5.0cm diameter x 9cm), and a diet cup containing adult insects was put into the polyethylene cup. This prevented direct contact of the test adults with the test compound. Each polyethylene cup was then either sealed with a lid. Controls received 100 µL acetone only.

All tests were carried out at 28±2ºC temperature, and 65±5% relative humidity. Mortality was ensured by probing insect body with a slender paintbrush. Dead insects were counted every 24 hrs for a total period of 72 hrs post treatment. There were five replicates per treatment while the tests were repeated 3 times on different date each time to avoid any day-to-day variation.

Data Analysis

Where it was considered necessary, mortality counts were corrected for control mortality as suggested by Abbott (1925). Statistical analysis of the toxicity data was performed using probit analysis to find out the LC_{50} and LC_{95}, (Finney, 1971). All experimental data were submitted to a one-way ANOVA to determine differences between samples, by using the Sigma stat ver 3.5 statistical software. Means were separated by using the Tukey`s HSD test at the 5% level.

RESULTS

Contact toxicity of the tested plant oils

The insecticidal activities of pine, eucalyptus and coriander oils against *S. oryzae*, *C. chienesis* and *C. cephalonica* adults were examined by direct contact application method (Table 1). Toxicity increase with increasing concentration, exposure period and insect species, indicated that all the three insects were significantly susceptible to the three essential oils after 24 hrs (F=69.037; df= 8; P< 0.001), 48 hrs (F= 87.041; df- 8; P< 0.001) and 72hrs (F=38.363; df=7; P< 0.001) of treatment. Coriander oil showed greater efficacy compared with that of eucalyptus and pine oil.

Table 1. Contact toxicity of three essential oils against three stored pests

Essential oils	Insects	Mean (%) Toxicity±SE ($130 µg/cm^2$)			LC_{50} (95% FLa) ($µg/cm^2$)	X^2 (df)	P-level
		24 hrs	48 hrs	72 hrs			
Pine oil	*S. oryzae*	45.2±3.1b	75.2±0.9a	80.8±0.7e	77.30 (40.7- >130.0)	8.28(3)	0.015
	C. chinensis	63.4±0.8c	76.6±2.1a	92.4±1.1b	74.95 (71.0- 78.7)	0.49(3)	0.779
	C. cephalonica	49.6±2.0b	78.8±2.0a	90.4±1.4b	64.16 (37.0- 111.0)	8.73(3)	0.012
Eucalyptus oil	*S. oryzae*	70.6±3.4c	76.2±1.1a	94.2±0.8b	52.77 (29.5- 94.1)	6.28(3)	0.043
	C. chinensis	56.0±1.2d	77.4±1.1a	85.0±1.2c	59.29 (53.2- 65.3)	1.33(3)	0.512
	C. cephalonica	57.6±4.0d	85.2±1.2e	91.2±0.9b	56. 47 (24.9- 127.7)	14.73(3)	0.000
Coriander oil	*S. oryzae*	77.6±1.2c	84.6±1.0e	100±0.0a	36.68 (15.9- 51.2)	0.50(3)	0.778
	C. chinensis	88.2±1.2a	100±0.0d	NT	27.26 (8.19- 90.7)	2.06(3)	0.355
	C. cephalonica	68.8±1.6c	81.2±1.2e	94.2±0.7b	47.93 (42.9- 52.6)	1.04(3)	0.593

[a] Fiducial limits; after 72 hrs after treatment

[b] Each column followed by the same letter is not significantly different from another (One-Way ANOVA; Tukey test at, P< 0.05)

Table 2. Fumigant toxicity of three essential oils against three stored pests by vapor toxicity method

Essential oils	Insects	Mean (%) Toxicity±SE (130µg/cm^2)			LC_{50} (95% FLa) (µg/cm^2)	X^2 (df)	P-level
		24 hrs	48 hrs	72 hrs			
Pine oil	*S. oryzae*	69. ±0.6b	77.0±1.3a	84.0±1.0d	47.88(23.6- 96.8)	10.18(3)	0.006
	C. chinensis	81.6±1.2e	89.0±1.7d	98.4±0.8c	33.11(30.1- 35.8)	0.32(3)	0.851
	C. cephalonica	78.6±2.2b	85.8±1.0a	94.0±1.4a	33.75(3.75- 52.0)	1.60(3)	0.448
Eucalyptus oil	*S. oryzae*	100±0.0a	NT	NT	30.29(8.42- 71.4)	6.70(3)	0.035
	C. chinensis	100±0.0a	NT	NT	21.70(14.5- 44.3)	3.11(3)	0.210
	C. cephalonica	100±0.0a	NT	NT	22.37(13.6- 32.1)	0.96(3)	0.618
Coriander oil	*S. oryzae*	100±0.0a	NT	NT	18.11(16.3- 19.9)	0.79(3)	0.671
	C. chinensis	100±0.0a	NT	NT	16.25(12.8- 28.3)	2.09(3)	0.350
	C. cephalonica	100±0.0a	NT	NT	18.25(16.3- 22.2)	1.00(3)	0.606

[a] Fiducial limits; after 72 hrs after treatment
[b] Each column followed by the same letter is not significantly different from another (One-Way ANOVA; Tukey test at, P< 0.05)
NT- indicates no toxicity to the insect

At the rate of 130 µg/cm^2, coriander oil caused 100% toxicity to *C. chinensis* and *S. oryzae* after 72 hrs of treatment. At the same concentration, pine and eucalyptus oils showed 90% toxicity to test insects. The test insect, *C. chinensis* initially after release, frantic movements were observed with immediate knockdown effect while at lower concentration (30 µg/cm^2) they recover after 15 min. During this method, insects moved up towards lid and they preferred to stay away from the treated discs suggesting the repellent activity of the oils tested. *C. cephalonica* exhibited < 90% mortality to the test essential oils at 130 µg/cm^2 were observed. All test compounds showed potent insecticidal activities at rates of 130 µg/cm^2. At 30 µg/cm^2, the toxicity of these essential oils to the test adults was significantly moderate in contact method.

Vapour toxicity of the tested plant oils

The fumigant action of essential oils of pine, eucalyptus and coriander was tested on adults of *S. oryzae*, *C. chinensis* and *C. cephalonica*. In all cases, a strong difference in mortality of the insects was observed as oil concentration was increased. Table 1 shows that eucalyptus and coriander oils were significantly (P< 0.001) more toxic against all the test insects at 100 µg/cm^2 within 24hrs of after treatment whereas, pine (F=430.21, df=2, P< 0.001) oil showed > 90% toxicity to *C. chinensis* and *C. cephalonica* and < 85% toxicity to *S. oryzae* after 72 hrs of treatment (Table 2). *C. chinensis* was the most sensitive insect, followed by *C. cephalonica* and *S. oryzae*. Also, coriander was more toxic than eucalyptus and pine oils (*C. chinensis*: F=234.18, df=2, P<0.001; *C. cephalonica*: F=254.18, df=2, P< 0.001; *S. oryzae*: F=1,974, df=2, P< 0.001). Based on LC_{50} values, of those essential oils tested, the stored-product pests were significantly the most susceptible to the all three essential oils in fumigation mode of action Table 2.

DISCUSSION

In the current study, the three essential oils obtained from pine, eucalyptus and coriander demonstrated fumigant and contact toxicity to *S. oryzae*, *C. chinensis* and *C. cephalonica*. The results indicated that the insecticidal activity of the essential oils varied depending on the insect species and the plant origin of essentials oils. The results showed that adults of *C. chinensis* among the three test insects were more susceptible to the test oils and lower doses were required to achieve 100% mortality. In contact toxicity method, tested oils showed significant mortality (P< 0.001) to the test insects at higher dosage (130 µg/cm^2) after 72 hrs of treatment, whereas in fumigation method toxic properties were more rapid to test insects within 24 hrs of treatment. The insecticidal activity of many plant essential oils might be attributed to monoterpenoids (Waliwitiya *et al.*, 2005; Tong, 2010). Due to the high volatility they have fumigant activity that might be of importance for controlling stored product insects (Konstantopoulu *et al.*, 1992; Koul, 2004). Monoterpenoids were reported earlier as fumigants and contact toxicants on various insect pests (Rice and Coats, 1994; Tsao *et al.*, 1995). Many studies have demonstrated differential susceptibility of stored product beetle species to the essential oils. *Callosobruchus* species was more susceptible to essential oils or their components than those of other insect species (Subramanyam *et al.*, 1994; Tripathi *et al*., 2003; Lee *et al*., 2004).

Coriander oil was found to be most effective among the oils tested and the treatments resulted in mortality to the test insects even at lower concentrations tested in fumigation (16 to 19 µg/cm^2) and contact (28 to 48 µg/cm^2) methods. These findings were supported by the results reported earlier by Su (1986). It is reported about toxic as well as repellent activity of the coriander oils against *Tribolium confusum*, *T. castatenum* (Islam *et al.*, 2009) and insecticidal properties to *S. oryzae* and *Rhyzopertha dominica* (Lopez *et al*., 2008). The toxic effects of coriander oil could be due to major constituents like terpenoids such as linalool, linolenic acid, 1, 8-cineole, alpha pinene, carvone, triacontane and phenolic acids, quercetin, caffeic acid and protocatechuic acid. The high toxicity of linalool, 1, 8-cineole was reported against *S. oryzae* and *R. dominica* (Rozman *et al*., 2007). Coriander oil was highly effective against *C. chinensis* both in fumigation and contact toxicity methods with LC_{50} values 16.25 and 27.26 respectively. Small scale farmers in Asia and South East Asia mix dried leaves of *C. sativum* with stored products for protection against post harvest damage (Islam *et al*., 2009).

The oil of eucalyptus, at lower concentrations showed highly repellent as well as immediate knockdown effects to the test insects. Earlier reports showed that different solvent extracts of eucalyptus leaf have repellent property against adults of S. oryzae (Lee *et al*., 2004). *Eucalyptus citriodora* is good applicant for use as repellents against *Tribolium castaneum* (Olivero-Verbal *et al*., 2010). The essential oil of *Eucalyptus* Species contains metabolic compounds such as terpenoids and phenolic compounds (Moore *et al*., 2004) and are toxic to stored product pests (Coleoptera) (Lee *et al*., 2004; Tapondjou *et al.*, 2005) and agricultural pests (Lepidoptera) (Isman, 2000) has already been reported. There were no reports for insecticidal properties of pine oil against stored product pests, but repellent effects against mosquito were noted (Ansari *et al*., 2005). Less toxicity to the test insects was observed in case of essential oil obtained from pine among the three oils. Perhaps the reason might be due to the absence of monoterpenoids and phenolic acids present in coriander and eucalyptus oil. The highest toxicity of the coriander oil followed by eucalyptus oil might be due to the presence of the large number of monoterpenoids and phenolic acids in coriander and comparatively in less number in case of *Eucalyptus*.

In fumigation method, all the three stored product pests showed high susceptibility to coriander and eucalyptus oils, even at low concentrations and less exposure period. Whereas in case of pine oil longer exposure was required to obtain 90% toxicity to test insects in fumigation method. The toxicity of essential oils to stored product insects is influenced by the chemical composition of the oil and plant part to be used (Don-Pedro, 1966; Lee *et al*., 2001). From the previous reports on the insecticidal and repellent properties of monoterpenoids and phenolic acids, it can be stated that common chemicals found in oil of coriander as well as eucalyptus such as monoterpenoids, 1,8-cineole, alpha pinene, carvone, linalool, etc., and phenolic acids, quercetin, caffeic acid, protocatechuic acid are responsible for the insecticidal activity of the essential oils. Tapondju *et al*. (2005) and other researchers demonstrated that essential oils consisting of 1, 8-cineole, terpineol and a-pinene as major constituents show toxic and repellent properties. Lee *et al.* (2004) reported 1, 8-cineole for its fumigant toxicity against major stored grain insects. Obeng-Ofori *et al*. (1997) found 8-cineole to be highly repellent and toxic to *Sitophilus granaries, S. zeamais, Tribolium confusum* and Ojimelukwe and Adler (1999) found a-pinene and terpineol to possess potent toxic effects to *T. confusum.*

The results obtained suggest good potential for the use of essential oils as both fumigant and contact toxic agents against *S. oryzae*, *C. chinensis* and *C. cephalonica* adults. The concentrations of oils used in our work is only about 130-150 µg/cm^2 which is very insignificant compared to the LD_{50}'s of oils that varied between 3200mg-5000mg/kg body weight for pine oil on rabbits, 2480 mg/kg for eucalyptus oil on rats and 4130 mg/kg for coriander oil on rats .They showed mild side effects of being irritant and permeator on long time exposure (MSDS data). Thus it is directly proved that Essential oils are far safer for mammals compared to toxic fumigants like methyl bromide and phosphine currently used across the globe which is posing problems like adverse environmental disturbances, the possibility of carcinogenicity and increasing development of resistance in target pests. Therefore, there is an urgent need for new strategies to focus on a search for alternative fumigants for the control of stored-product insects.

Though the examined essential oils had contact as well as fumigant activity, the fumigant toxicity of the oils were much more potential in shorter period (24 hrs). From this we conclude that the plant essential oils can be potential alternative to synthetic fumigants in future. Due to the presence of linalool, linolenic acid, quercetin, carvone, coriander oil could be used effectively against *C. chinensis* and *C. cephalonica.* The efficiency of essential oils and their constituents in protecting the stored commodities were already reported in the past (Shaaya *et al*., 1997) and the efforts to develop biodegradable chemicals are still continued endlessly. Several researchers reported the toxicity and protectant potential of essential oils extracted from different plants against major stored product insects (Talukder *et al*., 2004; Islam and Talukder, 2005; Isman, 2006; Rajendran and Sriranjini, 2008; Usha Rani, 2011). Further work would focus on its penetration into insect cuticle and grain, metabolic target in the insect body as well as its effects on mammals fed on treated materials and the usefulness for commercial application.

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