

Fumigant toxicity of seven essential oils against the cowpea weevil, *Callosobruchus maculatus* (F.) and the rice weevil, *Sitophilus oryzae* (L.)

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ABSTRACT

Essential oils of Tea tree (*Melaleuca alternifolia*), Cinnamon (*Cinnamomum zeylanicum*), Cloves (*Syzygium aromaticum*), lemongrass (*Cymbopogon flexuosus*), Thyme, (*Thymus vulgaris*), *Eucalyptus* (*Eucalyptus globulus*), Jojoba (*Simmondsia chinensis*) were tested for their fumigant activity against *Callosobruchus maculatus* (L.) and *Sitophilus oryzae* (L.) adults. Percentage mortality increased with increasing concentration of different oils and exposure period. *C. zeylanicum* and *M. alternifolia* gave 90.0 % mortality at 8.0 and 16.0 μl /50 ml air, respectively, at exposure period of 24 hour for *S. oryzae* . *C. zeylanicum* , *M. alternifolia* and *T. vulgaris* essential oils gave 100% mortality at 8.0 , 16.0 and 16.0 μl /50 ml air , respectively, at exposure period of 24 hour for *C. maculatus*. Both species were found to be highly susceptible to *C. zeylanicum* whereas the LC₉₅ values were 3.67 and 4.7 μl /50ml air for *S. oryzae* and *C. maculatus*, respectively, at exposure period of 72 hour. *C. maculatus* was more sensitive than *S. oryzae* to the essential oils of *S. aromaticum* and *E. globulus* whereas the LC₉₅ values were 1.032 and 3.66 μl /50ml air, respectively , while the LC₉₅ values to *S. aromaticum* and *E. globulus* were 4.07 and 8.73 μl /50ml air, respectively , for *S. oryzae* at the same exposure period.

Key words: *Callosobruchus maculatus* , *Sitophilus oryzae* , Essential oils

INTRODUCTION

The rice weevil, *Sitophilus oryzae* (L.) and the cowpea weevil, *Callosobruchus maculatus* (F.) are two of the most widespread and destructive primary insect pests of stored cereals and stored legumes (Park *et al.* 2003; Demitry *et al.* 2007). Control of these insect populations around the world primarily depends upon applications of organophosphorus, pyrethroid insecticides and the fumigants (i.e. methyl bromide and phosphine). These still the most effective treatments for stored food, feedstuffs and other agricultural commodities protection from insect infestation. Although effective, their repeated use for decades has disrupted biological control by natural enemies and led to outbreaks of other insect species and sometimes resulted in the development of resistance. It has had undesirable effects on non-target organisms, and fostered environmental and human health concerns

(Champ and Dyte 1976; Subramanyam and Hagstrum 1995; White and Leesch 1995).

he use of methyl bromide would be prohibited in the near future because of its ozone depletion potential and high toxicity (Anonymous 1993).

These problems have highlighted the need for the development of selective insect-control alternatives with fumigant action.

Different types of aromatic plant preparations such as powders, solvent extracts, essential oils and whole plants are being investigated for their insecticidal activity including their action as repellents, anti-feedants and insect growth regulators (Prakash and Rao 1997; Isman 2000; Weaver and Subramanyam 2000). Weaver and Subramanyam (2000) suggested that fumigant activity in botanicals could have a greater potential use than grain protectants in future on the basis of their efficacy, economic value and use in large-scale storages.

More current research showed that essential oils and their constituents may have potential as alternative compounds to currently used fumigants (Singh *et al.*, 1989; Shaaya *et al.* 1991, 1997; Regnault-Roger *et al.* 1993; Dunkel and Sears 1998; Huang and Ho 1998; Huang *et al.* 2000; Tunc *et al.* 2000; Lee *et al.* 2001). Major constituents from aromatic plants, mainly monoterpenes, are of special interest to industrial markets because of other potent biological activities in addition to their toxicity to insects (Kubo *et al.* 1994; Isman 2000; Weinzierl 2000).

Lee *et al.* (2001) reported toxicity of commercially available essential oils and their major compounds against *S. oryzae*. Based on the results of Lee *et al.* (2001), toxicity of 7 essential oils were tested against the 2 major stored-grain insects: *Sitophilus oryzae* (L.) and *Callosobruchus maculatus* (F.).

MATERIALS AND METHODS

Essential oils:

Tea tree (*Melaleuca alternifolia*) Fam: Myrtaceae, Cinnamon (*Cinnamomum zeylanicum*) Fam: Lauraceae, Cloves (*Syzygium aromaticum*) Fam: Myrtaceae, lemongrass (*Cymbopogon flexuosus*), Fam: Poaceae, Thyme (*Thymus vulgaris*) Fam: Labiatae, Eucalyptus (*Eucalyptus globulus*) Fam: Myrtaceae., Jojoba (*Simmondsia chinensis*) Fam: Simmondsiaceae, essential oils were obtained from T. Stanes Company Limited, India.

Culturing insects:

Cultures of the rice weevil, *S. oryzae* and the cowpea weevil, *C. maculatus*, were maintained under the laboratory conditions (28.0 ± 1.0 °C, 60.0± 5.0 % R.H) without exposure to any insecticide for several generations. They were reared on rice and cowpea grains, respectively, in plastic containers (26x30x20 cm³).

Fumigation bioassay:

Fumigation bioassays without grain were carried out with 10 adults exposed in 50 ml conical flasks sealed with glass adaptors fitted with injection septa. Filter papers (Whatman Number 1) were placed below the septa to capture the injected oil and to produce a large surface area for evaporation. Each flask had its volume measured by the amount of water it could

contain. Different volumes of each essential oil were injected through the septa into the conical flasks using a gas syringe. Flasks were held at 28.0±1.0 °C & 60.0± 5.0 % R.H. in a constant temperature room during the exposure periods. At least 4 concentrations were tested from 0.5- 4.0 µl (Eucalyptus and Cloves), 4.0-10.0 µl (lemongrass), 4.0-16.0 µl (Thyme), 2.0-8.0 µl (Cinnamon), 6.0-16.0 µl (Tea tree) and 4.0-16.0 µl (Jojoba) for each insect. Except, the concentrations which used from Thyme oil were from 4.0-16.0 µl for *C. maculatus*. Five replicates were prepared for each treatment and control.

Adults of *C. maculatus* and *S. oryzae* (1-3 days old) were exposed to treatments for 24, 48 and 72 hours for each oil/ concentration. After each exposure period, insects were removed and put into clean vials and mortality determined immediately. Similar units, conical flasks sealed with glass adaptors fitted with septa and filter papers (Whatman Number 1) were placed below the septa used as control containing the same number of insect and maintained at the same conditions. Insects showing any movement were considered to be alive. Mortality counts was recorded at the same exposure periods that conducted in treatments. The percentage mortality was calculated after each exposure period for each oil/ concentration by Abbott equation (1925). The LC₅₀ and LC₉₅ values were calculated by Probit analysis (Finney 1971).

RESULTS AND DISCUSSION

In all cases, a strong difference in mortality percentage of the insects was observed as oil concentration and exposure time was increased. Results in Table 1 show that the different oils were relatively more toxic against *C. maculatus* than *S. oryzae* adults. The plant essential oils, *C. zeylanicum* and *M. alternifolia* gave 90.0% mortality at 8.0 and 16.0 µl /50 ml air, respectively, at exposure period of 24 hour for *S. oryzae*. However, essential oils, *C. zeylanicum*, *M. alternifolia* and *T. vulgaris* gave 100% mortality at 8.0, 16.0 and 16.0 µl /50 ml air, respectively, at exposure period of 24 hour for *C. maculatus*. The present

findings in agreement with Negahban and Moharrampour (2007) who found that the fumigant action of essential oils of *E. intertexta*, *E. sargentii* and *E. camaldulensis* caused high mortality rate in *C. maculatus* compared with *S. oryzae*. Also, Papachristes and Stamopoulos (2002) who reported the higher susceptibility of the *C. maculatus* than *T. castaneum*. Moreover, the results indicated that higher concentrations of the oils for a relatively short period of time are much more effective than lower concentrations for a long period. However, each tested oils achieved 90-100 % mortality with *C. maculatus* at the highest concentrations at 24 hour exposure periods, except, *E. globulus* which achieved 56.0 % mortality at the highest concentration (4.0 μ l/50 ml air) at 24 hour exposure period.

Table (1): Fumigant activity of the selected essential oils against *C. maculatus* and *S. oryzae* adults during 42, 48 and 72 hour exposure periods.

Essential oils	Dose (μ l/50ml air)	Mortality (%) of <i>C. maculatus</i>			Mortality (%) of <i>S. oryzae</i>		
		Exposure periods (hour)					
		24	48	72	24	48	72
<i>Eucalyptus globulus</i>	0.5	18.0	34.0	48.0	22.0	30.0	50.0
	1.0	24.0	50.0	72.0	30.0	44.0	62.0
	2.0	44.0	76.0	86.0	34.0	50.0	80.0
	4.0	56.0	80.0	96.0	46.0	78.0	88.0
<i>Syzygium aromaticum</i>	0.5	58.0	74.0	84.0	28.0	40.0	60.0
	1.0	70.0	90.0	96.0	52.0	58.0	68.0
	2.0	84.0	100	----	68.0	72.0	84.0
	4.0	94.0	----	----	84.0	88.0	100
<i>Cinnamomum zeylanicum</i>	2.0	60.0	78.0	80.0	50.0	80.0	86.0
	4.0	86.0	90.0	92.0	70.0	90.0	96.0
	8.0	100	----	----	90.0	98.0	100
<i>Cymbopogon flexuosus</i>	4.0	0.0	14.0	72.0	0.0	40.0	80.0
	6.0	0.0	42.0	94.0	0.0	60.0	84.0
	8.0	0.0	82.0	100	0.0	70.0	90.0
	10.0	0.0	100	---	0.0	80.0	98.0
<i>Thymus vulgaris</i>	4.0	60.0	72.0	80.0	0.0	60.0	90.0
	8.0	82.0	88.0	92.0	0.0	80.0	94.0
	10.0	90.0	96.0	98.0	40.0	90.0	100
	16.0	100	---	---	---	---	---
<i>Simmondsia chinensis</i>	4.0	44.0	80.0	90.0	0.0	70.0	80.0
	8.0	60.0	94.0	94.0	0.0	86.0	96.0
	16.0	90.0	100	----	60.0	92.0	98.0
<i>Melaleuca alternifolia</i>	6.0	58.0	76.0	84.0	40.0	70.0	80.0
	8.0	82.0	90.0	94.0	70.0	90.0	94.0
	16.0	100	----	----	90.0	94.0	100

Insecticidal activity (90% mortality) was observed at concentrations 16.0, 16.0 and 8.0 μ l /50 ml air at 24 h after treatment with *M. alternifolia* and *C. zeylanicum*, respectively, for *S. oryzae*. In case of *C. maculatus*, the essential oils that achieved \geq 90% mortality were *S. aromaticum*, *C. zeylanicum*, *T. vulgaris*, *S. chinensis* and *M. alternifolia* at concentrations 4.0, 8.0, 10.0, 16.0 and 16.0 μ l /50 ml air, respectively, after 24 h exposure period. However, *C. flexuosus* had no potent effect after 24h exposure period against *C. maculatus* and *S.*

oryzae at the different concentrations used (Table2).

Tested essential oils used gave 90.0% mortality or above at the high concentrations and exposure period of 72 hour for *S. oryzae* and *C. maculatus*, respectively. except, *E. globulus* which achieved 88.0 % mortality to *S. oryzae* at 4.0 μ l /50 ml air after 72 h exposure period. Rozman *et al.* (2006) found that camphor acted as fumigant caused 100% mortality to *Cryptolestes pusillus* at a dose of 1 μ l /7.2ml vol.

Based on LC₅₀ and LC₉₅ values, the two species tested were significantly more susceptible to the essential oil of *S. aromaticum* whereas the LC₉₅ values were 1.032 and 4.07 μ l /50ml air for *C. maculatus* and *S. oryzae*, respectively (Table 2). Lee *et al.* (2004) found that essential oils of *Eucalyptus nicholii*, *E. codonocarpa*, *Callistemon sieberi*, *M. fulgens*, *E. blakelyi* and *M. armillaris*, had potent fumigant toxicity against *S. oryzae*. The LD_{50s} of these essential oils were 29.0, 19.0, 27.3, 28.6, 31.2 and 30.6 ml/l air, respectively. LD_{95s} of these essential oils were 48.2, 53.1, 43.0, 53.6, 51.4 and 56.0 ml/l air, respectively.

Results in Table (2) show that *S. oryzae* was more sensitive than *C. maculatus* to the essential oils of *C. zeylanicum* and *T. vulgaris*. The LC₉₅ values were 3.67 and 6.75 μ l /50ml air, respectively.

Table (2). Estimated fumigant toxicity values of 7 essential oils against *C. maculatus* and *S. oryzae* after 72 hour exposure period.

Insects	Essential oils	LC ₅₀	LC ₉₅	Slope	(X ²)
<i>C. maculatus</i>	<i>Syzygium aromaticum</i>	0.16	1.032	2.02	0.144
	<i>Eucalyptus globulus</i>	0.52	3.66	1.95	0.23
	<i>Cinnamomum zeylanicum</i>	0.87	4.7	2.5	0.43
	<i>Simmondsia chinensis</i>	1.71	9.04	2.27	0.001
	<i>Thymus vulgaris</i>	1.6	9.93	2.08	0.413
	<i>Cymbopogon flexuosus</i>	3.07	6.46	5.09	0.014
	<i>Melaleuca alternifolia</i>	2.91	9.16	3.3	0.46
<i>S. oryzae</i>	<i>Syzygium aromaticum</i>	0.42	4.07	1.67	4.97
	<i>Eucalyptus globulus</i>	0.53	8.73	1.35	0.545
	<i>Cinnamomum zeylanicum</i>	0.62	3.67	2.12	0.033
	<i>Thymus vulgaris</i>	0.8	6.75	1.76	2.37
	<i>Simmondsia chinensis</i>	1.55	9.1	2.14	0.91
	<i>Cymbopogon flexuosus</i>	1.8	10.4	2.15	2.88
	<i>Melaleuca alternifolia</i>	3.48	9.33	3.84	1.28

Unit LC₅₀, ₉₅= μ l/50 ml air, applied for 72 h at 28.0 \pm 1.0 $^{\circ}$ C & 60.0 \pm 5.0 % R.H.

In contrast, *C. maculatus* was more sensitive than *S. oryzae* to the essential oils of *S. aromaticum*, *E. globulus* and *C. flexuosus* whereas the LC₉₅ values were

1.03, 3.66 and 6.46 μl /50ml air, respectively. Generally adults of *Rhyzopertha dominica* and *Callosobruchus* spp. were more susceptible to essential oils or their components than those of other insect species (Ahmed and Eapen 1986; Tripathi *et al.* 2003; Lee *et al.* 2004). Negahban *et al.* (2006) showed that *Artemisia sieberi* Besser oil is more potent than that of ZP51 as well as *Artemisia tridentata* L. oils against the adults of *C. maculatus*, *S. oryzae* and *Tribolium castaneum*. These results are relatively different from the results previously reported by Lee *et al.* (2001) stated that 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. Also, Akram *et al.* (2010) found that the LT_{50} values *Thymus persicus* at the lowest and the highest concentrations were ranged from 28.09 to 13.47 h for *T. castaneum*, and 3.86 to 2.30 h for *S. oryzae*. It was found that *S. oryzae* adults were much more susceptible to the oil than *T. castaneum*. After 24 h of exposure, the LC_{50} values (95% fiducial limit) for *T. castaneum* and *S. oryzae* were estimated to be 236.9 (186.27–292.81) and 3.34 (2.62–4.28) $\mu\text{l/l}$ air, respectively.

The results of fumigant activity of some the essential oils against *S. oryzae*, and *C. maculatus*, serious pests of Croatian silos and storages, identified some possible alternatives for fumigants currently in use (Rozman *et al.*, 2007).

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السمية البخارية لبعض الزيوت العطرية ضد خنفساء اللوبيا وسوسة الأرز

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تم اختبار السمية البخارية لعدد سبعة زيوت عطرية هي زيت الشاي، القرفة، القرنفل، حشيشة الليمون، والزعتر، الكافور، الجوجوبا ضد حشرتي خنفساء اللوبيا وسوسة الأرز. النتائج اوضحت ان نسبة الموت تزداد مع زيادة تركيز الزيت وفترة التعريض. وقد وجد ان السمية البخارية لكلا من زيت القرفة والشاي قد حققت ٩٠.٠% موت لسوسة الارز عند التركيز ٨، ١٦، ١٦ ميكروليتر / ٥٠ مل هواء (حجم حيز التعريض) بعد فترة تعريض ٢٤ ساعة. بينما فى حالة خنفساء اللوبيا فقد حققت السمية البخارية للزيوت القرفة والشاي والزعتر ١٠٠% موت للحشرة عند التركيز ٨، ١٦، ١٦ ميكروليتر / ٥٠ مل هواء على التوالي وعلى نفس فترة التعريض. ايضا وجد ان كلا من الحشرتين قد تأثرا بشدة بالسمية البخارية لزيت القرفة حيث حقق ٩٥% موت لحشرة سوسة الارز عند التركيز ٣.٦٧ ميكروليتر/٥٠ مل هواء بينما حقق ٩٥% موت عند التركيز ٤.٧ ميكروليتر/٥٠ مل هواء لحشرة خنفساء اللوبيا عند فترة تعريض ٧٢ ساعة. ووجد ان خنفساء اللوبيا كانت اكثر حساسية من سوسة الارز للسمية البخارية للزيوت القرنفل والكافور حيث تحقق ٩٥% موت عند التركيز ١.٠٣٢، ٣.٦٦ ميكروليتر / ٥٠ مل هواء عند فترة تعريض ٧٢ ساعة بينما السمية البخارية لنفس الزيوت القرنفل والكافور حيث تحقق ٩٥% موت عند التركيز ٤.٠٧، ٨.٧٣ ميكروليتر / ٥٠ مل هواء على التوالي لسوسة الارز عند نفس فترة التعريض.