MIXED EFFECTS OF 1,8-CINEOLE, BOTANICAL CONSTITUENT, AND REDUCED ATMOSPHERE PRESSURE ON STORED PRODUCTS BEETLES IN LABORATORY CONDITION

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ABSTRACT: Controlled atmosphere is efficient way to control of stored-product insects. 1,8-Cineole as main component of *Eucalyptus* spp. has high toxicity on insects. Toxicity of 1,8-Cineole at low pressure was performed against two most common stored-product insects, *Callosobruchus maculatus* (F.) and *Tribolium Castaneum* (Herbst) in two time, at three different pressure (Normal pressure, 100 mm Hg, 150 mm Hg). LD_{50} values of 1,8-Cineole fumigant toxicity in 12 h and 24 h for *C. maculatus* and *T. Castaneum* in normal atmosphere pressure of Urmia (Iran) (653 mm Hg) were 275.6, 201.5, 452.2 and 265.1 µl/l air, respectively. LD_{50} values of 1,8-Cineole fumigant toxicity plus reduced atmosphere pressure in 100 mm Hg, in 12 & 24 h for mentioned insects were 193.5, 118.3, 331.7 and 176.8 µl/l air, and in 150 mm Hg were 210.3, 151.5, 381.4 and 214.7 µl/l air, respectively. Results have been shown that insects susceptibility to 1,8-Cineole, had significant enhancement by reduction in pressure of atmosphere. Toxicity of 1,8-Cineole at reduced pressures was strongly influenced by ambient time. Application of integrated management by essential oils and physical practices such as reduced atmosphere pressure has potential efficacy for stored-product pests control.

KEY WORDS: Reduced pressure, 1,8-Cineole, *Callosobruchus maculatus*, *Tribolium castaneum*.

Agricultural and animal stored products are attacked by more than 1200 species of pests (Rajendran, 2002). In many storage systems, fumigants are the most economical and common tool for managing stored product pests not only due to their ability to kill a broad spectrum of pests but because of their easy penetration into commodity with leaving minimal residue (Mueller, 1990). Because of these reasons, Methyl bromide and phosphine are widely common fumigants (Lee et al., 2004). But due to following reasons currently, few chemicals are available for use as fumigants that meet all of these constraints. Due to potential ozone-depleting property and high toxicity to warm-blooded animals including human kind of methyl bromide, this most effective fumigant except for control quarantine pest was restricted (Dansi et al., 1984; Anonymous, 1991). Fumigation by phosphine which is widely using may become increasingly districted in use as it makes resistance of stored product insects to this fumigant and some arguments about the genotoxicity potential of phosphine (Bell, 1995; Meaklim, 1998).

Essential oils are potential alternative material to currently used fumigants (Lee et al., 2001). Plant products, like essential oils and their components were used for fumigation since it is believed that extracts from plants may have the advantage over conventional fumigants in terms of low mammalian toxicity, Rapid degradation and local availability (Rajendran et al., 2008). Some of the plants which have medicinal properties are *Cupressus sempervirens, Eucalyptus spp.*, *Salvia hydrangea* and *Artemsia spp.*. Essential oils contain components that have ovicidal, repellent, antifeedant, sterilization and toxic effects in insects (Nawrot, 1994; Isman, 2006). Among extracts of various aromatic plants, *Eucalyptus* spp. extracts specially 1,8-Cineole as main component has high toxicity on insects (Lee et al., 2000).

In current study 1,8-Cineole was selected as fumigant material which their toxicity previously had been proved in small jars (250 ml). But this study was undertaken to investigate toxicity and amount of using of this fumigant in bigger containers (9000 ml).

In other hand controlled atmosphere including low oxygen, high carbon dioxide concentrations and reduced pressure are efficient ways to control stored-product insects especially on adult stage. Modified atmosphere treatments are healthy and environmental friendly ways for controlling pests that damage a large number of stored-products. In several developed countries, they have been adopted as feasible alternative treatments since the use of methyl bromide was phased out in 2005. Modified atmosphere was used for many years and were tested in the laboratory and under industrial conditions for the control of various insects and mites species (Fleurat-Lessard, 1990; Adler et al., 2000; Navarro, 2006). Low pressure through producing low O_2 concentration and high concentration of CO_2 can control stored-product insects in storages (Philips et al., 2010). Some stored-product insects, like *Sitophilus oryzae* (L.), *Sitophilus granaries* (L.), *Trogoderma granarium* (Everts), *Ephestia cautella* (Walker), *Tribolium castaneum* (Herbst), *Lasioderma serricorne* (F.), *Ephestia elutella* (Hubner) and *Callosobruchus maculatus* (F.) were previously investigated for mortality under low pressure (Bare, 1948; Calderon, 1983; Navarro & Donhaye, 1987; Locatelli & Daolio, 1993; Finkelman et al., 2004; Mbata et al., 2004, 2005, 2009).

Toxicity of propylene oxide (PPO) and methyl bromide at low pressure against some stored-product insects was investigated by Isikber *et al.* (2004) and Donhaye *and* Navarro (1989) but investigation of interaction between reduced pressure and essential oils was investigated firstly in this study. Due to this reason much information is not available. The aim of this study was increase the toxicity of 1,8-Cineole as main constituent of *Eucalyptus* spp. by reduction atmosphere pressure

MATERIALS AND METHODS

Tests were carried out on adult stage of two stored-product beetle pests, *Callosobruchus maculatus* and *Tribolium castaneum.*

Test insects

All experimented insects were obtained from cultures reared at 29±2°C and 65±5% relative humidity (r.h.) in darkness on a diet of bean for *C. maculatus* and wheat meal for *T. castaneum* using standard culture techniques**.**

In the first stage, 1,8-Cineole and reduced pressure were tested separately against insects in a without grain space. In the second stage the effect of 1,8-Cineole and reduced pressure together were determined. This study was carried out on adult stage 1-3 days old of *C. maculatus* and *T. castaneum*. Each test was replicated three times on three different days. All of experiments were done in two times, 12 and 24 h. Mortality was recorded, 24 h after termination of exposure. As Isikber et.al (2004) said, temperature and relative humidity were very effective on low pressure experiments, due to this reason all of experiments were carried out in incubator with 30±2°C and 40±5% relative humidity.

Materials of experiments

The tested 1,8-Cineole was 98% and supplied by Merck Co. Ltd. All doses used in this study are expressed as commercial formulations. Reduced pressures made by vacuum pump Model DSE42 in containers with 9 liters capacity and were equipped by two gates for input and output air and one manometer and one septum for injection fumigant which Number 1Whatman papers were placed below the septum to capture the injected essential oil and to produce a large surface area for evaporation.

Dosing and fumigation procedures

Preliminary dose-mortality tests were done before each experiment. Those insects that did not move when lightly probed or shaken in the light and mild heat were considered dead. 9000 ml tight containers were used as a fumigant and reduced pressure chamber.

Adults of *C. maculatus* and *T. castaneum* were fumigated by 1,8-Cineole for 12 and 24 h in 9000 ml containers, separately. Tested insects were confined in cages districted with 40 mesh wire gauze. Each cage was contained 30 insects and 2 g of food. Each container was capped with tight screwed lid. The appropriate amount of each concentration of 1,8-Cineole was injected in container with an oxford sampler through a septum, located in the center of the cap. In each test, the control container was treated identically except that no 1,8-Cineole was injected in container. After exposure, the insects were transferred to clean jars containing rearing medium.

To select appropriate pressures for interaction tests, preliminary tests carried out in containers which have been explained above and by vacuum pump. Normal pressure of experiments in Urmia city (Iran) (653 mm Hg) calculated by way offered by Evett et al. (1988). After preliminary tests, 100±5 mm Hg and 150±5 mm Hg was selected as co-experimenting factor. In last stage interaction tests between 1,8-Cineole and reduced pressure on adult stage of *C. maculatus* and *T. castaneum* carried out. For perform this experiment, air of containers was vacuumed by vacuum pump through a gate located on lid of container, then 1,8-Cineole was injected through a septum by an oxford sampler.

Data analysis

Mortality data were analyzed with SPSS software (SPSS Inc, 1993). Probit analysis was used to determinate LD_{50} and LD_{95} values. The values significance of χ^2 was estimated according to Robertson and Preisler (1992). Data were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test to estimate statistical differences between means at $\alpha = 0.05$.

RESULTS

Tables 1, 2, 3 and 4 shows probit mortality regression data for 1,8-Cineole lonely and at 100 and 150 mm Hg against adult stage of *C. mculatus* and *T. castaneum* at 12 h after exposure. There was a remarkable difference in susceptibility to 1,8-Cineole at different atmosphere pressures. In table 1 results shows that the LD_{50} ranged from 193.5 µl/l air to 275.6 µl/l air, reflecting increasing susceptibilities on the order of normal pressure, 100 mm Hg and 150 mm Hg. Similarly, LD_{95} ranged from 1416.7 µl/l air to 1931.9 µl/l air, reflecting increasing susceptibilities by atmosphere pressure reduction at 12 h after exposure on adults of *C. maculatus*.

Table 2 shows the LD_{50} values ranged from 118.3 µl/l air to 201.5 µl/l air, reflecting increasing susceptibilities on the order of normal pressure, 100 mm Hg and 150 mm Hg. Similarly, LD_{95} ranged from 702.7 µl/l air to 1178.8 µl/l air, reflecting increasing susceptibilities by atmosphere pressure reduction at 24 h after exposure on adults of *C. maculatus*.

In table 3 the LD_{50} values ranged from 331.7 µl/l air to 452.2 µl/l air, and LD_{95} ranged from 1167.4 µl/l air to 1401.3 µl/l air, reflecting increasing susceptibilities on the order of normal pressure, 100 mm Hg and 150 mm Hg is observable, which reflecting increasing susceptibilities by atmosphere pressure reduction at 12 h after exposure on adults of *T. castaneum*.

Table 4 shows probit analysis data of experiments on *T. castaneum* at 24h after exposure. This table shows that LD50 values ranged from 176.8 µl/l air to 265.1 µl/l air, reflecting increasing susceptibilities on the order of normal pressure, 100 mm Hg and 150 mm Hg. Similarly, LD95 ranged from 2109.2 µl/l air to 2908.9 µl/l air, reflecting increasing susceptibilities by atmosphere pressure reduction.

Percent reduction of LD50 values of 1,8-Cineole ranged from 18.5 to 41.2 and percent reduction of LD95 values of 1,8-Cineole from 12.4 to 33.8 in two experimented pests. This comparison shows that in all of experiments, drops in dosages to reach LD50 and LD95 in *C. maculatus* are more than *T. castaneum*. The most drops in LD50 and LD95 is depends on *C .maculatus* in treatment 24 h exposure in 100 mm Hg pressure. The lowest drops in LD50 and LD95 is depends on *C.maculatus* in treatment 12 h exposure in 150 mm Hg pressure.

DISCUSSION

Unfortunately, 1,8-Cineole despite advantages such as another essential oils and their components have low pressure (<1 mm Hg at 20° C) when compared with phosphine (vapour pressure (31.92 mm Hg at 23° C), methyl bromide (1250 mm Hg at 20° C) and sulphuryl floride (12087 mm Hg at 20° C) (Rajendran, 2002).

Earlier studies like as Lee *et al.* (2003) were tested 1,8–Cineole on *T. castaneum*, *sitophilus oryzae* and *Oryzaephilus surinamensis* and Stamopoulos *et al.* (2007) on *T. confusom*. Current study performed to determine fumigant toxicity of 1,8-Cineole. Obtained LC_{50} and LC_{95} values in this study were more than results of earlier studies. These differences were caused due to performing experiments in very high capacity relative by previously researches and because of 1,8-Cineole low pressure (Rajendran, 2002) this increasing LC_{50} and LC_{95} values seems rationale.

Previously, many studies were performed on mortality properties of modified atmosphere and reduced pressure on stored-product insects (Bare, 1948; El Nahal, 1953; Calderon, 1968; Calderon, 1983; Navarro, 1987; Locatelli, 1993; Finkelman et. al, 2004; Mbata et. al, 2004, 2005, 2009).

Reduced pressure or vacuum causes low O_2 and high CO_2 concentrations by metabolic arrest and losses water through opened spiracle is lethal for insects (Philips et al., 2010; Mitcham et al., 2006).

Influence of low pressure and CO_2 was studied by Navarro et al. (2004) and Isikber et al. (2002). Their results showed that the combination of propylene Oxide with low pressure or CO_2 can provide a potential alternative to methyl bromide for quarantine treatment of commodities where rapid disinfestations techniques and high level of insect mortality are essential.

Toxicity of methyl bromide alone and in combination with carbon dioxide or under reduced pressure was studied by Donhaye et al. (1989). Their results, showed significant difference between methyl bromide alone and mixed by CO_2 and reduced pressure.

In current study results showed that effect of 1,8-Cineole in reduced pressures were more than normal pressure. This occurrence was conducted due to opening insect's spiracles and increase the rate respiration to gain appropriate O_2 for perform enough metabolism to be alive (Mitcham, 2006) . This reaction to low level of O_2 causes to absorb more doses of fumigant. As can be expected in lower pressure (100 mm Hg) and more exposure time (24 h) LC_{50} and LC_{95} values due to more reduction of O_2 and increase CO_2 concentrations more reduced.

Preliminary experiments showed that *C. maculatus* is more susceptible than *T. castaneum*. This order causes most effectiveness by reduced pressure on consumed doses in *C. maculatus* than *T. castaneum*.

Another probability advantage of this combined method (Reduced pressure and 1,8-Cineole), is reduction of flammability of 1,8-Cineole in lower O_2 and higher CO_2 concentrations. Clearly, furthermore studies are needed to prove this claim.

1,8-Cineole was found to be effective against tested insects, however it was less toxic than methyl bromide and phosphine. The use of low pressure of 100 mm Hg appears to have synergetic effect on these species mortality as evidenced by significant reductions in LC_{50} and LC_{95} values. These results showed that the combination of 1,8-Cineole with low pressure can render this essential oil to potential replacement of methyl bromide and phosphine. More another research is needed to obtain toxicity data on other stored-product insects, on its absorption by different commodities, and on its power of penetration into bulk storage commodities.

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Table 1. Toxicity of 1, 8-cineole to *C. maculatus* in 3 pressure exposed 12 h at 29±2 in 9000 ml containers.

	Normal pressure		100 mm Hg		150 mm Hg	
Toxicity value	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal concentration	275.6	1931.9	193.5		210.3	1683.8
Upper 95 (%) FL	237.9	3572.9	1416.7		247.1	2804.5
Lower95 (%) FL	320.4	304.1	224.7	2640.9	179.8	1097.6
Slope ± SE	1.94±0.23		165.7	55.3	1.80±.22	
X^{2a}	0.23		1.9±0.23		2.135	
ρ	0.97		1.32		0.54	
F	202.2**		0.72		347.5**	
Reduced percent LD_{50} dose [b]			235.1**		23	12.8
			29.8	26.7		

Three replicates (30 insects per replicate) were tested in each treatment. [a] Pearson's X^2 goodness-of-fit tests: all values of ρ>0.05 and the data fits regression model. **significant in level %1. [b] LD_{50} percent which reduced in each controlled atmosphere pressure.

Table 2. Toxicity of 1, 8-cineole to *C. maculatus* in 3 pressure exposed 24 h at 29±2 in 9000 ml containers.

Toxicity value	Normal pressure		100 mm Hg		150 mm Hg	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal concentration	201.5	1178.8	118.3	702.7	151.5	82.1
Upper 95 (%) FL	231.04	2156.5	135.34	1276.9	179.12	1605.6
Lower95 (%) FL	176.47	811.4	103.19	485.6	129.13	608.2
Slope ± SE	2.14±0.27		2.12±0.27		1.82±0.22	
X^{2a}	0.24		0.73		1.74	
ρ	0.97		0.86		0.63	
F	281.7**		609.4**		205.8**	
Reduced percent LD^{50} dose [b]			41.2	33.8	24.8	16.9

Three replicates (30 insects per replicate) were tested in each treatment. [a] Pearson's X^2 goodness-of-fit tests: all values of $\rho>0.05$ and the data fits regression model. **significant in level %1. [b] LD_{50} percent which reduced in each controlled atmosphere pressure.

Table 3. Toxicity of 1, 8-cineole to *T. castaneum* in 3 pressure exposed 12 h at 29±2 in 9000 ml containers.

Toxicity value	Normal pressure		100 mm Hg		150 mm Hg	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal concentration	452.2	1401.3	331.7	1167.4	381.4	1227.6
Upper 95 (%) FL	492.4	2005.3	299	1548.8	446.5	1630.3
Lower95 (%) FL	414.3	1118	366.1	952.9	316.4	1016.3
Slope ± SE	3.35±0.41		3.01±0.22		3.174±0.23	
$X^{2\,a}$	0.432		6.93		1.299	
ρ	0.93		0.074		0.73	
F	293.1**		316.1**		220.1**	
Reduced percent LD_{50} dose [b]			26.6	16.7	18.5	12.4

Three replicates (30 insects per replicate) were tested in each treatment. [a] Pearson's X^2 goodness-of-fit tests: all values of $\rho>0.05$ and the data fits regression model. **significant in level %1. [b] LD_{50} percent which reduced in each controlled atmosphere pressure.

Table 4. Toxicity of 1, 8-cineole to *T. castaneum* in 3 pressure exposed 24 h at 29±2 in 9000 ml containers.

Toxicity value	Normal pressure		100 mm Hg		150 mm Hg	
	LD_{50}	LD_{95}	LD_{50}	LD_{95}	LD_{50}	LD_{95}
Lethal concentration	265.1	2908.9	176.8	2109.2	214.7	2473
Upper 95 (%) FL	316	6530.4	212.39	5043.8	261.2	5876.3
Lower 95 (%) FL	218.27	1781.8	145.17	1253	174.9	1471.3
Slope ± SE	1.58±0.21		1.53±0.2		1.53±0.2	
$X^{2\,a}$	1.64		0.066		1.02	
ρ	0.65		0.99		0.79	
F	155.5**		123.4**		309.6**	
Reduced percent LD_{50} dose [b]			33.3	27.5	19	14.9

Three replicates (30 insects per replicate) were tested in each treatment. [a] Pearson's X^2 goodness-of-fit tests: all values of $\rho>0.05$ and the data fits regression model. **significant in level %1. [b] LD_{50} percent which reduced in each controlled atmosphere pressure.