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ORIGINAL ARTICLE

Fumigant toxicity of volatile synthetic compounds and natural oils against red flour beetle *Tribolium castaneum* (Herbst) (Coleopetera: Tenebrionidae)

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Abstract Two synthetic volatile compounds (benzaldehyde and propionic acid) and two volatile oils (camphor and eucalyptus) were screened individually and in combinations against different life stages of *Tribolium castaneum*. Benzaldehyde–propionic acid combination recorded the maximum larvicidal (LC_{50} = 78.03 μl/l) and adulticidal (LC_{50} = 30.60 μl/l air) activities and this treatment was also effective in reducing the oviposition, egg hatchability and adult emergence of *T. castaneum*. Among the individual treatments benzaldehyde was found to be more toxic to adults (LC_{50} = 34.07 μl/l) and larvae (94.15 μl/l). The individual treatments of camphor and eucalyptus oils were less effective, but combinations of benzaldehyde–camphor oil were found to be effective. Benzaldehyde–propionic acid combination recorded 99.3% adult mortality inside a 1 m^3 wooden cage after 15 days and this mixture can be used as a fumigant in store houses.
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1. Introduction

The red flour beetle *Tribolium castaneum* (Herbst) is a major pest of stored products in many parts of the world particularly in the tropics. The adult beetles and larvae of *T. castaneum* spoil seeds, grains and milled products. Fumigation is a common method of stored product pest management. Phosphine and methyl bromide are the commonly used fumigants in store houses. Several studies clearly showed that *T. castaneum* has developed resistance to many chemical fumigants including phosphine (Dyte, 1970; Irshad and Gillani, 1989; Zettler and Cuperus, 1990). The synthetic chemical fumigants are also reported to be highly hazardous to human health as well as the environment and insects can develop resistance against the chemical fumigants like phosphine and methyl bromide

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(Zettler, 1993; Leesch, 1995). The unwanted effects of chemical fumigants have led to the exploration of less hazardous and environmentally friendly alternatives.

Several volatile oils and volatile compounds have been reported as attractants, repellents, insecticides, oviposition deterrents and growth inhibitors against many stored product insects including *T. castaneum* and are considered as promising pest control agents (Deshpande et al., 1974; Deshpande and Tipnis, 1977; Regnault-Roger and Hamraoui, 1995; Lee et al., 2004; Mondal and Khalequzzaman, 2006). Tunc et al. (2000) and Channoo et al. (2002) have studied the fumigant toxicity of eucalyptus oil on life stages of *T. castaneum*. Lee et al. (2004) have tested the fumigant toxicity of 42 essential oils extracted from 42 plant species including 25 *Eucalyptus* spp. against *Sitophilus oryzae* adults and found that six oils including three *Eucalyptus* spp. were potent against *S. oryzae*. Negahban and Moharramipour (2007) have reported that essential oils from *Eucalyptus intertexta*, *Eucalyptus sargentii* and *Eucalyptus camaldulensis* had fumigant toxicity against *Callosobruchus maculatus*, *S. oryzae* and *T. castaneum* adults. Paulraj and Ignacimuthu (2007) have reported that eucalyptus and camphor oils were toxic to eggs, larvae and adults of *Lasioderma serricorne*. Though volatile oils are efficacious their activity is slow and high concentrations are needed compared to conventional fumigants.

Some investigators have tested the toxicity of synthetic volatile compounds against stored product pests. In a fumigant toxicity experiment Lee et al. (2004) found that 1,8-cineole was toxic to *S. oryzae*, *T. castaneum* and *Rhyzopertha dominica* adults. Burkholder et al. (1973) have reported that propionic acid was toxic to larvae and eggs of *Trogoderma variabile* Ballion and *Attagenus megatoma* (F.) (Coleoptera, Dermestidae) when added to the insect's food at 2% concentration. Propionic acid is a naturally occurring carboxylic acid. It occurs in the blend of volatile compounds emitted by barley grains (Maga, 1978). It is commonly used by the food industry as a preservative in several food products. Benzaldehyde, the simplest aromatic aldehyde, was reported to be a safer fumigant to control stored grain insect pests (Lee et al., 2001).

Toxicity and grain protecting efficacy of several volatile oils and volatile compounds have been checked individually against *T. castaneum* by many investigators (Negahban and Moharramipour, 2007; Mohamed and Abdelgaleil, 2008). But the additive or synergistic effects of volatile oils and volatile compounds were not studied against *T. castaneum*. Hence the present study was undertaken to investigate the additive or synergistic effects of camphor and eucalyptus oils and benzaldehyde and propionic acid against *T. castaneum*.

2. Materials and methods

2.1. Insect

T. castaneum adults and larvae were obtained from a stock culture that was continuously maintained for several generations on wheat flour at the Entomology Research Institute insectary at laboratory conditions (29 ± 1 °C; 60–65% R.H.; 11 ± 0.5 h photoperiod). The adults used in the fumigation toxicity experiments were 5–7 day-old and the larvae were 12 day-old.

2.2. Volatile oils and volatile compounds

Camphor oil obtained from *Cinnamomum camphora* (also known as *Laurus camphora*) and eucalyptus oil obtained from *Eucalyptus globulus* Labill. were procured from the Central Institute of Medicinal and Aromatic plants, Lucknow, India. The two synthetic volatile compounds, benzaldehyde and propionic acid, were purchased from Ranbaxy Fine Chemicals Limited (RFCL), New Delhi, India.

2.3. Treatments and concentrations

The volatile oils and volatile compounds were tested individually as well as in different combinations. Besides a set of control, 12 different treatments were used as follows: (i) propionic acid (PA); (ii) benzaldehyde (B); (iii) eucalyptus oil (EO); (iv) camphor oil (CO); (v) PA + B (1:1 ratio); (vi) EO + CO (1:1 ratio); (vii) PA + EO (1:1 ratio); (viii) PA + CO (1:1 ratio); (ix) B + EO (1:1 ratio); (x) B + CO (1:1 ratio); (xi) PA + EO + CO (1:1:1 ratio) and (xii) B + EO + CO (1:1:1 ratio). Concentrations for fumigation studies were prepared based on the volume of the container used and expressed as μl/l of air. In the combination treatments the volatile oils and/or compounds were mixed in equal proportion and required concentrations were prepared. Five different concentrations viz., 20, 40, 80, 120 and 160 μl/l of air were screened in fumigation toxicity studies against larvae and adults. The ovicidal activity and adult emergence were studied at three different concentrations viz., 20, 40 and 80 μl/l of air. The fecundity studies were conducted using the sublethal concentrations viz., LC_{10}, LC_{20} and LC_{30}.

2.4. Fumigation toxicity on T. castaneum adults and larvae

The toxicity of the volatile oils (eucalyptus and camphor) and the volatile compounds (propionic acid and benzaldehyde) on *T. castaneum* adults was tested by filter paper dip method at laboratory conditions (30 ± 1 °C; 60–65% R.H.). Whatman No. 1 filter paper discs (2 cm diameter) were impregnated with different concentrations of essential oils or compounds separately and were attached to the under surface of the screw caps of glass vials (volume 45 ml) separately as described by Negahban et al. (2007). The cap was screwed tightly on the vial after the release of 10 adult beetles (1–7 days old) or larvae along with little amount of wheat flour as food. For comparison a set of control, without volatile oils and compounds, was maintained. Each treatment and control was replicated five times. Mortality was recorded after 4, 8, 12 and 24 h from the commencement of exposure. When no leg or antennal movements were observed, insects were considered dead. Percentage insect mortality was calculated using the Abbott's correction formula (Abbott, 1925).

2.5. Fecundity

Ten male and ten female *T. castaneum* beetles were taken in a 220 ml air tight petri dish and a little amount of wheat flour was added as food. Filter paper discs (Whatman No. 1; 2 cm diameter) impregnated with LC_{10} concentration of benzaldehyde were placed inside the petri dish. After 24 h of treatment the adults were transferred to an untreated petri dish and number of eggs laid was counted up to 2 days under a stereo microscope. Fecundity per female was calculated. The other treatments and other sublethal concentrations (LC_{20} and LC_{30}) were also tested by the same method. Five replications were maintained for control and treatment groups.

2.6. Egg hatchability, larval survival and adult emergence

Fresh eggs of *T. castaneum* were separated from insect culture vials and carefully transferred to 50 ml glass vials with the help of a smooth hair brush. About 15 eggs were separately treated with each concentration of each treatment by the same fumigation method described earlier. In the controls filter paper discs without volatiles were placed inside the vials. All treatments and control were replicated five times ($n = 50$). The egg hatchability was recorded daily and converted into percentage. Hatched larvae were maintained on wheat flour and the larval survival and percent adult emergence were recorded.

2.7. Fumigation toxicity inside 1 m^3 wooden cage

The efficacy of effective treatments was tested inside a 1 m^3 wooden cage. The wooden cage was used because it simulates a common storage structure. The wooden cage had a small opening with a sliding door. The cage was completely sealed with adhesive tape at all joints. Four treatments namely benzaldehyde, propionic acid, benzaldehyde + propionic acid and benzaldehyde + camphor oil were tested at their respective LC_{99} concentrations. The LC_{99} concentration of benzaldehyde solution required for 1 m^3 volume was calculated and taken in a glass bottle. The bottle with benzaldehyde solution was kept at the centre inside the cage. One hundred adult beetles were released on 50 g wheat flour taken in a plastic bottle (3.5 l) and the mouth of the vial was closed with a muslin cloth. The plastic bottle with the beetles was kept inside the cage. Three replications were maintained for one treatment. The door of the cage was closed and the gaps were completely sealed with adhesive tape. The plastic vials were taken out after every 15 days; the mortality was recorded and the dead insects were replaced with new live insects. The experiment was continued for 90 days. Similarly other treatments were also tested. All treatments were replicated nine times. The percentage mortality was calculated and corrected using Abbott's formula (Abbott, 1925).

2.8. Statistical analysis

The fumigation toxicity, fecundity, egg hatchability, larval survival and adult emergence were subjected to analysis of variance (ANOVA). Analysis of covariance (ANCOVA) was used to find treatment effects on egg hatchability and larval survival. Significant differences between treatments were determined by Tukey's HSD tests ($p \leqslant 0.05$). The median lethal concentration of LC_{50} and LC_{99} values were calculated using probit analysis (Finney, 1971).

3. Results

3.1. Adulticidal activity

Table 1 shows the lethal concentrations (LC_{50} and LC_{99}) of all the volatile oil and volatile compound treatments against *T. castaneum* adults. Among the various treatments benzaldehyde + propionic acid combination killed more beetles in less period of exposure compared to the other treatments. The LC_{50} and LC_{99} concentrations of benzaldehyde + propionic acid combination were calculated as 30.6 and 99.28 μl/l, respectively. Individual treatments of benzaldehyde and propionic acid were also more effective than volatile oils. The LC_{50} concentrations of propionic acid and benzaldehyde were 36.79 and 34.07 μl/l, respectively (Table 1).

The volatile oils were less effective than benzaldehyde and propionic acid treatments. However, camphor oil + benzaldehyde combination showed increased toxicity compared to individual camphor oil treatment and the increase was statistically significant ($p > 0.05$) after 24 h exposure ($LC_{50} = 40.98$ μl/l).

3.2. Larvicidal activity

Benzaldehyde + propionic acid combination recorded the maximum larvicidal activity and the LC_{50} value was 78.03 μl after 24 h exposure (Table 2). Benzaldehyde individual treatment was the second most effective treatment (LC_{50} =

Table 1 Lethal concentrations (LC_{50} and LC_{99}) of volatile oils, volatile compounds and their combinations against *T. castaneum* adults ($n = 50$).

Treatments	LC_{50} (μl/l air)	95% Confidence limits		LC_{99} (μl/l air)	95% Confidence limits		Chi-square	*p*-Value
		Lower	Upper		Lower	Upper		
PA	36.79	30.27	43.39	112.20	97.05	135.46	17.56	0.937
B	34.07	27.55	40.46	107.64	92.77	130.46	19.18	0.893
EO	54.95	46.11	63.74	171.63	150.99	201.58	23.83	0.691
CO	60.84	51.48	70.22	187.92	165.54	220.32	17.68	0.934
PA + B	30.60	24.40	36.72	99.28	85.14	121.58	27.68	0.466
EO + CO	57.97	42.02	67.00	178.87	159.66	209.50	19.82	0.871
PA + EO	50.51	41.48	59.29	167.96	147.28	198.17	23.96	0.684
PA + CO	49.18	40.18	57.81	163.62	143.62	193.38	23.79	0.692
B + EO	47.23	38.23	55.89	162.49	142.20	192.27	27.51	0.491
B + CO	40.98	33.44	48.41	132.21	115.09	157.82	22.38	0.763
P + EO + CO	51.29	41.75	60.46	176.77	154.77	209.03	24.15	0.674
B + EO + CO	44.33	36.22	52.26	145.33	126.96	172.48	19.97	0.866

PA, propionic acid; B, benzaldehyde; CO, camphor oil; EO, eucalyptus oil.

Table 2 Lethal concentrations of volatile oils and volatile compounds against larvae of *T. castaneum* after 24 h ($n = 50$).

Treatments	LC_{50} (μl/l air)	95% Confidence limits		LC_{99} (μl/l air)	95% Confidence limits		Chi-square	*p*-Value
		Lower	Upper		Lower	Upper		
PA	101.16	90.68	112.48	232.79	205.79	274.06	7.83	0.999
B	94.15	83.80	104.99	223.90	198.15	262.90	10.98	0.983
EO	131.34	119.53	147.45	272.78	237.38	331.27	9.33	0.995
CO	141.03	127.68	159.49	287.35	247.73	335.28	5.45	1.000
PA + B	78.03	68.43	87.51	191.15	170.25	221.94	11.80	0.973
EO + CO	134.39	121.67	160.99	280.00	242.44	324.93	9.55	0.994
PA + EO	126.91	115.02	141.85	268.58	234.12	324.88	8.08	0.998
PA + CO	123.65	112.07	137.86	263.13	230.10	316.38	8.78	0.997
B + EO	119.17	107.65	133.01	261.03	228.23	313.56	6.84	1.000
B + CO	110.38	99.36	122.97	248.84	218.59	296.24	11.50	0.977
P + EO + CO	138.40	123.82	158.88	305.23	259.71	384.58	8.36	0.998
B + EO + CO	121.10	109.56	135.09	262.26	229.28	315.26	8.13	0.998

PA, propionic acid; B, benzaldehyde; CO, camphor oil; EO, eucalyptus oil.

94.15 μl). The larvicidal activity of volatile oils, eucalyptus and camphor increased when they were mixed with benzaldehyde. The LC_{50} values of benzaldehyde + camphor oil and benzaldehyde + eucalyptus oil were 110.38 and 119.17 μl, respectively, after 24 h of exposure.

3.3. Fecundity

The exposure of gravid female *T. castaneum* beetles to sublethal concentrations (LC_{10}, LC_{20} and LC_{30}) of different treatments for 24 h resulted in reduced fecundity. The average fecundity of the beetle in control group was recorded as 4.7 eggs/female. In all the treatments the fecundity was reduced and it was directly proportional to the concentrations tested. Benzaldehyde + propionic acid combination recorded the lowest fecundity at LC_{10} (2.44 eggs/female), LC_{20} (1.32 eggs/female) and LC_{30} (0.8 eggs/female) concentrations (Table 3). Benzaldehyde individual treatment was also found to be effective; this treatment recorded 2.76, 1.38 and 0.92 eggs/female insect at the LC_{10}, LC_{20} and LC_{30} concentrations, respectively, in 24 h.

3.4. Egg hatchability and larval survival

The egg hatchability was found to be much reduced by the treatment with benzaldehyde + propionic acid combination in a highly significant manner compared with propionic acid (*F* value in ANCOVA = 18.00; $p < 0.001$) and benzaldehyde individually (*F* value in ANCOVA = 12.73; $p < 0.001$). Benzaldehyde + propionic acid combination and benzaldehyde individual treatments recorded 100% ovicidal activity at 80 μl concentration (Table 4). In the control group the egg hatchability and survival of the hatched larvae were recorded as 86.7% and 84.6%, respectively. Benzaldehyde + propionic acid treatment showed the lowest egg hatchability of 27.8% and 12.5% at 20 and 40 μl concentration, respectively, and the larval survival was also the lowest in this treatment. The larval survival in benzaldehyde + propionic acid treatment was recorded as 10% and 6.6% at 20 and 40 μl concentrations, respectively, whereas these variations are less significant (Table 5). Propionic acid treatment at 80 μl concentration registered only 6.6% egg

Table 3 Fecundity (number of eggs/insect/day) of *T. castaneum* at three sublethal concentrations (mean ± SE) ($n = 50$).

Treatment	Exposure concentrations (μl/l)		
	LC_{10}	LC_{20}	LC_{30}
PA	3.02 ± 0.04^{ef}	1.80 ± 0.09^{cd}	1.12 ± 0.04^{def}
B	2.76 ± 0.09^{fg}	1.38 ± 0.06^{f}	0.92 ± 0.04^{fg}
EO	3.68 ± 0.07^{bc}	1.86 ± 0.10^{cd}	1.22 ± 0.05^{cde}
CO	4.08 ± 0.09^{b}	2.24 ± 0.05^{b}	1.40 ± 0.04^{bc}
PA + B	2.44 ± 0.07^{g}	1.32 ± 0.05^{ef}	0.80 ± 0.03^{g}
EO + CO	4.06 ± 0.09^{b}	2.02 ± 0.07^{bc}	1.58 ± 0.04^{b}
PA + EO	3.54 ± 0.07^{cd}	2.04 ± 0.07^{bc}	1.36 ± 0.05^{bcd}
PA + CO	3.40 ± 0.07^{cde}	1.86 ± 0.04^{cd}	1.32 ± 0.06^{bcde}
B + EO	3.26 ± 0.09^{cde}	1.68 ± 0.06^{cd}	1.22 ± 0.07^{cde}
B + CO	3.18 ± 0.13^{def}	1.46 ± 0.05^{ef}	1.08 ± 0.06^{ef}
P + EO + CO	3.66 ± 0.08^{bc}	1.84 ± 0.21^{cd}	1.38 ± 0.05^{bcd}
B + EO + CO	3.48 ± 0.13^{cd}	1.62 ± 0.06^{def}	1.24 ± 0.08^{cde}
Control		4.7 ± 0.14^{a}	

Means followed by same letters within each column are not significantly ($p \leqslant 0.05$) different in Tukey's HSD test.
PA, propionic acid; B, benzaldehyde; CO, camphor oil; EO, eucalyptus oil.

Table 4 Egg hatchability (%) of *T. castaneum* in volatile oils and volatile compounds treatments (mean ± SE).

Treatments	Exposure concentrations (μl/l)		
	20	40	80
PA	39.1 ± 1.8^{bcd}	19.4 ± 3.4^{de}	6.6 ± 4.1^{d}
B	33.8 ± 3.0^{cd}	17.4 ± 2.8^{e}	0^{d}
EO	80.0 ± 6.3^{a}	66.6 ± 2.6^{b}	54.0 ± 4.9^{b}
CO	84.0 ± 7.4^{a}	68.0 ± 3.7^{b}	42.0 ± 2.0^{bc}
PA + B	27.8 ± 1.7^{d}	12.5 ± 1.4^{e}	0^{d}
EO + CO	76.5 ± 3.3^{a}	63.1 ± 2.3^{b}	50.6 ± 3.7^{b}
PA + EO	54.0 ± 3.6^{b}	39.7 ± 2.4^{c}	32.3 ± 2.0^{c}
PA + CO	50.1 ± 2.4^{bc}	43.8 ± 3.5^{c}	30.1 ± 2.0^{c}
B + EO	43.8 ± 4.1^{bcd}	35.2 ± 3.8^{c}	28.7 ± 1.0^{c}
B + CO	40.0 ± 3.2^{bcd}	33.7 ± 0.7^{cd}	27.3 ± 2.9^{c}
P + EO + CO	47.2 ± 1.2^{bc}	41.2 ± 3.4^{c}	35.0 ± 4.8^{c}
B + EO + CO	43.1 ± 1.4^{bcd}	36.5 ± 2.5^{c}	30.0 ± 3.4^{c}
Control	86.7 ± 2.4^{a}	86.7 ± 2.4^{a}	86.7 ± 2.4^{a}

Means followed by same letters within each column are not significantly ($p \leqslant 0.05$) different in Tukey's HSD test.
PA, propionic acid; B, benzaldehyde; CO, camphor oil; EO, eucalyptus oil.

Table 5 Larval survival (%) of *T. castaneum* in volatile oils and volatile compounds treatments (mean ± SE).

Treatments	Exposure concentrations (μl/l)		
	20	40	80
PA	25.6 ± 6.0^{c}	10.6 ± 6.8^{cde}	0^{c}
B	21.9 ± 3.5^{c}	10.0 ± 9.9^{de}	0^{c}
EO	48.6 ± 4.2^{b}	32.3 ± 2.4^{bcd}	23.0 ± 2.9^{b}
CO	50.6 ± 4.8^{b}	35.3 ± 2.3^{bc}	26.3 ± 2.9^{b}
PA + B	10.0 ± 6.1^{c}	6.6 ± 6.6^{e}	0^{c}
EO + CO	49.0 ± 0.9^{b}	35.9 ± 3.9^{b}	26.3 ± 2.9^{b}
PA + EO	26.5 ± 3.9^{c}	16.3 ± 4.6^{bcde}	14.0 ± 5.7^{bc}
PA + CO	22.3 ± 2.8^{c}	17.1 ± 1.7^{bcde}	10.0 ± 6.1^{bc}
B + EO	15.2 ± 4.5^{c}	14.6 ± 3.7^{bcde}	8.0 ± 4.8^{bc}
B + CO	14.5 ± 4.0^{c}	13.0 ± 5.6^{bcde}	9.0 ± 5.5^{bc}
P + EO + CO	21.9 ± 2.8^{c}	16.6 ± 4.5^{bcde}	15.6 ± 4.2^{bc}
B + EO + CO	15.4 ± 1.8^{c}	15.1 ± 4.1^{bcde}	13.3 ± 5.6^{bc}
Control	84.6 ± 2.1^{a}	84.6 ± 2.1^{a}	84.6 ± 2.1^{a}

Means followed by same letters within each column are not significantly ($p \leqslant 0.05$) different in Tukey's HSD test.
PA, propionic acid; B, benzaldehyde; CO, camphor oil; EO, eucalyptus oil.

Table 6 Percentage of *T. castaneum* adult emergence at different concentrations of volatile oils and volatile compounds treatments (mean ± SE).

Treatments	Exposure concentrations (μl/l)		
	20	40	80
PA	46.6 ± 4.1^{b}	20.0 ± 2.9^{cd}	0^{b}
B	30.0 ± 3.4^{b}	20.0 ± 2.9^{cd}	0^{b}
EO	53.3 ± 4.8^{b}	46.7 ± 4.1^{bcd}	30.0 ± 3.4^{b}
CO	66.6 ± 2.6^{b}	56.7 ± 3.9^{bc}	50.0 ± 2.4^{b}
PA + B	20.0 ± 2.9^{b}	0^{d}	0^{b}
EO + CO	65.0 ± 2.7^{b}	53.3 ± 3.6^{b}	40.0 ± 3.2^{b}
PA + EO	36.6 ± 2.6^{b}	30.0 ± 3.4^{bcd}	20.0 ± 2.9^{b}
PA + CO	43.3 ± 1.4^{b}	40.0 ± 3.2^{bcd}	20.0 ± 3.4^{b}
B + EO	40.0 ± 3.2^{b}	30.0 ± 3.4^{bcd}	20.0 ± 2.9^{b}
B + CO	30.0 ± 3.4^{b}	20.0 ± 2.9^{cd}	0^{b}
P + EO + CO	43.3 ± 1.4^{b}	40.0 ± 3.2^{bcd}	20.0 ± 3.4^{b}
B + EO + CO	40.0 ± 3.2^{b}	30.0 ± 3.4^{bcd}	20.0 ± 2.9^{b}
Control		80.2 ± 3.3^{a}	

Means followed by same letters within each column are not significantly ($p \leqslant 0.05$) different in Tukey's HSD test.
PA, propionic acid; B, benzaldehyde; CO, camphor oil; EO, eucalyptus oil.

hatchability and all the hatched larvae died. Camphor and eucalyptus oil when treated individually affected the egg hatchability drastically at higher concentrations viz. 40 and 80 μl. The egg hatchability in eucalyptus oil treatment was recorded as 80%, 66.6% and 54% at 20, 40 and 80 μl concentrations, respectively. The oils were also found to be toxic to the hatched larvae and the survival of larvae at 20 μl concentration of camphor oil and eucalyptus oil was nearly 50% of total hatched larvae. When camphor oil was mixed with benzaldehyde only 27.3% egg hatching was recorded at 80 μl concentration, which was less than individual camphor and eucalyptus oil treatments. At 20 μl/l concentration, egg hatchability was statistically same in the treatments of propionic acid, benzaldehyde + eucalyptus oil, benzaldehyde + camphor oil and benzaldehyde + eucalyptus oil + camphor oil.

3.5. Emergence of F1 adults

All the treatments including volatile oils significantly affected the adult emergence compared to control. In control 80.2% adult emergence was recorded. In benzaldehyde + propionic acid treatment some larvae survived in 20 and 40 μl concentration treatments; the F1 adult emergence was noticed only in 20 μl concentration as 20% (Table 6). In benzaldehyde, propionic acid and benzaldehyde + camphor oil treatments adults did not emerge at 80 μl concentration.

3.6. Fumigation toxicity inside confined wooden cage

The efficiency of the effective treatments namely benzaldehyde, benzaldehyde + propionic acid, propionic acid and benzaldehyde + camphor oil was tested inside a confined wooden cage (1 m^3) and the results are presented in Table 7. The LC_{99} concentrations of these effective treatments presented good fumigation toxicity effects against the adult beetles. On the first observation period (15th day), 99.3% mortality was noticed in benzaldehyde + propionic acid treatment and the difference was highly significant (F value by ANCOVA = 7.646; $p = 0.003$) compared with other treatments. The same treatment was found to be significantly effective throughout the study period; it recorded 61.1% mortality on the 90th day (last observation). Benzaldehyde treatment recorded 91% mortality in the beginning (15th day). The toxic effect of all the treatments decreased gradually at every consecutive observation period.

4. Discussion

Fumigation is a successful method of eradicating stored product pests present in food products. A number of studies have been carried out to show the fumigation effects of volatile oils against stored product pests (Lee et al., 2004; Germinara et al., 2007; Negahban et al., 2007; Paulraj and Ignacimuthu, 2007). Eucalyptus oil obtained from different species of *Eucalyptus* plant has been reported as a potential toxicant against many stored product insects. In the present investigation eucalyptus and camphor oils showed fumigation toxicity against eggs, larvae and adults of *T. castaneum*. But their effects were less than benzaldehyde, propionic acid and their combination treatments. A notable result of the present study was that the combination of the synthetic compounds, benzaldehyde and propionic acid, recorded higher toxicity against the eggs, larvae and adults of *T. castaneum* than individual synthetic compounds. However, the statistical analysis clearly indicated that the effect of benzaldehyde + propionic acid treatment was marginal compared to the individual treatments of benzaldehyde and propionic acid in many parameters.

Negahban et al. (2007) have studied the fumigant toxicity of *Artemisia sieberi* oil against *T. castaneum* adults and reported that *A. sieberi* oil killed 100% *T. castaneum* in 12 h at 444 μl/l concentration. Our study showed that eucalyptus and camphor oils killed more than 90% adults at the highest concentration of 160 μl. But benzaldehyde and propionic acid treatments killed 100% adults within 12 h at 160 μl concentrations.

Table 7 Fumigant efficiency (%) of effective volatile compounds and volatile oils at LC_{99} concentrations against *T. castaneum* adults inside a 1 m^3 wooden cage after different exposure periods (mean ± SE).

Treatment	Adult mortality after days					
	15th	30th	45th	60th	75th	90th
PA	80.7 ± 0.8^{c}	74.0 ± 0.9^{bc}	65.7 ± 0.8^{c}	55.2 ± 1.7^{b}	45.7 ± 0.8^{b}	33.9 ± 1.5^{b}
B	90.9 ± 0.8^{b}	84.5 ± 0.9^{b}	74.9 ± 1.0^{b}	62.3 ± 1.1^{b}	51.1 ± 0.9^{b}	40.7 ± 1.0^{b}
PA + B	99.3 ± 0.3^{a}	93.3 ± 0.9^{a}	85.5 ± 0.9^{a}	77.6 ± 1.6^{a}	69.6 ± 0.8^{a}	61.1 ± 0.8^{a}
B + CO	83.8 ± 1.0^{c}	75.8 ± 1.1^{c}	66.0 ± 1.1^{c}	56.2 ± 1.5^{b}	46.8 ± 0.5^{b}	354.0 ± 1.4^{b}

Means followed by same letters within each column are not significantly ($p \leqslant 0.05$) different in Tukey's HSD test.
PA, propionic acid; B, benzaldehyde; CO, camphor oil; EO, eucalyptus oil.

The activity of essential oils mainly depends upon the major volatile components they possess. In eucalyptus oil the major component is 1,8-cineole and this compound is responsible for the oil's biological activity (Maciel et al., 2010). The quantity and composition of volatile components of essential oils will vary in different species (Maciel et al., 2010). Previous studies have shown the effects of volatile oils from different *Eucalyptus* spp. against different stored product insects including *T. castaneum*. According to Negahban and Moharramipour (2007) the toxicity of *E. intertexta* was markedly high compared to *E. sargentii* and *E. camaldulensis* against *T. castaneum* adults. Lee et al. (2004) have explored the fumigant toxicity of 42 essential oils obtained from 42 plant species against *S. oryzae*. They identified six essential oils as more potent and reported that 1,8-cineole was the major constituent in most of the effective essential oils.

Benzaldehyde and propionic acid are gaining importance in the stored product pest management because of their favourable features like fast kill rate, safe usage among grains and cereals and efficient fumigant properties. Germinara et al. (2007) have tested the repellence and fumigant toxicity of propionic acid against *Sitophilus granarius* and *S. oryzae* adults. In the present study the combination of benzaldehyde and propionic acid recorded the highest toxicity against egg, larvae and adults of *T. castaneum*. Furthermore this treatment also interfered with the development of larvae into adult. From these findings we could understand that benzaldehyde and propionic acid had additive effect on *T. castaneum*. According to Germinara et al. (2007) the LC_{50} of propionic acid varied between 5 and 10 mg/l air at 23 and 30 °C, respectively, against *S. granarius* and *S. oryzae*. In the present experiment, the temperature was constant with a slight variation (30 ± 1 °C) and the LC_{50} values of propionic acid and benzaldehyde against *T. castaneum* were calculated as 36.79 and 34.01 μl/l air, respectively. According to Lee et al. (2001) benzaldehyde and its derivatives could be used as an alternative to the conventionally used chemical fumigants to control stored product pests. Propionic acid occurs in the blend of volatile compounds emitted by barley grains (Maga, 1978). Benzaldehyde is an important component of the almond oil and can be extracted from many nuts, seeds and leaves. Benzaldehyde is generally regarded as safe (GRAS) food additive and preservatives in the United States and is accepted as a flavouring substance in the European Union. Both benzaldehyde and propionic acid were organic compounds and are easily biodegradable.

In this study the toxicity revealed by eucalyptus oil was slightly higher than camphor oil against *T. castaneum* adults and larvae. Eucalyptus oil and camphor oil killed 50% adult population at 54.95 and 60.84 μl concentrations, respectively. Paulraj and Ignacimuthu (2007) also reported that eucalyptus oil was more toxic than camphor oil to *L. serricorne* (Fab) (Coleoptera: Anobiidae) beetles and killed 100% beetle population at 200 μl/l concentration in 24 h. When the volatile oils were mixed either with propionic acid or with benzaldehyde the toxicity increased significantly compared to individual oil treatments. In the present experiment the activity of camphor oil increased significantly when it was mixed with benzaldehyde. Volatile oils of many plants consist of alkanes, alcohols, aldehydes and terpenoids, especially monoterpenoids, and exhibit fumigant activity (Coats et al., 1991; Ahn et al., 1998; Kim and Ahn, 2001; Kim et al., 2003). From the present study it is clear that the compounds of volatile oils and benzaldehyde can give additive effect when both of them are mixed together and the activity of volatile oil could be enhanced by such combination. Another significant finding in this study was that the larval stage was more resistant than adult beetles of *T. castaneum* to the tested volatiles. However, the potential treatments, benzaldehyde + propionic acid combination and benzaldehyde alone, inhibited the egg hatchability and larval development into adults. Hence the application of these volatiles in store houses during the early stages of pest occurrence may eliminate *T. castaneum* population completely. The fumigation toxicity of benzaldehyde and propionic acid combination treatment lasted for 90 days with 50% adulticidal record inside the 1 m^3 cage. So this treatment can be used for *T. castaneum* management in store houses. Both benzaldehyde and propionic acid are biodegradable compounds and they will not pose any environmental pollution.

5. Conclusion

Benzaldehyde + propionic acid combination was toxic to eggs, larvae and adults of *T. castaneum*. Individual benzaldehyde and propionic acid treatments were also significantly effective against *T. castaneum*. These synthetic compounds either alone or as blend can be used as fumigants in store houses where *T. castaneum* is a severe problem.

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