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Insecticidal activities of natural volatile compounds against pulse beetle, *Callosobruchus chinensis* (Bruchidae)

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ABSTRACT. Two pure natural volatile compounds, carvacrol and menthone were investigated for repellent, insecticidal, ovipositional and egg hatching inhibition activities against pulse beetle, *Callosobruchus chinensis* (Coleoptera: Bruchidae). Carvacrol and menthone repelled bruchid adults in choice oviposition assay. Both compounds caused toxicity, reduced oviposition potential and viability of eggs significantly when fumigated. In chronic toxicity assay, both carvacrol and menthone reduced F_1 progeny production and weight loss in cowpea seeds. Reduced in grain damage was probably occurred due to inhibition of oviposition and egg hatching. Acetylcholine esterase enzyme activity was inhibited in adults when fumigated with carvacrol and menthone showing neurotic mode of action. Findings of the present study suggest that application of carvacrol and menthone can be effective in the management of *C. chinensis*.

Keywords: Carvacrol; menthone; oviposition deterrence index; hatching inhibition rate; insect pest management.

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Introduction

Insect infestation started with the beginning of storage practices damaging stored grains both quantitatively and qualitatively. These insects pest especially belonging to order Coleoptera and Lepidoptera cause damage grain under storage (Khare, 2006; Kuamar and Kalta, 2017). In developing countries, about 10-60% losses at post-harvest level are caused by these stored grain pests (Mihale, Selemani, Kamatenesi, Kidukili, & Ogendo, 2009). These losses have created food security issues especially in developing countries. To mitigate the losses due to insect infestation, several synthetic chemicals are in common practice, but, excessive and continuous use of these chemicals have developed resistance in insects and causes financial burdens (Elzen and Hardee, 2003; Benhalima, Chaudhary, Mills, & Price, 2004; Islam and Talukdar, 2005). These chemicals cause chromosomal aberrations, formation of DNA adducts (Le Goff et al., 2005; Muniz et al., 2008; Simoniello et al., 2008). These chemicals cause ozone depletion, neurotoxicity, carcinogenicity and teratogenicity in non-target animals as well (United Nations Environment Programme [UNEP], 2000; Beckel, Lorini, & Lazzari, 2002; Grandjean and Landrigan, 2003; Kalyabina, Esimbekova, Kopylova, & Kratasyuk, 2021). These serious issues have forced to change the insect pest management practices and include plant derived chemicals. Plant derived volatile chemicals are secondary metabolites and are characterized by their specific odour (Bakkali, Averbeck, Averbeck, & Idaomer, 2008). These chemicals are included in 'generally recognized as safe' category (Tripathi, Upadhyay, Bhuiyan, & Bhattacharya, 2009). These volatile products are produced in the members of Alliaceae, Apiaceae, Asteraceae, Cupressaceae, Myrtaceae, Lamiaceae, Lauraceae, Piperaceae, Poaceae, Rutaceae and Zinziberaceae. These chemicals are categorized in several classes like terpenes, terpenoids, phenols, ketones, lactones etc (Chaubey, 2019).

Carvacrol belongs to monoterpenoid family and found mainly in oregano essential oils (Fig. 1) (Hao, Li, & Shi, 2021). It shows antibacterial, antiplatelet, antitumor and antimutagenic activities (Aydin, Basaran, & Basaran, 2005; Son, Park, Kim, Chung, & Lee, 2005; Hao et al., 2021). Menthone belongs to monoterpene family and found as important constituent especially in essential oils derived from *Mentha* species (Fig. 1) (Makkar, Sharma, & Kaur, 2018). It shows antibacterial activities against *Staphylococcus aureus* by changing membrane potential and membrane integrity (Zhao et al., 2023). It is used in the preparation of herbal drugs for Schistomiasis treatment (Zaia et al., 2016).

Among several insect pest, *Callosobruchus chinensis* (Order: Coleoptera, Family: Bruchidae) commonly known as pulse beetle is a serious pests infesting beans, cowpea, gram, lentil and other pulses. Its grubs are

the damaging stages and can cause 100% loss under storage (Gbaye, Millard, & Holloway, 2011). Grubs make holes in undamaged grains, consume inner part and leave empty kernel. The grains damaged lose quantity and quality both and become incapable to sprout losing nutritional and economic value. The present study has been conducted to evaluated repellent, insecticidal, ovipositional and egg hatching inhibitory activities of carvacrol and menthone against pulse beetle, *C. chinensis* (Figure 1).

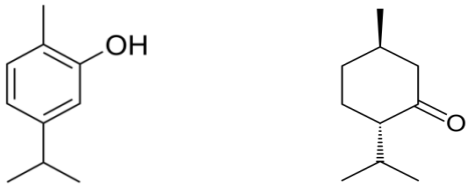

Figure 1. Structure of carvacrol and menthone

Materials and methods

Compounds

Pure compounds viz. carvacrol [2-Methyl-5-(propan-2-yl)phenol] and menthone [(2S,5R)-2-Isopropyl-5-methyl cyclohexanone] were purchased from Sigma Chemicals, USA.

Insect

Pulse beetle, *Callosobruchus chinensis* was used to investigate the insecticidal activities of carvacrol and menthone. The insects were reared on cowpea seeds in laboratory at 30±2°C, 75±5% RH in a photo period of 10:14 (L:D) h.

Repellency assay

In plastic box (10 cm diameter and 13 cm height), two transparent glass vials (3 cm in diameter and 10 cm in height) interconnected by a plastic tube (2 cm long and 1.5 cm diameter) at the base of vials were taken. A filter paper disc (1.5 cm diameter) treated with 0.5 mL aliquot prepared by dissolving carvacrol and menthone was pasted under the cover of one vial. In other vial of the box, filter paper treated with acetone only was applied as in the treated. Before use, solvent was allowed to evaporate from filter paper discs applied. Neck of vial was blocked by a piece of plastic mesh to avoid direct contact of insect with filter paper disc. In each vial, twenty cowpea seeds along with 0-24 hours old ten adults of mixed sexes were taken. After 96 hours, counted the number of eggs laid on cowpea seeds and calculated Percent Repellency (PR) using the formula:

$$PR = \frac{N_{UT} - N_T}{N_{UT} + N_T} X\ 100$$

N_{UT} = Number of eggs in untreated vial
N_T = Number of eggs in treated vial

Fumigant toxicity assay

Fumigant toxicity of carvacrol and menthone was determined against 2-4 days old bruchid adults. For this, made test solutions of different concentration by dissolving carvacrol and menthone in acetone, impregnated filter paper strip (1.5 cm diameter) with 100 µL aliquot, evaporated solvent and pasted impregnated filter paper strip on the inner side of cap. Neck of vial (3 cm diameter and 10 cm height) was blocked with a piece of plastic mesh to avoid contact of adults to test solution. Now, ten adult bruchids along with twenty cowpea seeds were introduced in each vial and closed by screw cap. Experiment was carried out in conditions maintained for insect culture. Mortality in adult insects was recorded after 24, 48, 72 and 96 hours of the start of the treatment. Filter paper strip treated with acetone only was used in control group.

Oviposition inhibition assay

In oviposition inhibitory assay, ten 0-24 hours old *C. chinensis* adults were fumigated with 40 and 80% of 96 hours-LC_{50} of carvacrol and menthone for 96 hours. Number of eggs laid over the cowpea seeds was counted

at the end of the exposure period. Six replicates were set for each concentration of carvacrol, menthone as well as control group. Percent Oviposition Deterrence Index (%ODI) was calculated using formula:

%ODI = (C-T/C+T) X 100

C = Number of eggs in control
T = Number of eggs in test

Ovicidal assay

In ovicidal assay, *C. chinensis* eggs were fumigated with carvacrol and menthone. Prepared test solutions by dissolving in acetone, applied 100 µL aliquot of test solution on filter paper strip (2.5 cm diameter), evaporated solvent from filter paper strip, pasted it on inner surface of vial's screw cap (3 cm diameter and 10 cm height), crewed vials with caps and fumigated eggs for 96 hours in conditions used in fumigant toxicity. Now, eggs were allowed to hatch and develop number of eggs hatched was recorded after 14 days of treatment. For fumigation, two different concentrations were used and six replicates were set for each concentration and control group. Percent Hatching Inhibition Rate was calculated using formula:

%HIR = (C-T/C) X 100

C = Number of adults in control

T = Number of adults in test

Chronic toxicity assay

In chronic toxicity assay, 100 gm of fresh cowpea seeds was taken into a plastic box (7 cm diameter and 11 cm height) and mixed well with 2 mL of test solution prepared by dissolving carvacrol and menthone in acetone. Twenty 0-24 hours old *C. chinensis* adults were used for fumigation. After 24 days of initiation of the experiment, number of F_1 progeny emerged was counted and removed for 5 days continuously. The potency of carvacrol and menthone was estimated as percent protection (PP) using formula:

$PP = (N_{UT} - N_T/N_{UT}) X 100$

N_{UT} = Number of progeny in untreated group
N_T = Number of progeny in treated group

After 90 days, weight loss in cowpea seeds was estimated.

Acetylcholine esterase activity

Acetylcholine esterase activity was determined by Ellman, Courtney, Andres and Featherstone, (1961) method. *C. chinensis* adults were fumigated with two sub-lethal concentrations viz. 40 and 80% of 24 hours-LC_{50} carvacrol and menthone. After 24 hours of fumigation, adults were homogenized in phosphate buffer saline (50 mM, pH8), then the homogenate was centrifuged at 1000 rpm for 3 min. The supernatant was re-centrifuged at 5000 rpm for 5 min and the supernatant was used as enzyme source. Now, 0.1 mL of enzyme source was taken and into it 0.1 mL substrate acetylthiocholine iodide (ATChI)(0.5 mM), 0.05 mL chromogenic reagent, 5,5-Dithio-bis 2-nitrobenzoic acid (DTNB) (0.33 mM) and 1.45 ml phosphate buffer (50 mM, pH 8). After 3 min of incubation at 25°C enzyme activity was determined by measuring changes in the optical density at 412 nm. Enzyme activity was expressed as mmol of 'SH' hydrolysed $min^{-1}mg^{-1}$ protein.

Data analysis

Median lethal concentration (LC_{50}) was calculated by POLO programme (Russel, Robertson, & Savin, 1977). Regression analysis was performed to test the significancy of data (Sokal and Rohlf, 1973). One-way analysis of variance (ANOVA) and Tukey's test is carried out to figure out 'Honestly Significant Difference' (HSD) to compare every mean with every other mean (http://wwww.socscistatistics.com/tests/anova/default2.aspx.).

Results

Repellency assay

Carvacrol and menthone reduced oviposition in repellency assay which was estimated in terms of percent repellency (PR). PR was found 14.19, 46.85, 75.25 and 91.85; and 10.46, 33.66, 55.48 and 81.24 at 0.028, 0.042,

0.056 and 0.070; and 0.030, 0.045, 0.060 and 0.075056, 0.085, 0.113 and 0.169 µL cm^{-3} concentration of carvacrol and menthone respectively (Table 1).

Table 1. Repellent activity of carvacrol and menthone in choice oviposition assay in *Callosobruchus chinensis* adults.

Treatment	Concentration (µL cm^{-3})	Oviposition in untreated vial (Mean±SD)	Oviposition in treated vial (Mean±SD)	Percent Repellency (PR)
Control	0	34.50±1.03	35.50±0.28	-
Carvacrol	0.028	39.50±0.73	29.50±1.06	14.19
	0.042	48.83±1.59	17.33±0.95	46.85
	0.056	56.66±2.31	8.00±0.68	75.25
	0.070	62.66±2.68	2.66±0.28	91.85
Menthone	0.030	38.00±1.38	30.80±1.34	10.46
	0.045	42.33±1.69	24.00±1.26	33.66
	0.060	47.16±2.07	13.50±0.96	55.48
	0.075	56.33±2.37	5.83±0.18	81.24

*PR was calculated using formula: PR = [(C-T)/(C+T)] ×100, C = number of insects in the untreated halves and T = number of insects in treated halves; * Significant (P<0.01)

Toxicity assay

Carvacrol and menthone caused fumigant toxicity in *C. chinensis* adults. Median lethal concentrations (LC_{50}) for carvacrol and menthone were determined 0.164, 0.137, 0.112 and 0.097 µL cm^{-3}; and 0.183, 0.152, 0.124 and 0.101 µL cm^{-3} air after 24, 48, 72 and 96 hours respectively (Table 2). Regression analysis showed concentration-dependent correlation between pure compounds, carvacrol and methone, and mortality in bruchid adults (Table 2).

Table 2. Fumigant toxicity assays to study the effect of carvacrol and menthone against *Callosobruchus chinensis* adults.

Toxicity	Exposure period	LC_{50} (µL cm^{-3})	Intercept	Slope	Regression equation	Regression coefficient
Carvacrol	24h	0.164	5.32	8.40	Y=-5.32+8.40X	0.995
	48h	0.137	7.56	6.35	Y=-7.56+6.35X	0.993
	72h	0.112	3.81	7.66	Y=-3.81+7.66X	0.989
	96h	0.097	9.32	9.67	Y=-9.32+9.67X	0.991
Menthone	24h	0.183	2.31	7.62	Y=-2.31+7.62X	0.993
	48h	0.152	6.50	6.11	Y=6.50+6.11X	0.987
	72h	0.124	7.39	2.52	Y=7.39+2.52X	0.985
	96h	0.101	6.21	5.68	Y=-6.21+5.68X	0.989

Oviposition inhibition assay

Carvacrol and menthone significantly reduced oviposition potential oil of bruchid adults when exposed. In oviposition inhibition assays, oviposition was reduced to 59.69 and 21.95%; and 56.25 and 23.49% of the control when bruchid adults were fumigated with 40 and 80% of 96h-LC_{50} of carvacrol and menthone (Table 3a). Per cent ODI was calculated 25.23 and 63.99; and 27.98 and 61.94 when adults were fumigated with 40 and 80% of 96h-LC_{50} of carvacrol and menthone (Table 3a). Post Hoc Tukey's Honestly Significant Difference (HSD) analysis shows significant (P<0.01) (Table 3b).

Table 3a. Effect of 40 and 80% 96h-LC_{50} of carvacrol and menthone on oviposition potential of *Callosobruchus chinensis*.

Compound	Treatment	Number of eggs laid (Mean±SD)	%ODI	F (df =2;15)*
Control	-	97.16±2.33 (100)	-	-
Carvacrol	40% 96h-LC_{50}	58.00±1.64 (59.69)	25.23	119.16
	80% 96h-LC_{50}	21.33±1.02 (21.95)	63.99	
Menthone	40% 96h-LC_{50}	54.66±1.58 (56.25)	27.98	106.17
	80% 96h-LC_{50}	22.83±0.97 (23.49)	61.94	

Values in parentheses represent per cent change with respect to control group taking as 100%; *Significant (P <0.01)

Table 3b. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of effect of of carvacrol and menthone on oviposition inhibitory activities in *C. chinensis*

Compound	Pair wise comparison		$HSD_{0.05}$ = 12.76 $HSD_{0.01}$ = 16.79	$Q_{0.05}$ = 3.67 $Q_{0.01}$ = 4.83
Carvacrol	T_1:T_2	M_1 = 97.17, M_2 = 58.00	39.17	11.27
	T_1:T_3	M_1 = 97.17, M_3 = 21.33	75.83	21.83
	T_2:T_3	M_2 = 58.00, M_3 = 21.33	36.67	10.55
Menthone	T_1:T_2	M_1 = 97.17, M_2 = 54.67	42.50	11.74
	T_1:T_3	M_1 = 97.17, M_3 = 22.83	74.33	20.54
	T_2:T_3	M_2 = 54.67, M_3 = 22.83	31.83	8.80

*Significant (P <0.01) difference between the means

Ovicidal assay

Carvacrol and menthone significantly reduced hatching rate in *C. chinensis* eggs when fumigated. Mean number of eggs hatched per twenty five eggs was reduced to 19.5, 14.67 and 11.50; and 19.83, 15.67 and 11.83 when fumigated with 0.30, 0.60 and 0.90 µL cm^{-3}; and 0.35, 0.70 and 1.05 µL cm^{-3} air of carvacrol and menthone respectively as compared to 23.17 eggs hatched in control (Table 4a). Percent HIR was increased to 84.16, 36.69 and 50.37; and 14.42, 32.37 and 48.95 when fumigated with 0.30, 0.60 and 0.90 µL cm^{-3}; and 0.35, 0.70 and 1.05 µL cm^{-3} air of carvacrol and menthone respectively. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis shows significant (*P*<0.01) (Table 4b).

Table 4a. Effect of different concentration of carvacrol and menthone on egg hatching rate of *Callosobruchus chinensis.*

Compound	Concentration (µL cm^{-3})	Number of eggs hatched (Mean±SD)	%HIR	F (df =2;15)*
Control	-	23.17±1.68 (100)	-	-
Carvacrol	0.30	19.50±1.29 (84.16)	15.84	102.41
	0.60	14.67±1.18 (63.31)	36.69	
	0.90	11.50±0.98 (49.63)	50.37	
Menthone	0.35	19.83±1.49 (85.58)	14.42	104.86
	0.70	15.67±1.08 (67.63)	32.37	
	1.05	11.83±1.01 (51.05)	48.95	

Values in parentheses represent per cent change with respect to control group taking as 100%; *Significant (P <0.01)

Table 4b. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of ovicidal property of carvacrol and menthone in *Callosobruchus chinensis*.

Compound	Pair wise comparison		$HSD_{0.05}$ = 2.02 $HSD_{0.01}$ = 2.56	$Q_{0.05}$ = 3.96 $Q_{0.01}$ = 5.02
Carvacrol	T_1:T_2	M_1 = 23.17, M_2 = 19.50	3.67	7.19
	T_1:T_3	M_1 = 23.17, M_3 = 14.67	8.50	16.68
	T_1:T_4	M_1 = 23.17, M_3 = 11.50	11.67	22.89
	T_2:T_3	M_2 = 19.50, M_3 = 14.67	4.83	9.48
	T_2:T_4	M_2 = 19.50, M_4 = 14.67	8.00	15.70
	T_3:T_4	M_3 = 14.67, M_4 = 11.50	3.17	6.21
Menthone	T_1:T_2	M_1 = 23.17, M_2 = 19.83	3.33	6.92
	T_1:T_3	M_1 = 23.17, M_3 = 15.67	7.50	15.57
	T_1:T_4	M_1 = 23.17, M_3 = 11.83	11.33	23.53
	T_2:T_3	M_2 = 19.83, M_3 = 15.67	4.17	8.65
	T_2:T_4	M_2 = 19.83, M_4 = 11.83	8.00	16.61
	T_3:T_4	M_3 = 15.67, M_4 = 11.83	3.83	7.96

*Significant (P <0.01) difference between the means

Chronic toxicity assay

Progeny production and weight loss in cowpea seeds was reduced significantly in comparison to untreated group when *C. chinensis* adults were exposed to carvacrol and menthone for longer period. F_1 progeny was reduced to 69.78, 46.96 and 20.58%; and 77.57, 54.88 and 25.06% at 0.3, 0.6 and 0.9; and 0.4, 0.8 and 1.2 µL gm^{-1} concentration of carvacrol and menthone respectively (Table 5a). Per cent weight loss in cowpea seeds was recorded 4.66%, 3.81%, 2.32% and 0.58%; and 14.83%, 10.16%, 2.83% and 1.33% when *C. chinensis* adults were exposed to carvacrol and menthone at concentrations of 0.3, 0.6 and 0.9; and 0.4, 0.8 and 1.2 µL gm^{-1} respectively (Table 5a). This weight loss in cowpea seeds was due to reduction in F_1 progeny. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis shows significant (*P*<0.01) (Table 5b, c).

Table 5a. Effect of carvacrol and menthone on F_1 progeny emergence and grain weight loss during chronic exposure of *Callosobruchus chinensis* adults.

Compound	Concentration (µL gm^{-1})	F_1 progeny emergence Mean±SD	PP	F value*	Grain weight loss Mean±SD	F value*
Control	-	126.33±2.65 (100)		-	15.87±1.02 (100)	-
Carvacrol	0.30	88.16±2.26 (69.78)	30.21	240.96	11.95±0.64 (75.29)	89.74
	0.60	59.33±2.1 (46.96)	53.04		8.29±0.79 (52.23)	
	0.90	26.00±1.08 (20.58)	79.42		4.36±0.036 (27.47)	
Menthone	0.40	98.00±2.68 (77.57)	22.43	190.82	12.76±0.59 (80.40)	87.34
	0.80	69.33±2.19 (54.88)	45.12		9.36±0.46 (58.97)	
	1.20	31.66±1.38 (25.06)	74.94		5.21±0.67 (32.82)	

Values in parentheses represent per cent change with respect to control group taking as 100%; *Significant (P <0.01)

Table 5b. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of effect of carvacrol and menthone on F_1 progeny emergence and weight loss caused by *Callosobruchus chinensis* in chronic toxicity assay.

F_1 progeny emergence							
Carvacrol				Menthone			
Pair wise Comparison		$HSD_{0.05}$ = 10.87 $HSD_{0.01}$ = 13.78	$Q_{0.05}$ = 3.96 $Q_{0.01}$ = 5.02	Pair wise comparison		$HSD_{0.05}$ = 10.87 $HSD_{0.01}$ = 13.78	$Q_{0.05}$ = 3.96 $Q_{0.01}$ = 5.02
T_1:T_2	M_1 = 126.33, M_2 = 88.17	38.17	9.87	T_1:T_2	M_1 = 126.33, M_2 = 98.00	28.33	9.67
T_1:T_3	M_1 = 126.33, M_3 = 59.33	67.00	19.86	T_1:T_3	M_1 = 126.33, M_3 = 69.33	57.00	19.46
T_1:T_3	M_1 = 126.33, M_3 = 26.00	100.33	33.57	T_1:T_3	M_1 = 126.33, M_3 = 31.67	94.67	32.31
T_2:T_3	M_2 = 88.17, M_3 = 59.33	28.83	9.98	T_2:T_3	M_2 = 98.00, M_3 = 69.33	28.67	9.78
T_2:T_4	M_2 = 88.17, M_4 = 59.33	62.17	23.54	T_2:T_4	M_2 = 98.00, M_4 = 31.67	66.33	22.64
T_3:T_4	M_3 = 59.33, M_4 = 26.00	33.33	13.86	T_3:T_4	M_3 = 69.33, M_4 =31.67	37.67	12.86

*Significant (P <0.01) between the means.

Table 5c. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of effect of carvacrol and menthone on F_1 progeny emergence and weight loss caused by *Callosobruchus chinensis* in chronic toxicity assay.

Weight loss							
Carvacrol				Menthone			
Pair wise Comparison		$HSD_{0.05}$ = 2.11 $HSD_{0.01}$ = 2.67	$Q_{0.05}$ = 3.96 $Q_{0.01}$ = 5.02	Pair wise comparison		$HSD_{0.05}$ = 2.11 $HSD_{0.01}$ = 2.67	$Q_{0.05}$ = 3.96 $Q_{0.01}$ = 5.02
T_1:T_2	M_1 = 15.88, M_2 = 11.96	3.92	7.36	T_1:T_2	M_1 = 15.88, M_2 = 12.77	3.11	6.34
T_1:T_3	M_1 = 15.88, M_3 = 8.29	7.58	14.23	T_1:T_3	M_1 = 15.88, M_3 = 9.36	5.62	13.30
T_1:T_3	M_1 = 15.88, M_3 = 4.36	11.52	21.61	T_1:T_3	M_1 = 15.88, M_3 = 5.22	10.66	21.76
T_2:T_3	M_2 = 11.96, M_3 = 8.29	3.66	6.88	T_2:T_3	M_2 = 12.77, M_3 = 9.36	3.41	6.96
T_2:T_4	M_2 = 11.96, M_4 = 4.36	7.60	14.26	T_2:T_4	M_2 = 12.77, M_4 = 5.22	7.55	15.41
T_3:T_4	M_3 = 8.29, M_4 = 4.36	3.93	7.38	T_3:T_4	M_3 = 9.36, M_4 =5.22	4.14	8.46

*Significant (P <0.01) between the means.

Acetylcholinesterase (AchE) activity:

Fumigation of *C. chinensis* adults with 40 and 80% of 24h-LC_{50} of carvacrol and menthone reduced acetylcholine esterase activity to 69.59 and 46.96%; and 71.28 and 49.09% of control respectively (Table 6a). Post Hoc Tukey's Honestly Significant Difference (HSD) analysis shows significant (P<0.01) (Table 6b).

Table 6a. Effect of carvacrol and menthone on acetylcholine esterase inhibitory activities in *Callosobruchus chinensis*.

Compound	Treatment	Acetylcholine esterase activity (Mean±SD)	F (df =2;15) *
Control	-	0.0888±0.0024 (100)	
Carvacrol	40% of 24h-LC_{50}	0.0618±0.0018 (69.59)	163.25
	80% of 24h-LC_{50}	0.0417±0.0009 (46.96)	
Menthone	40% of 24h-LC_{50}	0.0633±0.0016 (71.28)	157.48
	80% of 24h-LC_{50}	0.0436±0.0010 (49.09)	

Enzyme activity has been represented as mmol of 'SH' hydrolysed min^{-1}mg^{-1} protein; Values in parentheses represent per cent change with respect to control group taking as 100%; *Significant (P <0.01).

Table 6b. Post Hoc Tukey's Honestly Significant Difference (HSD) analysis of acetylcholine esterase inhibitory activities of carvacrol and menthone in *Callosobruchus chinensis*.

Compound	Pair wise comparison		$HSD_{0.05}$ = 0.0068 $HSD_{0.01}$ = 0.0089	$Q_{0.05}$ = 3.67 $Q_{0.01}$ = 4.83
Carvacrol	T_1:T_2	M_1 = 0.09, M_2 = 0.06	0.03	14.61
	T_1:T_3	M_1 = 0.09, M_3 = 0.04	0.05	25.48
	T_2:T_3	M_2 = 0.06, M_3 = 0.04	0.02	10.87
Menthone	T_1:T_2	M_1 = 0.09, M_2 = 0.06	0.03	14.14
	T_1:T_3	M_1 = 0.06, M_3 = 0.04	0.05	25.00
	T_2:T_3	M_2 = 0.06, M_3 = 0.04	0.02	10.87

*Significant (P <0.01) difference between the means.

Discussion

Since synthetic insecticides cause several environmental, economic and human health issues, natural products especially natural volatile chemicals are gaining significant importance in stored grain insect management (Koul, Singh, Singh, & Singh, 2007; Chaubey, 2015). Essential oils contain diverse group of chemicals viz. terpenes, terpenoids, phenols, aldehydes, ketones and lactones (Chaubey, 2019). Among these, terpenes and terpenoids constitute 90% of essential oils (Bakkali et al.,2008). Many of these essential oils and chemical components have been evaluated for their role in insect pest management programme (Koul et al., 2007; Chaubey, 2012, 2015). Anethole, β-caryophyllene and α-pinene have been established as fumigants against *C. chinensis* (Koul et al., 2007; Chaubey, 2015). The two compounds viz. β-caryophyllene and α-pinene cause toxicity in insects by inhibiting acetylcholine esterase activity (Chaubey, 2022).

Attempts have been made in the past to explore the insecticidal nature of volatile chemicals against *C. chinensis* and some of them have been proved to exert antifeedant, repellent, oviposition inhibitory, ovicidal and progeny production inhibitory activities in insects by disrupting metabolic pathways (Chaubey, 2015). In the present study, carvacrol and menthone significantly repelled the adult bruchids. These two compounds caused fumigant toxicity and reduced egg laying capacity in *C. chinensis* adults when fumigated. Both carvacrol and menthone reduce oviposition and egg hatching in *C. chinensis* when fumigated.

In chronic toxicity assay, emergence of F_1 progeny was reduced in *C. chinensis* which could either be due to the reduction in egg hatching rate or death of larva. Jilani and Saxena (1988) have reported that larvae must penetrate seeds to ensure survival while eggs not firmly attached to the seeds are unable to do so (Jilani and Saxena, 1988). Damage in cowpea seeds and loss of weight have been found to reduced when exposed to carvacrol and menthone as compared to untreated group due to the reduction in F_1 progeny. Similar results have been observed in case of *Allium sativum* essential oil which has been reported to protect cowpea seeds by reducing oviposition and egg viability in *C. chinensis* (Chaubey, 2014). Since the emergence of adults depends on the proportion of hatched eggs, the results suggest that both carvacrol and menthone vapours probably cross the seed coat and therefore, interfere with the larvae development (Braga et al., 2007). The mechanism of action of essential oils and constituents has not been known clearly, however, these are known to affect ion transport and release of acetylcholine esterase (Re et al., 2000). Both carvacrol and menthone reduced acetylcholine esterase activity in *C. chinensis* adults. Toxicity of essential oils and several constituents have been reported due to its effect on acetylcholine esterase activity (Lee, Choi, Lee, & Park, 2011; Chaubey, 2022). However, Kostyukovsky, Rafaeli, Gileadi, Demchenko, & Shaaya, (2002) reported that besides acetylcholine esterase, octopamine is another target of volatile chemicals which interfere with neuromodulator octopamine or GABA-gated chloride channels (Enan, 2005; Tong and Coats, 2012). Some of these act on octopaminergic system of insects. Disruption in its activity breaks down the nervous system in insects (Hollingworth, Johnstone, & Wright, 1984).

Conclusion

It can be concluded from the present study indicate that carvacrol and menthone cause toxicity, repellency, oviposition inhibitory and developmental inhibitory activities in insects. These act on various targets in insects reducing the possibility of generating resistance. These can be considered as natural alternatives in insect pest management. However, before its application, it must be kept in mind that volatile chemicals should be toxic to target insects only. There are several other factors like risk associated to users, mode of exposure, degradation in the environment and chronic toxicity must be considered in mind during formulation of plant volatile chemicals based insecticides.

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