

Effect of Carvacrol and Menthone as Insecticidal Tool on Red Flour Beetle, *Tribolium Castaneum* (Coleoptera: Tenebrionidae)

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Abstract

Insect pests infest and damage agricultural stored grains and processed food products both qualitatively and quantity since time long before. Several synthetic chemical have been developed and applied as insecticides to manage these losses, but, extensive uses of these on this strategy have created serious environmental and health issues. To mitigate these problems, several natural volatile organic chemicals have been explored since last three decades and received significant results. In this continuation, two volatile organic chemicals, carvacrol and menthone have been evaluated for repellent, toxic, oviposition, developmental and feeding inhibitory activities against wheat flour insect, *Tribolium castaneum* (Coleoptera: Tenebrionidae). These two volatile organic chemicals repelled the adults of *T. castaneum* in filter paper repellency assay and cause acute toxicity in larvae and adults of *T. castaneum* in fumigant and contact toxicity assays. These two organic chemicals reduce oviposition and interfere with the transformation of larva to pupa and pupa to adult. Besides, deterrence in feeding habit of adults has also been recorded. These two chemicals inhibit acetylcholine esterase activity in adult insects suggesting probable neurotoxic mode of action in insects. These outcomes of the study suggest carvacrol and menthone as the key components in eco-friendly insecticide formulation based on volatile organic chemicals.

Keywords: Carvacrol, Menthone, *Tribolium castaneum*, Oviposition inhibition, Antifeedant activity, Acetylcholine esterase.

INTRODUCTION

Stored grain insect pests have been damaging our economy by infesting agricultural stored grains and products and are responsible for worldwide loss of 10-40% annually.¹ Simultaneously, the continuous increasing pressure of expanding human population has also raised food demand

across the globe. In such situation, management of stored grains and other agricultural products from insect infestation is prime task. In this connection, various synthetic insecticides have been formulated and applied with great success in managing insect's infestations and damages across the globe. But, the uncontrolled use of these synthetic insecticides causes great hazard for environment like ozone layer depletion, contamination of air, water and

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soil.²⁻⁴ Insecticides reduce biodiversity in aquatic as well as terrestrial ecosystems affecting food webs and ecosystems.⁵ These chemicals cause cancer, neurological disorders, teratogenic development and mutagenic effects in non-target animals including human.⁵⁻⁶ These insecticides induce cross- and multi resistance in target as well as non-target insects creating a serious problem of another dimension.⁷⁻⁹ Besides, residual property of insecticides increases the occurrence of different diseases like aging, asthma, atherosclerosis, cancer, diabetes, fatigue, nephropathies, neurological diseases, rheumatoid arthritis etc in humans¹⁰. These issues develop the idea to restrict the use of synthetic insecticides and to develop new alternatives that must be ecologically sound with no residual and adverse effect on other non-target animals. In this regard, several plant derived volatile oils and volatile chemicals have been evaluated for insecticidal properties and received great success^{11,12}. The essential oils are the complex mixture of volatile organic compounds produced as the secondary metabolites in plants as tool of communication and protection. Besides complete oils, toxic effects of many volatile organic constituents have also been determined against many insect pests with promising outcomes¹³⁻¹⁵.

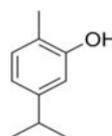
Tribolium castaneum is a major secondary pest of wheat grain flour¹⁶. It infest stored grains including cereals, beans and nuts as well as processed commodities like biscuits, flours and pasta. It is an important pest because of its broad host range and rapid growth in population. In spite of cannibalism in larva and adult, it has one of the highest population growth rates of all stored product beetles due to its high reproductive rate and long reproductive life¹⁷. It is unable to feed on intact grains, but, feeds on grains already damaged by other insects and flours¹⁸. Adult and larva both represent feeding stages.

Carvacrol is a monoterpenoid found mainly in oregano essential oils¹⁹. It has been known to bear antibacterial, antimutagenic, antiplatelet and antitumor properties^{20,21}. Menthone is a monoterpene found especially in essential oils of *Mentha* species²². It shows antibacterial activities against *Staphylococcus aureus* and has been used in the preparation of herbal drugs for Schistosomiasis treatment^{23,24}. In the present study, laboratory studies on the repellent, toxic and developmental inhibitory effects of two volatile chemicals, carvacrol and menthone against wheat flour pest *T. castaneum* have been reported.

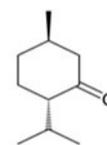
MATERIALS AND METHODS

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.



Carvacrol



Menthone

Insects

Red flour beetle, *T. castaneum* was used to determine the insecticidal nature of volatile organic compounds, carvacrol and menthone. The insects were reared on wheat flour in laboratory at $30 \pm 2^\circ\text{C}$, $75 \pm 5\%$ RH and a photoperiod of 10:14 (L: D) h.

Repellency

Repellency assays of volatile organic chemicals were carried out in 80 mm glass petri plates. Test solutions were prepared by dissolving carvacrol and menthone in acetone. Whatman filter paper was cut into two halves of 80 mm discs and each compound solution was applied to a filter paper half as uniform as possible using a micropipette. The other half of the filter paper was treated with acetone only. The treated and untreated halves were dried to evaporate the solvent completely. After that both treated and untreated halves were attached with cellophane tape and placed at the bottom in the petriplate. Twenty adults of *T. castaneum* were released at the centre of the filter paper disc and then Petri plates were covered and kept in dark. Four replicates were set for each concentration of compound. Number of the insects on both the treated and untreated halves was recorded after four hours in mild light.

Fumigant toxicity

Experimental solutions of carvacrol and menthone were made in acetone. Ten *T. castaneum* adults/larvae taken from laboratory culture were placed in glass petri dish (diameter 8.5 cm, height 1.2 cm) and added 2 gm of wheat flour. Experimental solution was applied in a filter paper strip (2 cm diameter) and left for few minutes to evaporate solvent. Now, treated filter paper strip was pasted on the inner surface of the petri dish

cover, air tightened with parafilm and kept in rearing conditions in dark. After 24h of exposure, mortality in adults/larvae was recorded.

Contact toxicity

Experimental solutions of carvacrol and menthone were made in acetone. Experimental solution was applied on the inner surface of glass petri dish (diameter 8.5 cm, height 1.2 cm). Petri dish was left open for few minutes to evaporate solvent completely. Now, ten *T. castaneum* adults/larvae were released in petri dish, covered and kept in rearing conditions in dark. After 24h of exposure, recorded the mortality in adults/larvae.

Oviposition inhibition

Oviposition inhibitory activity carvacrol and menthone was tested against *T. castaneum* by fumigation. Twenty 1-2 week old adults of mixed sexes were placed in 2 gm of wheat flour in glass petri dish. Flour was spread uniformly along the whole surface of the petri dish. A paper strip (2 cm diameter) treated with different sub-lethal concentrations viz. 40 and 80% of 24h-LC₅₀ was pasted on the inner surface of the cover of each petri dish. All the closed petri dishes were kept in dark and six replicates were set for each concentration. After 24h of fumigation, the treated adults were transferred to fresh petri dish having fresh wheat flour. After 7 days of treatment, the adults were removed and discarded. The number of the larvae hatched was counted for the treated as well as for control groups. The counting was done for four days continuously.

Developmental inhibition

Developmental inhibitory activity of carvacrol and menthone was tested against 4th instar larvae of *T. castaneum*. Ten larvae were fumigated with different sub-lethal concentrations viz. 40 and 80% of 24h-LC₅₀ of volatile compounds in petri dish for 24h as was done in larvicidal assay and then the treated larvae were transferred to fresh wheat flour in other petri dish. Number of survived larvae, transformed pupae from treated larvae and emerged adults from transformed pupae was recorded. Six replicates were set for each concentration.

Antifeedant activity

Antifeedant activity of carvacrol and menthone was determined in *T. castaneum* adults²⁵. Flour discs were prepared by making suspension of 10 gm of wheat flour in 50 ml water. Pipetted out 200µl of the flour suspension onto a plastic sheet

and dried at 60°C for one hour. Each flour disc was treated with 1, 2 and 4µl carvacrol and menthone, weighed, placed in glass petri dish and twenty five *T. castaneum* adults were introduced into it and allowed to feed. After four days of feeding, antifeedant activity of carvacrol and menthone was calculated by reweighing flour discs.

Acetylcholine esterase activity

T. castaneum adults were fumigated with 40 and 80% of 24h-LC₅₀ of carvacrol and menthone for 24h. After fumigation, adult insects were collected and used for acetylcholine esterase enzyme activity estimation²⁶. Acetylcholine esterase activity was estimated in terms of mmol of 'SH' hydrolysed min/mg protein.

Data analysis

Chi-square test was applied to establish the repellent activity of the volatile organic compound tested²⁷. The LC₅₀ and EC₅₀ were calculated by POLO programme²⁸. Correlation and linear regression analysis were conducted to define all concentration-response relationships²⁷. Analysis of variance was performed to test the equality of regression coefficient²⁷.

RESULTS AND DISCUSSION

In early attempts for the management of economic losses caused by *T. castaneum*, several volatile products of botanical origin have been reported for their repellent, toxic and developmental inhibitory activities. The rapid mode of action of the volatile organic chemicals shows their low persistence in the environment. The persistence of the insecticidal activity depends on the chemical composition of volatile oils and nature and position of the functional groups in oil constituents²⁹. Volatile oils possessing high content of hydrogenated compound loss their activity as compared to those containing high content of oxygenated compounds^{30,31}.

In repellency assay, chi-square analysis indicated that carvacrol and menthone were repellent to *T. castaneum* adults. These two volatile compounds showed significant repellent activity even at low concentrations as the hypothesis of the ratio 1:1 was rejected (Table 1). These two volatile compounds caused fumigant and contact toxicity in *T. castaneum* adults and larvae. In fumigant toxicity assay, median lethal concentrations were recorded 0.38 and 0.42 µlcm⁻³ air against *T. castaneum* adult for carvacrol and menthone after

24h exposure period respectively (Table 2). On the other hand, median lethal concentrations were 0.31 and 0.34 μcm^{-3} air against *T. castaneum* larvae for carvacrol and menthone after 24h exposure period respectively (Table 2). The index of significance of potency estimation, g-value indicates that the mean value is within the limits of all probability levels ($P < 0.1, 0.5$ and 0.01) as it is less than 0.5. Values of t-ratio greater than 1.6 indicate the significance of regression. Values of heterogeneity factor less than 1.0 denotes that model fits the data adequate. Regression analysis shows concentration-dependent mortality in *T. castaneum* adults and larvae (Table 2). In contact toxicity assay, median lethal concentrations were 0.31 and 0.34 μcm^{-2} area against *T. castaneum* adult for carvacrol and menthone after 24h exposure period respectively (Table 3). On the other hand, LC_{50} values were 0.26 and 0.27 μcm^{-2} against *T. castaneum* larvae for carvacrol and menthone after 24h exposure period respectively (Table 3). Regression analysis showed concentration-dependent mortality in *T. castaneum*

adults by carvacrol and menthone (Table 3). The two volatile chemicals cause fumigant as well as contact toxicity in adult and larvae of *T. castaneum* showing acute toxicity. These two volatile oil constituents inhibit acetylcholine esterase activity. Fumigation of *T. castaneum* adults with 40 and 80% of 24h LC_{50} of carvacrol and menthone significantly reduced acetylcholine esterase activity to 75.82 and 50.54; and 71.42 and 51.64% of control respectively (For carvacrol, $F=33.97$; For menthone, $F = 35.19$; $P < 0.05$; Table 4). This shows that the two volatile chemicals cause neurotoxicity in adults of *T. castaneum*. Other volatile compounds have also been reported to inhibit acetylcholine esterase activity causing paralysis and death in insects^{14,32}. These oils interfere with neuromodulator octopamine or GABA-gated chloride channels^{33,34}. The octopaminergic system of insects uses octopamine as neurotransmitter, neurohormone and neurohormone-neuromodulator. Disruption in its activity breaks down the nervous system in insects³⁵.

Table 1: Repellent effect of carvacrol and menthone on *Tribolium castaneum*

Concentration (vol:vol)	Carvacrol			Menthone		
	Treated Mean \pm SE	Untreated Mean \pm SE	χ^2 - value	Treated Mean \pm SE	Untreated Mean \pm SE	χ^2 - value
0.6	0.0 \pm 0.0	20.00 \pm 0.0	60.00*	0.0 \pm 0.0	20.00 \pm 0.0	60.00*
0.3	0.86 \pm 0.31	19.16 \pm 0.31	50.70*	1.00 \pm 0.51	19.00 \pm 0.51	49.40*
0.15	4.17 \pm 0.31	15.83 \pm 0.31	20.70*	5.37 \pm 0.33	14.66 \pm 0.33	13.40**
0.075	7.17 \pm 0.31	12.83 \pm 0.31	5.10#	7.37 \pm 0.21	12.66 \pm 0.21	4.40#

*Significant ($P < 0.01$), **Significant ($P < 0.05$), #Non-significant

Table 2: Fumigant toxicity of carvacrol and menthone on *Tribolium castaneum*

Compound	Parameter	LC_{50} ($\mu\text{l cm}^{-3}$)	g-value	t-ratio	Heterogeneity	Regression Equation	Correlation Coefficient
Carvacrol	Adult mortality	0.38	0.26	3.05	0.35	$Y = 7.52 + 1.67X$	0.98
	Larval mortality	0.31	0.29	4.11	0.34	$Y = -3.09 + 2.97X$	0.99
Menthone	Adult mortality	0.42	0.26	3.56	0.39	$Y = 2.67 + 4.65X$	0.99
	Larval mortality	0.34	0.28	4.21	0.29	$Y = -2.33 + 4.39X$	0.98

The oviposition potential of the *T. castaneum* was decreased significantly when fumigated with the carvacrol and menthone. The reduction in oviposition was 73.74 and 49.16%; and 70.50 and 55.75% when *T. castaneum* adults were fumigated with 40 and 80% of 24h- LC_{50} of carvacrol and menthone respectively (For carvacrol $F = 36.18$; and for menthone $F = 36.34$; $P < 0.01$; Table 5). The percentage of larvae transformed into the pupae and the percentage of pupae transformed

into the adult stage were decreased significantly with an increase in concentration of volatile compounds. Pupal transformation was reduced to 53.3 and 40.0%; and 56.7 and 41.7% of the control when larvae were fumigated with 40 and 80% of 24h- LC_{50} of carvacrol and menthone respectively (For carvacrol $F = 167.5$; and for menthone $F = 112.64$; $P < 0.01$; Table 6). Pupal transformation was reduced to 49.94 and 32.78%; and 44.77 and 31.02% of the control when larvae were

fumigated with 40 and 80% of 24h-LC₅₀ of carvacrol and menthone respectively (For carvacrol F = 205.16; and for menthone F = 265.83; P<0.01; Table 6). Similar results have been reported when α-pinene and β-caryophyllene have been applied as fumigants¹⁴.

The mode of action of these volatile chemicals is yet to be confirmed but it appears that oviposition inhibition and development inhibition may be due to the suffocation and inhibition of different biosynthetic processes of the insect metabolism³⁶.

Table 3: Contact toxicity of carvacrol and menthone on *Tribolium castaneum*

Parameter	Compound	LC ₅₀ (µl cm ⁻²)	g-value	t-ratio	Heterogeneity	Regression Equation	Correlation Coefficient
Adult mortality	Carvacrol	0.31	0.24	4.21	0.33	Y = 7.96+1.67X	0.99
Larval mortality		0.26	0.26	3.97	0.38	Y = -2.87+1.32X	0.99
Adult mortality	Menthone	0.34	0.29	3.69	0.34	Y = 3.78+4.67X	0.98
Larval mortality		0.27	0.25	3.28	0.36	Y = -2.89+4.83X	0.99

Table 4: Effect of carvacrol and menthone on acetylcholine esterase activity in adults of *Tribolium castaneum*

Compound	Concentration	Enzyme activity* (Mean±SE)	F-value**
Control	-	0.091±0.007 (100)	-
Carvacrol	40% 24h-LC ₅₀	0.069±0.005 (75.82)	33.97
	80% 24h-LC ₅₀	0.046±0.002 (50.54)	
Menthone	40% 24h-LC ₅₀	0.065±0.006 (71.42)	35.19
	80% 24h-LC ₅₀	0.047±0.003 (51.64)	

*mmol of 'SH' hydrolysed min/mg protein, Values in parentheses indicate per cent change with respect to control taken as 100%, **Significant at P<0.01 (df = 2,15)

Table 5: Effect of carvacrol and menthone on oviposition potential of *Tribolium castaneum*

Compound	Concentration	Number of larvae emerged/adult (Mean±SE)	POD*	F-value**
Control	-	10.17±0.26 (100)	-	-
Carvacrol	40% 24h-LC ₅₀	7.50±0.19 (73.74)	26.26	36.18
	80% 24h-LC ₅₀	5.00±0.14 (49.16)	50.84	
Menthone	40% 24h-LC ₅₀	7.17±0.21 (70.50)	29.50	36.34
	80% 24h-LC ₅₀	5.67±0.13 (55.75)	44.25	

Values in parentheses indicate per cent change with respect to control taken as 100%

* Percentage of oviposition deterrence (POD) = [(E_C-E_T)/E_C] ×100, E_C = number of adults emerged in control and E_T = number of adults emerged in test, **Significant at P<0.01

Carvacrol and menthone reduced in feeding in *T. castaneum* adults. Consumption of flour disc by *T. castaneum* adults and larvae was reduced to 74.47, 39.37 and 17.63%; and 66.75, 37.19 and 14.19% of the control at 1, 2 and 4 µl of carvacrol and menthone respectively (For carvacrol F = 153.21; For menthone F = 194.23; P<0.01; Table 7). This may be due to repellent behaviour of the two chemicals. Further studies have to be carried out to explore antagonistic/synergistic

relationship of these two volatile organic chemicals³⁷. Its effectiveness against targets only but not against non-targets including humans should also be considered. Issues like risk associated to users, mode of exposure, degradation in the environment and chronic toxicity should also be considered and evaluated for compatible use of volatile organic chemicals for insect pest management.

Table 6: Effect of carvacrol and menthone on development of *Tribolium castaneum*

Compound	Concentration	Number of pupa transformed (Mean±SE)	F-value*	Number of pupa transformed (Mean±SE)	F-value*
Control		10.00±00	-	9.67±0.21	-
Carvacrol	40% 24h-LC ₅₀	5.33±0.23 (53.30)	167.50	4.83±0.13 (49.94)	205.16
	80% 24h-LC ₅₀	4.00±0.09 (40.00)		3.17±0.09 (32.78)	
Menthone	40% 24h-LC ₅₀	5.67±0.13 (56.70)	112.64	4.33±0.11 (44.77)	265.83
	80% 24h-LC ₅₀	4.17±0.12 (41.70)		3.00±0.07 (31.02)	

Values in parentheses indicate per cent change with respect to control taken as 100%, *Significant at P<0.01

Table 7: Effect of carvacrol and menthone on feeding behaviour of *Tribolium castaneum*

Compound	Concentration (µl/disc)	Consumption of flour disc (Mean±SE)	AFA*	F-value**
Control	-	11.91±0.26 (100)	-	-
	1	8.87±0.13 (74.47)	25.53	
	2	4.69±0.12 (39.37)	60.67	153.21
Carvacrol	4	2.10±0.02 (17.63)	82.37	
	1	7.95±0.16 (66.75)	33.25	
Menthone	2	4.43±0.09 (37.19)	62.81	194.23
	4	1.69±0.04 (14.19)	85.81	

Values in parentheses indicate per cent change with respect to control taken as 100%

*Antifeedant activity was calculated using AFA = $[(C-T)/C] \times 100$, Where, C = consumption of flour disc in control group, and T = consumption of flour disc in treated group. **Significant at P<0.01

CONCLUSION

In conclusion, it can be stated that carvacrol and menthone possess repellent, toxic as well as oviposition, developmental and feeding inhibitory activities against *T. castaneum*. These two volatile organic chemicals act at multiple target sites which minimize the possibility of resistance development. Thus, carvacrol and menthone can be considered as a natural alternative in preparation of plant based insecticides for the management of stored grains insects.

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