Role of carvacrol and menthone in maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) management

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ABSTRACT: Insecticides of synthetic origin used indiscriminately to manage insect pest populations are known for genotoxicity, neurotoxicity and teratogenicity in non-target organisms as well as the development of resistance in target insects. These issues have focused insect pest management research towards the use of plant-based chemicals of a volatile nature. In this study, two plant-origin volatile compounds, carvacrol and menthone have been evaluated for their potential insecticidal properties against the maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae). These two natural volatile chemicals repelled adults and caused lethality in adults as well as larvae. These two volatile chemicals inhibited acetylcholine esterase enzyme activity in adults when fumigated with sub-lethal concentrations. Both volatile chemicals reduced oviposition potential, progeny production and feeding behavior as well as prolonged the developmental period of the insect. Therefore, it can be concluded that these two natural volatile chemicals can be used in the preparation of volatile chemical-based formulations in the management of maize weevil *S. zeamais*.

Keywords: Carvacrol; Menthone; *Sitophilus zeamais*; Oviposition inhibition; Antifeedant activity.

1. INTRODUCTION

Insect pests of stored grains under storage damage grains across the globe both qualitatively and quantitatively causing huge economic loss annually, especially in tropical and subtropical countries. To reduce these losses, insecticides of synthetic nature have been applied in various forms. But, the continuous, unlimited and indiscriminate application of these insecticides damages the health of humans as well as the environment. These include depletion of the ozone layer, neurotoxic, carcinogenic, mutagenic, and teratogenic effects in non-target organisms as well as cross- and multi-resistance in target insect populations [1-3]. Besides, these synthetic insecticides especially organochlorines have entered and accumulated in our ecosystems causing mass killings of bees, fishes, amphibians, birds and mammals [4-5]. Thus, scientific communities have shifted their core of research towards the use of plant-derived volatile chemicals in insect pest management. These volatile chemicals are synthesized in a number of plant families as secondary metabolites. Most of these volatile

chemicals have been known for larvicidal, adulticidal, antifeedant, oviposition, developmental and adult emergence inhibitory properties in a variety of insects [6-10].

Carvacrol is a monoterpenoid found mainly in oregano essential oils [11]. It has been known to bear antibacterial, antimutagenic, antiplatelet and antitumor properties [12-14]. Menthone is a monoterpene found especially in essential oils of *Mentha* species [15]. It shows antibacterial activities against *Staphylococcus aureus* and has been used in the preparation of herbal drugs for schistomiasis treatment [16,17].
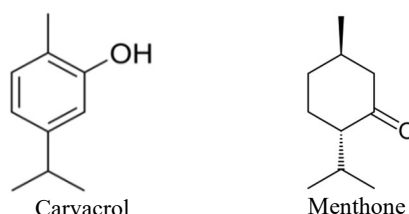

Figure 1. Chemical structures of carvacrol and menthone.

Sitophilus zeamais (Coleoptera: Curculionidae) is a serious pest of barley, cottonseed, maize, peas, maize, rice, sorghum, oats, wheat etc [18]. It is commonly found in tropical and subtropical areas of the world where maize is cultivated at large as food. Adults attack whole grains, but larvae feed cryptically and develop inside grains [19]. The present study aims to evaluate potential repellent, insecticidal, oviposition inhibitory, developmental inhibitory, antifeedant and acetylcholine esterase inhibitory activities of carvacrol and methone against maize weevil, *S. zeamais*.

2. MATERIALS AND METHODS

2.1. 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.

2.2. Insects

Maize weevil, *S. zeamais* was cultured on whole maize grain at 28 ± 4^0C, $50\pm5\%$ RH and photoperiod of 10:14 (L:D) hours to evaluate insecticidal properties of carvacrol and menthone.

2.3. Repellent activity

The repellent activity of carvacrol and menthone was determined by the filter paper disc method. Experimental solutions of carvacrol and menthone were prepared in acetone. Whatman filter papers were cut into two halves: one half was treated with test solution and the other half was treated with solvent only. Treated and untreated halves were dried to evaporate solvent, attached with cellophane tape and placed in each glass petri dish (diameter 8.5 cm, height 1.2 cm). Now, forty *S. zeamais* adults were released in petri dishes, covered and kept in the dark for 4h. Counted the number of adults in treated as well as untreated halves and calculated percent repellency and preference index.

2.4. Fumigant toxicity

Experimental solutions of carvacrol and methone were made in acetone. Ten adults/larvae taken from laboratory culture were placed in glass Petri dishes and added 2 g of maize flour. The experimental solution was applied in a filter paper strip (2 cm diameter) and left for a few minutes to evaporate the solvent. Now, the

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 the dark. After 24 and 48h of exposure, recorded the mortality in adults/larvae.

2.5. Contact toxicity

Experimental solutions of carvacrol and menthone were made in acetone. An experimental solution was applied on the inner surface of the glass Petri dish (diameter 8.5 cm, height 1.2 cm). The Petri dish was left open for a few minutes to evaporate the solvent completely. Now, ten *S. zeamais* adults/larvae were released in a Petri dish, covered and kept in rearing conditions in the dark. After 24 and 48h of exposure, recorded the mortality in adults/larvae.

2.6. Acetylcholine esterase activity

S. zeamais adults were fumigated with 40 and 80% of 24h-LC_{50} of carvacrol and menthone for 24h. After 24h of fumigation, adults were homogenized with 5 ml phosphate buffer (pH 8), then the homogenate was centrifuged at 1000 rpm for 30 min. at 4°C and the supernatant was used as an enzyme source [20]. To 0.1 ml of enzyme source, added 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). Enzyme activity was determined by measuring changes in the optical density at 412 nm by incubating the reaction mixture for 3 min at 25°C. Enzyme activity was expressed as mmol of 'SH' hydrolyzed /min/mg protein.

2.7. Oviposition inhibition

Oviposition inhibitory activity of carvacrol and menthone was determined using Vanmathi et al. method [21]. In this assay, *S. zeamais* adults of mixed sex were fumigated with 40 and 80% of 24h-LC_{50} of carvacrol and menthone for 24h. After fumigation, the treated adults were reared on maize grain. After 10 days, discarded adults and counted the number of F_1 progeny after 45 days.

2.8. Developmental inhibition

The developmental inhibitory activity of carvacrol and menthone was determined using Tapondju et al. method [22]. Ten *S. zeamais* adults of mixed sex were placed in a 250 ml plastic container with 20 g of maize grains and allowed to mate and lay eggs for seven days in conditions applied for the rearing of insects. Now, adults were removed and a filter paper strip (2 cm diameter) impregnated with carvacrol and menthone was pasted on undercover of the container. The eggs and juveniles were fumigated with three concentrations (0.4, 0.8 and 1.2 µl cm^{-3}) of carvacrol and menthone. Recorded the duration from the end of fumigation till the emergence of F_1 adults and the number of adults who emerged in control as well as in the test.

2.9. Antifeedant activity

The antifeedant activity of carvacrol and menthone was determined in *S. zeamais* adults by Suthisut et al. method [23]. Flour discs were prepared by making a suspension of 10 g of maize flour in 50 ml water. Pipetted out 200 **µl** of the flour suspension onto a plastic sheet and dried at 60^0 C for one hour. Each flour disc was treated with 40 and 80% of 96h-LC_{50} of carvacrol and methone, weighed, placed in a glass Petri dish and twenty-five *S. zeamais* adults were introduced into it and allowed to feed. After four days of feeding, calculated antifeedant activity of carvacrol and menthone by reweighing flour discs.

2.10. Statistical analysis

The median lethal concentration was estimated by POLO program [24]. One-way analysis of variance,

correlation and regression was conducted to study the concentration-response relationship [25].

3. RESULTS

3.1. Repellent activity

Percent repellency and preference index were increased as the concentration of carvacrol and menthone was increased and recorded maximum at 0.8% concentrations (Table 1).

Table 1. Repellent activity of carvacrol and menthone against *S. zeamais* adults.

Compound	Concentration (%)	Percent Repellency (PR)*	Preference Index (PI)**
Carvacrol	0.1	27.50	-0.27
	0.2	4.00	-0.49
	0.4	80.50	-0.80
	0.8	100.00	-1.0
Menthone	0.1	24.50	-0.24
	0.2	43.50	-0.43
	0.4	79.00	-0.79
	0.8	100.00	-1.0

*Percent repellency (PR) was calculated using the formula: PR = [(C-T)/(C+T)] ×100, C = number of insects in the untreated halves and T = number of insects in treated halves; **Preference index (PI) was calculated using formula: PI = (percentage of insects in treated halves - percentage of insects in untreated halves) / (percentage of insects in treated halves + percentage of insects in untreated halves). PI value between -1.0 to -0.1 indicates repellant chemical, -0.1 to +0.1 neutral chemical and +0.1 to +1.0 attractant chemical.

3.2. Fumigant toxicity

In adults, median lethal concentrations were recorded at 0.37 and 0.26 µl cm^{-3} air; and 0.38 and 0.29 µl cm^{-3} air for carvacrol and menthone after 24 and 48h exposure period, respectively (Table 2). On the other hand, median lethal concentrations were 0.32 and 0.21 µl cm^{-3} air; and 0.33 and 0.23 µl cm^{-3} air against larvae when fumigated with carvacrol and menthone for 24 and 48 h exposure periods, respectively (Table 3). 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 denote that the model fits the data adequately. Regression analysis shows concentration-dependent mortality in *S. zeamais* adults and larvae (Tables 2, 3).

Table 2. Fumigant and contact toxicity of carvacrol and menthone against *S. zeamais* adults.

Compound	Toxicity	Exposure period (h)	LC$_{50}$*	g-value	Heterogeneity	t-ratio	Regression Equation	Correlation Coefficient
Carvacrol	Fumigant toxicity	24	0.37	0.23	0.32	3.04	Y = - 4.76+4.69X	0.99
		48	0.26	0.21	0.31	3.97	Y = 3.48+4.68X	0.98
	Contact toxicity	24	0.32	0.24	0.32	4.32	Y = - 4.08+3.98X	0.99
		48	0.23	0.23	0.33	3.95	Y = -5.32+5.34X	0.98
Menthone	Fumigant toxicity	24	0.38	0.26	0.34	3.38	Y = - 7.95+3.87X	0.99
		48	0.29	0.25	0.35	4.17	Y = 6.84+3.07X	0.98
	Contact toxicity	24	0.33	0.24	0.33	4.36	Y = -7.02+5.30X	0.99
		48	0.25	0.23	0.32	3.46	Y = -5.22+4.36X	0.98

*µl cm^{-3} for fumigant toxicity and µl cm^{-2} for contact toxicity.

Table 3. Fumigant and contact toxicity of carvacrol and menthone against *S. zeamais* larvae.

Compound	Toxicity	Exposure period (h)	LC$_{50}$*	g-value	Heterogeneity	t-ratio	Regression Equation	Correlation Coefficient
Carvacrol	Fumigant toxicity	24	0.32	0.23	0.31	3.78	Y = - 4.90+3.84X	0.98
		48	0.21	0.21	0.33	4.09	Y = 4.90+4.12X	0.98
	Contact toxicity	24	0.31	0.22	0.32	4.31	Y = 4.97+3.27X	0.98
		48	0.25	0.23	0.36	3.97	Y = -5.19+5.32X	0.99
Menthone	Fumigant toxicity	24	0.33	0.22	0.31	3.24	Y = - 7.20+3.09X	0.99
		48	0.23	0.24	0.34	3.84	Y = - 5.06+4.08X	0.98
	Contact toxicity	24	0.34	0.23	0.32	4.01	Y = - 6.37+3.20X	0.98
		48	0.23	0.24	0.36	3.64	Y = 3.31+6.20X	0.98

*µl cm^{-3} for fumigant toxicity and µl cm^{-2} for contact toxicity.

3.3. Contact toxicity

In adults, median lethal concentrations were 0.32 and 0.23 µlcm^{-2}; and 0.33 and 0.25 µlcm^{-2} area for carvacrol and menthone after 24 and 48h exposure period, respectively (Table 2). On the other hand, LC$_{50}$ values were 0.31 and 0.25 µlcm^{-2}; and 0.34 and 0.23 µlcm^{-2} area for carvacrol and menthone when larvae were exposed for 24 and 48h respectively (Table 3). Regression analysis showed concentration-dependent mortality in *S. zeamais* adults and larvae by carvacrol and menthone (Tables 2, 3).

3.4. Acetylcholine esterase enzyme activity

Fumigation of *S. zeamais* adults with 40 and 80% of 24h LC$_{50}$ of carvacrol and menthone reduced acetylcholine esterase activity to 71.27 and 54.25%; and 75.82 and 52.12% of control, respectively (for carvacrol F=167.32; for menthone F = 159.87; P<0.01; Table 4).

Table 4. Effect of carvacrol and menthone on acetylcholine esterase activity in *S. zeamais*.

Compound	Concentration	Enzyme activity* (Mean±SD)	F-value (df = 2,15)
Carvacrol	Control	0.094±0.0021(100)	
	40% of 24h-LC$_{50}$	0.067±0.0016(71.27)	167.32**
	80% of 24h-LC$_{50}$	0.051±0.0011(54.25)	
Menthone	Control	0.094±0.0021(100)	
	40% of 24h-LC$_{50}$	0.069±0.0013(75.82)	159.87**
	80% of 24h-LC$_{50}$	0.049±0.0010(52.12)	

*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).

3.5. Oviposition inhibition

In *S. zeamais* adults, oviposition capacity was reduced to 81.28 and 62.56%; and 83.56 and 59.31% of the control, respectively when *S. zeamais* adults were fumigated with 40 and 80% of 24h-LC$_{50}$ of carvacrol and menthone (for carvacrol F = 27.64; for menthone F = 38.95; P<0.01; Table 5).

3.6. Developmental inhibition

Progeny production was reduced to 78.86, 64.67 and 48.80%; and 81.34, 62.29 and 46.29% of control when fumigated with 0.4, 0.8 and 1.2 µl cm^{-3} of carvacrol and menthone respectively (for carvacrol F=46.32; for menthone F = 53.29; P<0.05; Table 6). The duration of larval phase to adult emergence was increased

significantly to 129.60% and 133.55% of the control when fumigated with 1.2 µl cm^{-3} of carvacrol and menthone respectively (for carvacrol F = 69.32; for menthone 73.58; P<0.01; Table 6).

Table 5. Oviposition inhibitory activities of carvacrol and menthone in *S. zeamais*

Compound	Concentration	No. of progeny emerged (Mean±SD)	POD*	F-value
Carvacrol	Control	97.50±3.18 (100%)	-	27.64**
	40% of 24h-LC$_{50}$	79.25±2.33 (81.28)	18.72	
	80% of 24h-LC$_{50}$	61.00±2.01 (62.56)	37.44	
Menthone	Control	97.50±3.18 (100%)	-	38.95**
	40% of 24h-LC$_{50}$	81.50±1.84 (83.56)	16.44	
	80% of 24h-LC$_{50}$	57.83±1.66 (59.31)	40.69	

Values in parentheses indicate percent change with respect to control taken as 100%; * Percentage of oviposition deterrence (POD) = [(EC-ET)/EC] ×100, EC = number of adults emerged in control and ET = number of adults emerged in test; **Significant at P<0.01 (df = 2,15).

Table 6. Effect of carvacrol and menthone on development of *S. zeamais*.

Compound	Concentration	No. of progeny emerged (Mean±SD)	IR*	F-value	Duration of developmental period in days (Mean±SD)	F-value
Carvacrol	Control	84.00±3.64 (100)	-	46.32**	25.33±0.36 (100)	69.32**
	0.4 µlcm^{-3}	66.25±2.85 (78.86)	22.14		27.83±0.26 (109.86)	
	0.8 µlcm^{-3}	54.33±2.24 (64.67)	35.33		30.66±0.31 (121.04)	
	1.2 µlcm^{-3}	41.00±1.73 (48.80)	51.20		32.83±0.36 (129.60)	
Menthone	Control	84.00±3.64 (100)	-	53.29**	25.33±0.36 (100)	73.58**
	0.4 µlcm^{-3}	68.33±2.32 (81.34)	18.64		28.33±0.26 (111.84)	
	0.8 µlcm^{-3}	52.33±2.15 (62.29)	37.71		30.83±0.38 (121.71)	
	1.2 µlcm^{-3}	39.33±1.78 (46.82)	53.18		33.83±0.34 (133.55)	

Values in parentheses indicate percent change with respect to control taken as 100%; * Inhibition rate (IR) = [(Cn-Tn)/Cn] ×100, Cn = number of adults emerged in control and Tn = number of adults emerged in test; **Significant at P<0.01 (df = 3,20).

3.7. Antifeedant activity

Reduction in feeding was observed in *S. zeamais* adults. Consumption of flour disc by *S. zeamais* adults was reduced to 56.07 and 27.77%; and 53.68 and 28.74% of the control at 40 and 80% of 96h-LC$_{50}$ of carvacrol and menthone respectively (for carvacrol F = 164.87; for menthone F = 161.35; P<0.01; Table 7).

Table 7. The antifeedant activity of carvacrol and menthone against *S. zeamais*.

Concentration	Carvacrol		Menthone	
	Consumption of flour disc (mg) (Mean±SD)	AFA*	Consumption of flour disc (mg) (Mean±SD)	AFA*
Control	11.27±0.18 (100)	-	11.27±0.18 (100)	-
40% of 96h-LC$_{50}$	6.32±0.14 (56.07)	43.93	6.05±0.16 (53.68)	46.32
80% of 96h-LC$_{50}$	3.13±0.11 (27.77)	72.23	3.24±0.10 (28.74)	71.26
	F = 164.87**		F = 161.35**	

Values in parentheses indicate percent change with respect to control taken as 100%; *Antifeedant activity was calculated using AFA = [C-T/C] × 100, Where, C = consumption of flour disc in control group, and T = consumption of flour disc in treated group. Six replicates were set for each concentration of compound and control; **Significant at P<0.01 (df = 2, 15).

4. DISCUSSION

Plant volatile oils, as well as their individual constituents like linalool, linalyl acetate, menthol, menthone, limonene, α-pipene, β-pipene, β-caryophyllene and linalool, have been well known for their insecticidal properties against several insect pests including those of stored grains [9,10,26-31]. These volatile oils and constituents show repellent, toxic, oviposition inhibitory and developmental inhibitory activities against diverse coleopteran insects of stored grains. These volatile oils and chemicals cause mortality in insects by inhibiting the activity of acetylcholine esterase enzyme [9,32].

In this study, two pure plant volatile chemicals, carvacrol and menthone were evaluated for their potential repellent, toxic, oviposition inhibitory, developmental inhibitory and feeding inhibitory activities in *S. zeamais*. Both carvacrol and methone repel and cause acute toxicity in *S. zeamais* adults. The rapid mortality shows the neurotoxic mode of action of the two volatile chemicals under investigation as these inhibit the activity of the acetylcholine esterase enzyme activity. Several other volatile chemicals have also been reported to inhibit acetylcholine esterase activity, causing paralysis and death in insects [9-27]. These volatile chemicals interfere with neuromodulator octopamine or GABA-gated chloride channels, disrupting the nervous system in insects [33-35].

Oviposition and progeny production were reduced in *S. zeamais* when fumigated with carvacrol and methone. This reduction in oviposition potency of insects could be due to disruption in mating and sexual communication in *S. zeamais* adults. These two volatile chemicals inhibit the development of juvenile phases and increase the developmental period in *S. zeamais*. Reduced adult emergence could be due to the death of egg and larval phases, while delay in development could be due to inhibition of metabolic processes or disturbances in hormonal networking and tissue responsiveness. Both carvacrol and methone reduce feeding potency in *S. zeamais*. This reduction may be due to the repellent behavior of these volatile chemicals. Similar results have been obtained with other volatile chemicals [10]. The rapid mode of action of the two volatile compounds under investigation shows their low persistence in the environment, which depends on the nature and position of the functional groups in the chemical [36]. Highly saturated compounds lose their activity as compared to those containing a high content of oxygen [37,38].

Further studies have also been needed to explore the insecticidal activity of oil constituents and its role in antagonistic and synergistic relationships [39]. It must be kept in mind that these constituents should be effective against targets only. Issues associated with the environment and human health should also be considered and evaluated for the compatible use of volatile chemicals for insect pest management.

5. CONCLUSION

The present study reports that carvacrol and menthone possess repellent, toxic, oviposition, developmental and feeding inhibitory activities against *S. zeamais*. These two volatile compounds act at multiple target sites which minimize the possibility of resistance development. Since carvacrol and menthone are the parts of our food, these oils are safe for humans. For these reasons, carvacrol and menthone can be considered as a natural alternative in the management of stored grains insects.

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