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RESEARCH ARTICLE

Insecticidal Potential of Two Monoterpenes against *Tribolium Castaneum* (Herbst.) and *Sitophilus Oryzae* (L.) Major Stored Product Insect Pests

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ABSTRACT

In the present study, two pure monoterpenes: Citronellol and geraniol were tested for their fumigant toxicity, repellent activity, and antifeedant activity against two stored product insect pests, *Sitophilus oryzae* (L.), and *Tribolium castaneum* (Herbst.) Monoterpenes tested showed varying degrees of toxicity against different species of stored product pests but were highly dependent on dosage and exposure duration. Geraniol was found to be highly effective against both *S. oryzae* and *T. castaneum* than citronellol. 0.02 µl/ml geraniol produced a mortality of 26.30 ± 0.11 after a short duration of 6 h that reached 52.76 ± 0.28% after an increased exposure of 72 h against *S. oryzae* while citronellol showed least activity producing a mortality of 52.76 ± 0.28 at 0.02 after 72 h of exposure. Similarly for *T. castaneum* geraniol produced 68.75 ± 0.55% mortality at 0.2 µl/ml after 72 h and citronellol even at a highest dose of 0.2 µl/ml caused 30.77 ± 0.49% mortality after 12 h. Geraniol produced 42.56 ± 1.9% repellent activity at 1 µl/cm^2 after 1 h whereas repellency of 48.60 ± 1.4% was obtained by citronellol against *T. castaneum* after same concentration and time period citronellol and geraniol at a highest concentration of 1 µl/cm^2 gave 56.61 ± 3.4 and 50.56 ± 1.1% repellency, respectively, after 5 h against *S. oryzae.* Feeding deterrence index (FDI) of 78.95 ± 0.09 and 67.59 ± 0.17% was obtained for citronellol and geraniol at a high concentration of 30 µl/g against *T. castaneum.* Citronellol showed 52.80 ± 0.32% FDI, followed by geraniol with 49.28 ± 0.17 FDI % against *S. oryzae*.

Keywords: Antifeedant, fumigant, insect pests, monoterpenes, repellent activity

INTRODUCTION

Insect pests attacking stored grain products often lead to losses such as weight loss, volume reduction, germination impairment, feces contamination, and overall loss of quality. These pests are numerous and a large number of them belong to the order Coleoptera, the largest in the animal kingdom. Among these *Sitophilus* and *Tribolium* species are major stored products pests in the tropical countries.[1] During the last many decades different synthetic chemicals were tried and used for the protection of stored grain products from pests. Even today, they are the major contributors in the control process. As a result, the application of these chemicals is rather substantiated by their efficacy. The results, however, have come along with a number of undesirable consequences such as toxicity to parasites among others. A decline in the population of the natural enemies of the pests is an uncalled for cue pointing at the severity and non-targeted action of the synthetic pesticides.[2] Other problems such as pesticide resistance[3,4] susceptibility of crop plant to insect pests[5] and increased environmental and social cost[6] are other indirect consequences. The above factors imply the need to develop and use alternate pesticides. Research done recently shows the efficacy of natural products against insect invasion on stored grains and therefore indicates their possible use by farmers.[7] Essential oils are

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complex natural mixtures containing about 20–60 different components at different concentrations. Two or three major components characterize them which are found at fairly high concentrations (20–70%) as compared to others components found in trace amounts. Essential oils are known to play an important role in the protection of the plants by acting as antibacterial, antiviral, antifungal, insecticides, and against herbivores by reducing their appetite. Monoterpenes are major constituents isolated from essential oils found in plants and are known to be biologically active compounds.[8,9] These compounds are considered a potential pest control agent because they are highly toxic to insects and possess repellent and antifeedant activity.[10]

The present study was undertaken to investigate the effect of two monoterpenoids on the red flour beetle, *Tribolium castaneum* (Herbst) and rice weevil, *Sitophilus oryzae* (L.) serious pests of stored products worldwide. Citronellol and geraniol were tested for their fumigant toxicity, repellent activity, and antifeedant activity against these insect pests.

MATERIALS AND METHODS

The following monoterpenes were tested: Citronellol and geraniol were provided by Sigma-Aldrich, India. Most of them were identified as major components of essential oils which showed a strong insecticidal effect.[11]

Test insects

Laboratory cultures of *S. oryzae* and *T. castaneum* (5–10 days each) were maintained at 30 ± 20C and 68 ± 2% relative humidity. Test insects of *S. oryzae* were reared on rice kernels, and wholemeal wheat flour plus brewer's yeast (19:1) was used to rear *T. castaneum*.

Fumigant toxicity of monoterpenes

Vapor toxicity of monoterpenes against the adult insects was determined through impregnated paper assay following the method of Park *et al*.[12] with some modifications. Plastic jars of 250 ml capacity with screw lids were used as exposure chambers. Different doses of 5, 10, 30, and 50 µl of monoterpenes were diluted with 1 ml methanol and aliquots of 1 ml of each solution were applied to a circular filter paper (Whatman No. 1, 3 cm diameter). The treated filter paper discs were then introduced into the plastic jars (250 ml capacity) to achieve final concentrations of 0.02, 0.04, 0.12, and 0.2 µl/ml for monoterpenes with respect to volume of the jars. After allowing the solvent to evaporate for 10–15 min, the filter paper was attached to the inner surface of the screw lid of the jar using adhesive tape. At the bottom of each jar, ten individuals of each insect (5–10 day old) along with their food source were placed and exposed to the various concentrations. The insects had no contact with the diffuser and stayed at the bottom of the chamber throughout the experiment. Insect mortalities were determined and calculated after different exposure periods to the day of complete mortality of all insects according to the formula of Abbott.[13] Three replicates were set up for each dose and control.

Repellent activity of monoterpenes

Repellency tests were carried out according to the experimental method described.[14] Test solutions were prepared by dissolving 10, 30, and 50 µl of monoterpenes in 1 ml methanol. Whatman filter papers (diameter 8 cm) were cut into two equal halves one half of each dish was treated with monoterpenes as uniform as possible using micropipette. The other half of the filter paper was treated with methanol alone as a control. The treated and control half discs were dried to evaporate the solvent completely. Treated and untreated halves were attached to their opposite ends using adhesive tape and placed in Petri dishes. Twenty adult beetles of each insect species (5–10 day old) were released at the center of each filter paper. The Petri dishes were then covered and sealed with parafilm. Three replications were used for each concentration. Observations on the number of insects present on both the treated and untreated halves were recorded after 1, 3, 5, and 24 h. Percentage repellency (PR) was calculated as follows.[15]

$$PR = \frac{Nc - Nt}{Nc + Nt} \times 100 \quad (1)$$

Nc was the number of insects on the untreated area after the exposure interval and Nt was the number of insects on the treated area after the exposure interval.

Antifeedant activity of monoterpenes

To determine antifeedant activity of monoterpenes a no-choice test was carried out as described[16,17] with some modifications. 1 ml of prepared concentrations of 10 and 30 µl of monoterpenes dissolved in methanol and 1 ml solvent alone as control were applied on to a 5 g grinded mixture of pulses and rice kernels. The treated mixture of food media was placed in Petri dishes after evaporating the solvent. Ten adults of *T. castaneum* and *S. oryzae* were transferred to each pre-weighed food media in Petri dishes. After feeding for 72 h, under laboratory conditions food media were re-weighed and mortality of insects was recorded. Three replicates of each treatment were prepared, including the control. Nutritional indices and weight loss were calculated as previously described.[17,18] Weight loss (%WL) = (IW–FW) × 100/IW, where the IW is the initial weight and FW is the final weight. The grain protection due to application of compounds was observed by calculating the feeding deterrence index (FDI).[19,20] Using the formula, FDI (%) = (C – T) / (C + T) × 100, where C is weight loss of control rice kernels and T is weight loss of treated rice kernels.

Statistical analysis

Data obtained from each dose-response bioassay for toxicity of monoterpenes were subjected to probit analysis in which probit-transformed mortality was regressed against log10-transformed dose and LC_{50} values were generated. Tests for fumigant toxicity, repellency, and antifeedant activity were performed in triplicate and data presented are mean ± SE. The mean values were compared by one-way ANOVA and Tukey's multiple comparison tests using software SPSS, version 11.5.

RESULTS

Fumigant toxicity of monoterpenes against *S. oryzae* and *T. castaneum*

Monoterpene geraniol was found to be highly effective against both *S. oryzae* and *T. castaneum* than citronellol. At a lowest concentration of 0.02 µl/ml geraniol produced a mortality of 26.30 ± 0.11 after a short duration of 6 h that reached 52.76 ± 0.28% after an increased exposure of 72 h against *S. oryzae,* whereas 0.2 µl/ml geraniol resulted in a highest mortality of 40.44 ± 0.49, 48.22 ± 0.39, and 64.72 ± 0.39% at 24, 48, and 72 h, respectively. Citronellol showed least activity producing a mortality of 52.76 ± 0.28 and 64.72 ± 0.39% at 0.02 and 0.2 µl/ml after 72 h of exposure against *S. oryzae* [Table 1]. Similarly for *T. castaneum* geraniol produced 68.75 ± 0.55% mortality at 0.2 µl/ml after 72 h, followed by 65.89 ± 0.21 (0.12 µl/ml), 62.76 ± 0.39 (0.04 µl/ml), and 57.53 ± 0.51 (0.02 µl/ml). Citronellol even at a highest dose of 0.2 µl/ml caused 30.77 ± 0.49 and 38.44 ± 0.44% mortality after 12 and 24 h, respectively, followed by 48.66 ± 0.29 and 58.76 ± 0.37% mortality after an increased exposure of 48 and 72 h while at a lowest concentration of 0.02 µl/ml caused 32.09 ± 0.08, 38.65 ± 0.34, and52.76 ± 0.28% mortality after an interval of 24, 48, and 72 h, respectively, against *T. castaneum* [Table 2].

Citronellol and eugenol showed fumigant toxicity having LC_{50} value of 5.2 µl/ml and 3.0 µl/ml air after 6 h treatment whereas similar compounds exhibit LC_{50} values of 1.53 µl/ml and 0.24 µl/ml air after 24 h of treatment, respectively, against *T. castaneum*. Similarly, LC_{50} of 4.5 and 3.0 was obtained at 6 h, followed by LC_{50} values of 1.14 and 0.14 after an increased exposure of 24 h for *S. oryzae* [Table 3].

Repellent activity of monoterpenes against *S. oryzae* and *T. castaneum*

Geraniol produced 42.56 ± 1.9% repellent activity at 1 µl/cm^2 after 1 h, followed by 40.18 ± 1.8 (0.6 µl/cm^2) and 35.38 ± 1.8 (0.2 µl/cm^2) whereas % repellency of 48.60 ± 1.4 (1 µl/cm^2), 45.44 ± 3.1 (0.6 µl/cm^2), and 38.28 ± 2.8 (0.2 µl/cm^2) was

Table 1: Fumigant toxicity of two monoterpenes against *Sitophilus oryzae*

Monoterpenes	Doses µl/ml	% Mortality±SE				
		6 h	12 h	24 h	48 h	72 h
	0.02	26.30±0.11[a]	30.56±0.33[b]	32.09±0.08[a]	38.65±0.34[b]	52.76±0.28[a]
Citronellol	0.04	28.53±0.14[a]	30.55±0.33[b]	33.54±0.20[a]	40.77±0.45[b]	55.77±0.33[a]
	0.12	28.63±0.24[a]	31.45±0.45[b]	36.35±0.41[b]	44.54±0.51[b]	58.87±0.21[a]
	0.2	30.57±0.23[b]	31.56±0.50[b]	40.44±0.49[b]	48.22±0.39[a]	64.72±0.39[b]
	0.02	30.44±0.12[b]	33.45±0.45[b]	39.32±0.28[b]	47.52±0.53[a]	61.75±0.38[a]
Geraniol	0.04	32.54±0.08[b]	34.76±0.54[b]	45.65±0.39[b]	55.76±0.26[a]	64.88±0.49[b]
	0.12	32.38±0.32[b]	38.55±0.33[a]	48.76±0.40[c]	58.89±0.44[c]	66.70±0.61[b]
	0.2	34.67±0.29[b]	40.87±0.56[a]	52.50±0.44[c]	63.33±0.50[c]	70.66±0.67[c]
Control		0.00±0.00[ab]	0.00±0.00[ab]	0.00±0.00[ab]	0.00±0.00[ab]	0.00±0.00[ab]

% values are mean (n=3)±SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests

Table 2: Fumigant toxicity of two monoterpenes against *Tribolium castaneum*

Monoterpenes	Doses µl/ml	% Mortality±SE				
		6 h	12 h	24 h	48 h	72 h
	0.02	25.45±0.09[a]	28.54±0.36[b]	30.66±0.12[a]	36.57±0.31[a]	50.49±0.27[b]
Citronellol	0.04	25.65±0.32[a]	28.60±0.36[b]	32.56±0.08[a]	38.77±0.49[a]	52.54±0.39[b]
	0.12	28.87±0.18[a]	30.65±0.39[b]	35.46±0.19[a]	44.55±0.57[b]	54.17±0.42[b]
	0.2	28.66±0.34[a]	30.77±0.49[b]	38.44±0.44[b]	48.66±0.29[b]	58.76±0.37[a]
	0.02	28.58±0.09[a]	30.56±0.33[b]	38.77±0.27[b]	49.43±0.65[b]	57.53±0.51[a]
Geraniol	0.04	30.45±0.21[b]	32.43±0.42[b]	43.66±0.39[b]	53.78±0.39[b]	62.76±0.39[a]
	0.12	30.67±0.07[b]	35.87±0.21[b]	45.32±0.53[b]	54.65±0.19[b]	65.89±0.21[c]
	0.2	30.77±0.19[b]	38.23±0.56[a]	50.55±0.45[c]	60.66±0.45[c]	68.75±0.55[c]
Control		0.00±0.00[ab]	0.00±0.00[ab]	0.00±0.00[ab]	0.00±0.00[ab]	0.00±0.00[ab]

% Values are mean (n=3)±SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests

Table 3: LC_{50} values of two monoterpenes against insect pests on different exposure intervals

LC_{50} µl/ml air	6 h	12 h	24 h
Citronellol			
S. oryzae	4.5	2.3	1.14
T. castaneum	5.2	2.8	1.53
Geraniol			
S. oryzae	3.0	1.48	0.14
T. castaneum	3.0	1.87	0.24

S. oryzae: Sitophilus oryzae, T. castaneum: Tribolium castaneum

obtained by citronellol against *T. castaneum* after same time period. At 1 µl/cm^{2}59.42 ± 4.2 and 63.54 ± 1.2% repellency was produced by geraniol after 3 and 5 h while 62.28 ± 2.2 and 68.41 ± 3.3% repellent activity was obtained by citronellol at same concentration and time intervals toward *T. castaneum* [Table 4]. Moreover, the repellent activity decreased after 24 h of exposure for all the treatments at respective concentrations. Citronellol and geraniol at a highest concentration of 1 µl/cm^2 gave 56.61 ± 3.4 and 50.56 ± 1.1% repellency, respectively, after 5 h that further decreased to 43.16 ± 4.6 and 39.10 ± 3.2% after an increased exposure of 24 h against *S. oryzae* [Table 5].

Antifeedant activity of monoterpenes against *T. castaneum* and *S. oryzae*

8.32 ± 0.16 and 9.42 ± 0.08% grain damage was observed for citronellol and geraniol at a high concentration of 30 µl/g as compared to 70.32 ± 0.28% damage under control and FDI was 78.95 ± 0.09 and 67.59 ± 0.17% for *T. castaneum.* While 10.15 ± 0.27 and 11.23 ± 0.11% grain damage and 73.17 ± 0.15 and 72.41 ± 0.32% FDI were obtained at a lower concentration of 10 µl/g for similar treatments and insect pest [Table 6]. Citronellol showed 52.80 ± 0.32% FDI with 25.21 ± 0.18% grain damage, followed by geraniol with 49.28 ± 0.17% FDI and 26.05 ± 0.09% grain damage

Table 4: Percentage repellency of two monoterpenes against *Tribolium castaneum* at different time intervals (values are mean±SE)

Monoterpenes	Time (h)	Doses µl/cm²		
		0.2 µl/cm²	0.6 µl/cm²	1 µl/cm²
Citronellol	1	38.28±2.8^{cd}	45.44±3.1^{a}	48.60±1.4^{ab}
	3	42.34±1.9^{cd}	40.32±2.5^{a}	62.28±2.2^{bc}
	5	53.12±3.6^{a}	58.32±1.5^{d}	68.41±3.3^{b}
	24	18.46±2.1^{bc}	18.56±1.2^{ab}	45.29±1.1^{ab}
Geraniol	1	35.38±1.8^{cd}	40.18±1.8^{a}	42.56±1.9^{ab}
	3	40.48±2.2^{cd}	36.26±3.5^{bc}	59.42±4.2^{c}
	5	50.28±1.6^{a}	52.32±1.5^{a}	63.54±1.2^{bc}
	24	15.25±3.1^{bc}	15.56±4.2^{ab}	40.52±3.5^{ab}

% Values are mean (*n*=3)±SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests

Table 5: Percentage repellency of two monoterpenes against *Sitophilus oryzae* at different time intervals (values are mean±SE)

Monoterpenes	Time (h)	Doses µl/cm²		
		0.2 µl/cm²	0.6 µl/cm²	1 µl/cm²
Citronellol	1	28.13±2.4^{c}	36.65±2.8^{bc}	40.54±1.4^{ab}
	3	35.45±1.9^{c}	41.63±1.6^{d}	46.45±2.5^{d}
	5	40.51±2.4^{c}	49.36±2.2^{d}	56.61±3.4^{c}
	24	15.24±1.4^{ab}	18.52±4.1^{ab}	43.16±4.6^{d}
Geraniol	1	25.63±1.4^{c}	32.45±1.8^{bc}	36.24±3.2^{bc}
	3	30.25±2.9^{c}	35.43±2.8^{bc}	42.25±1.5^{ab}
	5	36.33±2.1^{bc}	45.56±1.2^{d}	50.56±1.1^{d}
	24	15.24±1.4^{ab}	18.5±4.1^{ab}	39.10±3.2^{ab}

% Values are mean (*n*=3)±SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests

at 10 µl/g against *S. oryzae,* whereas 21.45 ± 0.32 and 23.32 ± 0.25% grain damage and 63.82 ± 0.09 and 58.97 ± 0.09% FDI were calculated at concentration of 30 µl/g for same pest and monoterpenes, respectively [Table 7].

DISCUSSION

The present study demonstrated that the monoterpenes have varying degrees of fumigant toxicity, repellent activity, and antifeedant activity against two species of stored product pests but dependent on the dosage and duration of treatment. Monoterpene geraniol was found to be more effective than citronellol against both *S. oryzae* and *T. castaneum*. At a lowest concentration of 0.02 µl/ml geraniol produced a mortality of 26.30

Table 6: Antifeedant activity of two monoterpenes against *Tribolium castaneum* (values are mean±SE)

Monoterpenes	Doses µl/g	Grain damage (%)	Weight loss (%)	FDI (%)
Citronellol	10	10.15±0.27^{c}	7.01±0.28^{c}	73.17±0.15^{b}
	30	8.32±0.16^{c}	5.32±0.32^{d}	78.95±0.09^{b}
Geraniol	10	11.23±0.11^{d}	7.24±0.23^{c}	72.41±0.32^{b}
	30	9.42±0.08^{c}	5.48±0.19^{d}	67.59±0.17^{d}
Control		70.32±0.28^{ab}	45.25±0.32^{ab}	-

% Values are mean (*n*=3)±SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests

Table 7: Antifeedant activity of two monoterpenes against *Sitophilus oryzae* (values are mean± SE)

Monoterpenes	Doses µl/g	Grain damage (%)	Weight loss (%)	FDI (%)
Citronellol	10	25.21±0.18^{d}	18.32±0.34^{d}	52.80±0.32^{bc}
	30	21.45±0.32^{c}	13.10±0.21^{a}	63.82±0.09^{a}
Geraniol	10	26.05±0.09^{d}	20.15±0.18^{d}	49.28±0.17^{cd}
	30	23.32±0.25a	15.31±0.09^{c}	58.97±0.09^{d}
Control		85.36±0.09^{ab}	59.32±0.26^{ab}	-

% Values are mean (*n*=3)±SE. The means within a column followed by same letter are not significantly different from each other according to ANOVA and Tukey's comparison tests

± 0.11 after a short duration of 6 h against *S. oryzae* whereas citronellol showed least activity producing a mortality of 52.76 ± 0.28 and 64.72 ± 0.39% at 0.02 and 0.2 µl/ml after 72 h of exposure against *S. oryzae.* Similarly for *T. castaneum* geraniol produced 68.75 ± 0.55% mortality at 0.2 µl/ml after 72 h, whereas citronellol even at a highest dose of 0.2 µl/ml caused 30.77 ± 0.49 and 38.44 ± 0.44% mortality after 12 and 24 h, respectively, followed by 48.66 ± 0.29 and 58.76 ± 0.37% mortality after an increased exposure of 48 and 72 h. *S. oryzae* was found to be more susceptible for all the treatments than *T. castaneum*. The previous studies also evaluated the insecticidal activities of variable magnitude in monoterpenes against various insect species. Some monoterpenes, namely, limonene, terpinen-4-ol, 1,8-cineole, menthol, carvacrol, myrcene, and α-pinene were shown to be more toxic than others.[21-23] Due to their high volatility many plant derived materials including monoterpenoids have fumigant action against a variety of insect pests.[24] The present work supports the results discussed previously. In the repellency tests geraniol produced 42.56 ± 1.9% repellent activity at 1 µl/cm² after 1 h whereas repellency of 48.60 ± 1.4% was obtained by citronellol against *T. castaneum* after same concentration and time period. Citronellol and geraniol at a highest concentration of 1 μl/cm^2 gave 56.61 ± 3.4 and 50.56 ± 1.1% repellency, respectively, after 5 h against *S. oryzae.* Moreover, the repellent activity decreased after 24 h of exposure for all the treatments at respective concentrations. The previous studies showed that essential oil extracted from *Piper nigrum* (L.) caused repulsion in the adults of *T. castaneum* at low concentration.[25] Insecticidal activity against *T. castaneum* was also reported in essential oils isolated from *Trachyspermum ammi*, *Anethum graveolens*, and *Nigella sativa*[26] and different insecticidal activity against *Sitophilus zeamais* and *T. castaneum* by leaf essential oil of *Melaleuca cajuputi* in case of *T. castaneum* 100% repellency was reported.[27] FDI showed that the tested monoterpenes had antifeedant action against the two insect pests at different concentrations. 8.32 ± 0.16 and 9.42 ± 0.08% grain damage was observed for citronellol and geraniol at a high concentration of 30 μl/g as compared to 70.32 ± 0.28% damage under control and FDI was 78.95 ± 0.09 and 67.59 ± 0.17% for *T. castaneum.* Citronellol showed 52.80±0.32% FDI with 25.21 ± 0.18% grain damage followed by geraniol with 49.28 ± 0.17% FDI and 26.05 ± 0.09% grain damage at 10 μl/g against *S. oryzae*. In a related study, the adults of *S. zeamais* and larvae of *T. castaneum* showed antifeedant activity in media treated with cinnamaldehyde, a benzene derivative from the essential oil of cinnamon.[28] A feeding deterrent index of 91.51, 97.26, 98.02, and 6.18% of essential oil of *Aegle marmelos* for *C. chinensis*, *Rhyzopertha dominica, S. oryzae,* and *T. castaneum* with 100% grain damage in *T. castaneum* was recorded while in *C. chinensis, R. dominica,* and *S. oryzae* infested grains 7.0, 3.67, and 1.67% grain damage were found, respectively.[29] Oils containing mainly oxygenated monoterpene compounds were reported to lose their activity slower than those with high content of hydrocarbon monoterpenes compounds.[28]

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