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RESEARCH ARTICLE

Bioactivity of an Ionic Liquid against Two Major Coleopteran Stored Grain Insect Pests

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ABSTRACT

Introduction: Insecticidal efficacy of a monoterpene-based ionic liquid was evaluated against two Coleopteran insect pests, viz., the red flour beetle, *Tribolium castaneum* Herbst. and the drug store beetle, *Stegobium paniceum* (L). **Methods:** Bioassays for contact activity against adult insects, larvicidal activity, repellent activity, and antifeedant activity were conducted in the laboratory for the above said insect pests. **Results:** Ionic liquid at a highest concentration of 1.0 µl/cm^2 proved to be most effective against adults of *S. paniceum* producing 76.23 ± 1.9% mortality while 70.12±3.5% mortality was obtained against *T. castaneum* after 72 h, respectively. *T. castaneum* larvae were most susceptible to all the doses of ionic liquid at a highest dose of 100 µl/ml of ionic liquid 80.08 ± 2.2 mortality was obtained followed by a mortality of 68.32±1.8% for *S. paniceum* among 8-10 day old larvae. Ionic liquid showed remarkable repellency at different doses of 2, 6 and 10 µl/cm^2 against both insect pests. For *S. paniceum* 75.45 ± 1.9, 80.28 ± 2.4 and 56.35 ± 4.8% repellency was observed at 10 µl/cm^2 of ionic liquid after 3, 5 and 24 h. Ionic liquid proved to be effective seed protectant against both the insect pests. 87.99 ± 0.14% FDI was recorded with 5.10 ± 0.30 grain damage at 300 µl/g of ionic liquid, respectively, while 72.32 ± 0.18% grain damage was observed in control for *T. castaneum.*

Keywords: Antifeedant, insect pests, ionic liquid, larvicidal, repellent activity

INTRODUCTION

Severe damage in weight and nature of the stored products is caused by many insects.[1] Adult and juvenile insects form attack many flour factories, distribution centers and supermarkets and feed on an extremely wide assortment of dry vegetable substances, for example, processed cereal items. Interest in the utilization of monetarily accessible bio insecticide sprays and future possibilities for the improvement of new natural arrangements in plant protection has fundamentally expanded as of late. Bio insecticide sprays of business accessibility have produced a great deal of interest in the territory of plant protection with respect to new organic planning advancement. The characteristic items dependent on plants are generally less unsafe to climate and mammalian wellbeing when contrasted with manufactured synthetic pesticides. Thusly to lessen the negative effects on human wellbeing and climate regular items are the best option in contrast to engineered pesticides. Pesticides derived from plant fundamental oils or their constituents have set up extraordinary proficiency against the expansive scope of stored product damage. Monoterpenes containing ten carbons are a class of plant obtained metabolites. As the fundamental constituents of essential oil, these impart plants their exceptional odoriferous properties. Usually containing 1–3 terpenes as significant parts and numerous others as minor segments the oil constitution is unique for each plant species.[2] In the case of *Sitophilus oryzae*, the red flour beetle, *Tribolium castaneum* and the sawtoothed grain insect, *Oryzaephilus surinamensis* some usual monoterpenoids have been demonstrated to exhibit fumigant toxicity.[3] Four stored product

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pests *Callosobruchus analis*, *S. oryzae*, *Stegobium paniceum*, and *T. castaneum* were put up for fumigant toxicity studies involving the use of monoterpenes: citronellol, geraniol, linalool, eugenol, thymol, cinnamaldehyde, p-cymene and α-pinene.[4]

Among the many synthetic compounds which are labeled as green pesticides ionic fluids (ILs) are novel natural salts with a wide liquid range. These chemicals have great potential for industrial use as ecofriendly chemicals. The cationic and anionic segments of ionic liquids can be modified thereby affecting their properties and its toxic levels. Ionic fluids with surfactant properties have an extraordinary potential as pesticides. Insecticidal potential of citronellol, ionic surfactant and a nonionic surfactant was assessed for their insecticidal activity towards *C. analis* (F) and *S. oryzae*. Both the surfactants altogether deflected most of females of *C. analis* from laying eggs and in decreasing Fl offspring creation.[5]

The oils and surfactants are less inclined for selection by resistance and may provide safer options in contrast to synthetic chemical-based insecticides.[6,7] Surfactants have for quite some time been utilized as wetting, spreading, emulsifying and adhering specialists to improve the adequacy and inclusion of numerous pesticides.[8] Accordingly, the present examination was directed to decide insecticidal action of monoterpene-based ionic fluid against two Coleopteran pests the red flour beetle, *T. castaneum* Herbst and drug store beetle, *S. paniceum* (L).

MATERIALS AND METHODS

Synthesis of an Ionic Liquid

Ionic liquid was synthesized by simple esterification of citronellol with chloroacetic acid resulted in formation of intermediate, that is, 3,7-dimethyloct-6-en-1-yl 2-chloroacetate. Finally, intermediate was quaternized with n-methylimidazole at 80°C for one hour resulted in respective citronellol based ionic liquid, viz., 3-(2-((3,7-dimethyloct-6-en-1-yl)oxy)-2-oxoethyl)-1-methyl-1*H*-imidazol-3-ium chloride. The respective amphiphile was purified by recrystallization from diethyl ether followed by drying of the viscous material under vacuum at 40°C to get the pure final product. The ionic liquid possesses properties of surfactant was tested for its insecticidal efficacy by conducting different bioassays against two test insects.

Test Insects

Laboratory cultures of *S. paniceum*, *T. castaneum* (5–10 days each) was maintained at 30 ± 2°C and 68±2% relative humidity. Whole meal wheat flour plus brewer's yeast (19:1) was used to rear *S. paniceum* and *T. castaneum*, respectively.

Bioassays

Contact toxicity of ionic liquid against adult insect pests

Contact activity of ionic liquid against adults of insect pests was determined by direct contact application as cited in literature.[9,10] 5, 10, 30, and 50 µl of ionic liquid was dissolved in 1 ml of water to prepare final concentrations of 0.1, 0.2, 0.6 and 1 µl/cm^2. Control was taken as 1ml methanol. Each dried paper was placed at the bottom of a Petri dish and 10 adults (5–10 day old) of each insect along with their food source were placed in each Petri dish and covered with a lid. Each set of treatment was repeated three times and number of dead insects in each Petri-dish was counted at different intervals of time.

Larvicidal Activity of Ionic Liquid against Insect Pests

T. castaneum larvae were external feeders and obtained by carefully examining the food media. Larvae of *S. paniceum* devoured into the seeds so for *S. paniceum* 10 days after egg laying the seeds were observed for larval stages. A stock solution of ionic liquid was prepared by dissolving 100 µl of it in 1 ml of water. Plastic jars of 250 ml capacity with screw lids were used as exposure chambers. Different doses of ionic liquid 10 30, 50 and 100 µl prepared in solvent were applied to a circular filter paper (Whatman No. 1) and after evaporating the solvent for 5–10 minutes the treated filter paper discs were then introduced into the plastic jars and attached to inner surface of screw lid of the jar using adhesive tape. 1 ml methanol alone was used as control. In each jar, a small glass Petri dish was containing about 10 larvae (8–10 day old) of *T. castaneum* and 10 seed grains containing *S. paniceum* larvae of same age were placed carefully. After 48 h of exposure, the larvae were transferred to clean vials and larval mortality was recorded after 120 h. For *S. paniceum*, seeds or grains were subsequently dissected after five days to count the dead larvae and larval mortality was calculated accordingly. Three replicates were set up for each dose.

Percentage mortality was calculated using the Abott formula.[11]

$$\text{Corrected mortality \%} = \frac{1 - \text{n in T after treatment}}{\text{n in Co after treatment}} \times 100$$

n = Total number of insects, T – treated, Co – control.

Repellent Activity of Ionic Liquid against Insect Pests

Repellency tests of ionic liquid was carried out according to the experimental method described by Jilani and Saxena.[12] Test solutions were prepared by dissolving 100, 300 and 500 µl of ionic liquid in 1 ml water to obtain final doses of 2, 6 and 10 $\mu l/cm^2$ Whatman filter papers (diameter 8 cm) were cut into two equal halves one half of each dish was treated with essential oil solution as uniform as possible using micro pipette. 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.[13]

$$PR = = \frac{Nc - Nt}{Nc + Nt} \times 100$$

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 Ionic Liquid against Insect Pests

The bioassay experiment was conducted for evaluating ionic liquid as potential seed protectants against *T. castaneum and S. paniceum* (5–10 day old) adults. To determine antifeedant activity of essential oils a no-choice test was carried out as described[14,15] with some modifications. 1 ml of prepared concentrations of 100 and 300 µl of compound dissolved in water and 1 ml methanol alone as control were applied on to a 5 g grinded mixture of pulses and rice kernels. The treated mixture of food media were placed in Petri dishes after evaporating the solvent. 10 adults of *T. castaneum* and, *S. paniceum* were transferred to each pre-weighed food media in Petri dishes. After feeding for 72 h under laboratory conditions food media was 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.[16]

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 using the formula.[17,18]

FDI (%) = (C–T)/(C+T)×100, where C is weight loss in control and T is weight loss in treatment.

RESULTS

Contact Toxicity of Ionic Liquid against Adult Insect Pests

The toxicity of cationic surfactant (3-(2-((3,7-dimethyloct-6-en-1-yl)oxy)-2-oxoethyl)-1-

methyl-1*H*-imidazol-3-ium chloride) against *S. paniceum*, and *T. castaneum* adults exposed to direct contact has been summarized in Table 1. Ionic liquid at a highest concentration of 1.0 µl/cm² proved to be most effective against *S. paniceum* producing 64.40 ± 1.6 and 76.23 ± 1.9% mortality after 48 and 72 h, respectively, while 62.32 ± 3.4 and 70.12 ± 3.5% mortality was obtained against *T. castaneum* at similar concentration and time intervals. Ionic liquid at 0.1 µl/cm² obtained 34.21 ± 2.1% mortality against *S. paniceum* that reached to 42.17 ± 2.1 % at an increased concentration of 1.0 µl/cm² after 12 h of treatment, whereas 51.27 ± 1.8, 62.32 ± 3.2 and 70.12 ± 3.5% mortality was obtained at 1.0 µl/cm² followed by 42.29 ± 3.7, 55.29 ± 1.1 and 60.28 ± 1.3% after 24, 48 and 72 h at 0.1 µl/cm² of ionic liquid against *T. castaneum*. No mortality was obtained in control at the same time intervals.

Larvicidal Activity of Ionic Liquid against Insect Pests

Different concentrations of 10, 30 50 and 100 µl/ ml of ionic liquid resulted in larval mortality of all insect pests. *T. castaneum* larvae were most susceptible to all the doses of ionic liquid. At a highest dose of 100 µl/ml of ionic liquid 80.08 ± 2.2 mortality was obtained among 8–10 day old larvae followed by 68.17 ± 4.3% mortality for 18–20 day old larvae of *T. castaneum*. The mortality decreased to 75.30 ± 4.5% at 50 µl/ml followed by 72.34 ± 3.6 (30 µl/ml) and 70.24 ± 2.7 (10 µl /ml) against 8-10 day old larvae while mortality of 64.32 ± 2.5 (50 µl/ml), 56.28 ± 4.4 (30 µl/ml) and 52.12 ± 5.1% (10 µl/ml) was observed against 18–20 day old larvae of *T. castaneum.*

For *S. paniceum,* 100 µl/ml of ionic liquid resulted in 68.32 ± 1.8% mortality among 8–10 day old larvae followed by 45.42 ± 3.5% mortality of 18-20 day old larvae. Whereas 60.12 ± 2.2, 58.02 ± 3.7 and 52.24 ± 5.5% mortality was obtained against 8–10 day old larvae at 50, 30 and10 µl/ml of ionic liquid followed by 42.18 ± 1.5, 38.12 ± 3.9 and 34.15 ± 4.4% mortality respectively against 18-20 day old larvae of *S. paniceum* at similar concentrations. Ionic liquid proved to be less effective against *S. paniceum* than *T. castaneum* [Table 2].

Repellent Activity of Ionic Liquid against Insect Pests

Ionic liquid showed remarkable repellency at different doses of 2, 6 and 10 µl/cm² against both insect pests. For *S. paniceum* 75.45 ± 1.9, 80.28 ± 2.4 and 56.35 ± 4.8% repellency was observed at 10 µl/cm² of ionic liquid after 3, 5 and 24 h followed by a repellence of 66.42 ± 2.8, 72.26 ± 1.4 and 35.56 ± 1.6% at a dose of 6 µl/cm² while 55.30 ± 1.8, 67.22 ± 2.4 and 26.45 ± 1.6% repellency was obtained at 2 µl/cm² after same time period. In *T. castaneum* 55.64 ± 1.2, 72.35 ± 1.6 and 77.23 ± 2.8% repellency was obtained at 2, 6 and 10 µl/cm² of ionic liquid after 3 h while 66.51 ± 2.1, 78.26 ± 1.8 and 81.40 ± 3.6% repellence was observed after 5 h of exposure at same concentrations. After 24 h of treatment

Table 1: Contact toxicity of ionic liquid against insect pests

Insect pests	Doses µl/cm²	% Mortality±SE				
		6 h	12 h	24 h	48 h	72 h
Stegobium paniceum	0.1	34.27±4.3ᵃ	34.21±2.1ᵈ	45.34±4.3ᵈ	55.04±1.3ᶜᵈ	65.25±4.1ᵈ
	0.2	36.03±2.1ᵃ	38.11±1.9ᵈ	50.07±1.8ᵃ	60.34±2.2ᵇ	70.06±3.4ᵃ
	0.6	36.33±1.3ᵃ	40.39±2.7ᵇ	52.10±2.4ᶜ	62.35±1.9ᵇ	72.43±2.5ᵃ
	1.0	38.34±3.1ᵃ	42.17±2.1ᵇ	54.27±3.5ᶜ	64.40±1.6ᵃ	76.23±1.9ᵃ
Tribolium castaneum	0.1	30.33±3.3ᶜ	35.29±2.4ᵈ	42.293.7ᵇᶜ	55.29±1.1ᶜᵈ	60.28±1.3ᵇᶜ
	0.2	32.06±1.8ᶜ	36.23±1.4ᵈ	45.30±1.4ᵇᶜ	57.16±2.9ᶜᵈ	64.08±45ᵈ
	0.6	35.19±2.9ᵃ	38.11±1.1ᵈ	48.04±3.6ᵃ	59.34±1.4ᵇ	66.28±1.6ᵈ
	1.0	38.30±1.6ᵃ	40.21±2.1ᵇ	51.27±1.8ᵃ	62.32±3.4ᵇ	70.12±3.5ᵃ
Control		0.00±0.00ᵃᵇ	0.00±0.00ᵃᵇ	0.00±0.00ᵃᵇ	0.00±0.00ᵃᵇ	0.00±0.00ᵃᵇ

% 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 repellence decreased to 30.40 ± 1.6, 40.47 ± 1.2 and 56.46 ± 1.2% at 2, 6 and 10 µl/ cm^2 of ionic liquid against *T. castaneum* [Table 3].

Antifeedant Activity of Ionic Liquid against Insect Pests

The data in Table 4 showed that ionic liquid at concentrations of 100 and 300 µl/g proved to be effective seed protectant against both the insect pests. In case of *T. castaneum* 87.99 ± 0.14 and 80.67 ± 0.21% FDI was recorded with 5.10 ± 0.30 and 7.15 ± 0.21% grain damage at 300 and 100 µl/g of ionic liquid, respectively, while 72.32 ± 0.18% grain damage was observed in control for *T. castaneum.* 65.59 ± 0.18 and 60.56 ± 0.30% FDI was recorded with 7.36±0.21 and 18.15 ± 0.18% grain damage at 300 and 100 µl/g of ionic liquid as compared to control with 75.41 ± 0.35% grain damage for *S. paniceum.*

Table 2: Larvicidal activity of ionic liquid against insect pests (Values are mean±SE)

Insect pests	Doses µl/ml	(% Mortality) 8–10 day old larvae	18–20 day old larvae
Tribolium castaneum	10	70.24±2.7[b]	52.12±5.1[a]
	30	72.34±3.6[b]	56.28±4.4[b]
	50	75.30±4.5[a]	64.32±2.5[c]
	100	80.08±2.2[c]	68.17±4.3[c]
Stegobium paniceum	10	52.24±5.5[bc]	34.15±4.4[bc]
	30	58.02±3.7[d]	38.12±3.9[bc]
	50	60.12±2.2[d]	42.18±1.5[bc]
	100	68.32±1.8[d]	45.42±3.5[d]
Control		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: Percentage repellency (mean±SE) of ionic liquid against insect pests at different time intervals

Insect pests	Time (h)	Doses µl/cm^2 2 µl/cm^2	6 µl/cm^2	10 µl/cm^2
Stegobium paniceum	1	50.14±4.2[b]	60.56±3.5[c]	68.50±3.6[d]
	3	55.30±1.8[d]	66.42±2.8[c]	75.45±1.9[d]
	5	67.22±2.4[bc]	72.26±1.4[b]	80.28±2.4[a]
	24	26.45±1.6[cd]	35.56±1.6[bc]	56.35±4.2[bc]
Tribolium castaneum	1	46.32±2.2[b]	60.46±2.5[c]	68.37±1.5[d]
	3	55.64±1.2[d]	72.35±1.6[b]	77.23±2.8[d]
	5	66.51±2.1[bc]	78.26±1.8[cd]	81.40±3.6[a]
	24	30.40±1.6[cd]	40.47±1.2[bc]	56.46±1.2[bc]

% 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

DISCUSSION

Different concentrations of 0.1, 0.2, 0.6 and 1 µl/cm^2 of ionic liquid were tested for contact activity against insect pests. Ionic liquid at a highest concentration of 1.0 µl/cm^2 proved to be most effective against *S. paniceum* producing 76.23 ± 1.9% mortality after 72 h while 70.12 ± 3.5% mortality was obtained against *T. castaneum* at similar concentration and time intervals. Ionic liquid at a lowest concentration of 0.1 µl/cm^2 obtained 35.29 ± 2.4% mortality against *T. castaneum* after 12 h while showed 34.21 ± 2.1 mortality against *S. paniceum*. Result of this investigation were also similar and[5] reported that ionic surfactant at concentrations of 5 and 10 µl showed 68–88% mortality towards *C. analis* and 63 to 76% for *S. oryzae* after 24 h of treatment.[19-22] reported insect mortality under laboratory conditions using various surfactants. In a related study of ionic and nonionic surfactants, Triton GR-7, Pronon 505, Catanac SN, and Retzanol M-139 resulted in significant green peach aphid mortality.[23] Ionic liquid showed remarkable repellency at different doses of 2, 6 and 10 µl/cm^2 against all insect pests. At a highest dose of 10 µl/cm^2 showed highest repellency of 80.28 ± 2.4 and 81.40 ± 3.6 after 5 h that further decreased to 53.35 ± 4.2 and 56.46 ± 1.2% after 24 h against *S. paniceum* and *T. castaneum* and

Table 4: Antifeedant activity of ionic liquid against insect pests (Values are mean±SE)

Insect pests	Doses µl/g	Grain damage (%)	Weight loss (%)	FDI (%)
Tribolium castaneum	100	7.15±0.21[a]	5.16±0.15[c]	80.67±0.21[c]
	300	5.10±0.30[b]	3.08±0.32[b]	87.99±0.14[a]
Control		72.32±0.18[bc]	48.25±0.37[bc]	-
Stegobium paniceum	100	18.15±0.18[c]	12.32±0.2[cd]	60.56±0.30[b]
	300	7.36±0.21[a]	10.42±0.19[d]	65.59±0.18[d]
Control		75.41±0.35[bc]	50.16±0.19[bc]	-

% 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 respectively. Larvicidal activity of ionic liquid was also tested against insect pests. Highest mortality of 80.08 ± 2.2% was obtained for 8–10 day old larvae of *T. castaneum* followed by *S. paniceum* (68.32 ± 1.8) and *S. oryzae* (64.31 ± 3.5) at 100 µl/ml of ionic liquid. The addition of surfactant Silwet to a range of products aimed at controlling Tetranychidae on grapevine increased the efficacy against eggs, nymphs and adults between 4 and 22%.[24] It also revealed good antifeedent activity toward all insect species and 300 µl/g of ionic liquid obtained 87.99 ± 0.14 and 65.59 ± 0.18 FDI for *T. castaneum and S. paniceum*, respectively, and a less grain damage was recorded as compared to controls. Citronellol and ionic surfactant showed better antifeedant action against *S. oryzae* adults, a concentration of 10 µL/g rice kernels, resulted in 65.21 and 60.24% reduction in feeding, respectively.[5,25] tested the effectiveness of ILs as stored product insect antifeedants and also compared the impact of cation and anion on the antifeedant activity. Ionic surfactant exhibited a more potent effect on food consumption rate than nonionic surfactant.

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