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Research Article

INSECTICIDAL EFFICACY OF ESSENTIAL OILS FROM *ARTEMISIA MARITIMA* L. AND *ZANTHOXYLUM ARMATUM* DC. AND THEIR TWO MAJOR CONSTITUENTS AGAINST *PLODIA INTERPUNCTELLA* (HUBNER)

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Abstract: Essential oils isolated from *Artemisia maritima* L. and *Zanthoxylum armatum* DC were analyzed by mass spectroscopy (MS) and their main constituents were identified. Fumigant activity, repellent activity, progeny deterrency and antifeedant activity of essential oils and two constituents viz., alpha pinene and linalool were examined against the major stored product insect pest, *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae). 100% mortality was achieved by alpha pinene, linalool, *A. maritima* and *Z. armatum* oils at all concentrations within 120 hrs against P. *interpunctella*. Alpha pinene showed the highest repellent activity of 80.18±1.9, 88.36±1.4, 93.15±4.1% after 1, 3 and 5 hrs respectively followed by linalool and *Z. armatum* and showed remarkable activity at 6 µl/cm2 against *P. interpunctella* with 76.24±1.1 and 66.42±2.8% repellence after 3 hrs followed by 80.46±1.8 and 72.26±1.4% after an interval of 5 hrs. In progeny deterrence tests alpha pinene and linalool were more potent than others producing 10.25±1.2 and 13.45±2.2 F1 progeny of *P. interpunctella* with 74.66 and 66.74% deterrent activity even at a lowest concentration of 10 µl/ml, whereas 40.45±4.8 adults emerged successfully in control. Similarly, highest antifeedant activity was revealed for alpha pinene followed by linalool while both the essential oils showed less FDI against the given pest. Responses varied with respect to doses of compounds and exposure time. Further, alpha pinene showed higher toxicity than linalool and it may be attributed to its chemical structure.

Keywords: *Fumigant toxicity, Essential oil, Repellency, Antifeedant activity, Insect pests*

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Introduction

Stored-product insects are serious pests of dried, stored, durable agricultural supplies and of many value-added food products and nonfood derivatives. According to FAO, approximately 10% world - wide loss of all stored grains occurs in store, i.e., 13 million tons of grain lost due to insects and 100 million tons due to improper storage [1]. The most economically important post-harvest insect pests belong to two major groups; Coleoptera (beetles) and Lepidoptera (moths and butterflies). Several lepidopteran larvae through silky secretion entangle the feeding media which turns products into entwined lumps. Indian meal moth, *Plodia interpunctella* (Lepidoptera: Pyralidae), is a serious pest of stored products like grains, seeds, flour and other milled products and has a universal distribution [2]. The webbing formed is noticeably dense and tough, adding to the damage caused [3, 4]. Chemical pesticides are considered as the most common and cost effective method of protecting grains and controlling insect infestations in field and storage conditions. Although synthetic pesticides have an immense contribution in increasing the agricultural productivity and food supply but indiscriminate use of synthetic insecticides such as methyl bromide and phosphine have many negative effects causing severe environmental problems like ozone depletion, environmental pollution, toxicity to non-target organisms and pesticide residues due to their high toxicity and non-biodegradable properties [5,6]. Recently, there has been growing interest in research concerning the possible use of plant extracts as alternatives to synthetic insecticides. Plant essential oils (EOs) are among the best known substances tested against insects. These compounds may act as fumigants, contact insecticides, repellents, and antifeedants and may affect some biological parameters such as growth rate, life span and reproduction [7-9]. The present study was undertaken to investigate the effect of essential oils of *Artemisia maritima* L. and *Zanthoxylum armatum* DC. and their prime components alpha pinene and linalool, towards *Plodia interpunctella* (Hubner) adults for their toxicity, repellent activity, progeny deterrency and antifeedant activity.

Materials and Methods

Extraction of essential oils

Essential oils were extracted from leaves of *A. maritima* and *Z. armatum* collected from the local areas of Shimla district of Himachal Pradesh, India. The leaves were dried in shade at room temperature (30±5°C) and grounded by domestic mixer. The dried powdered material was hydro-distilled in Clevenger apparatus. Conditions of extraction were: 50 g of air-dried sample in 1:10 plant material/water volume ratio for 4 hrs distillation. Oil yield (2.9% w/w) was calculated on a dry weight basis.

Test insects

Laboratory cultures of *P. interpunctella* were maintained at 28±2°C and 68±2% relative humidity. *P. interpunctella* was reared on a diet of 80% ground rice, 10% glycerin, 5% yeast in plastic containers. Mouth of the containers was covered with fine mesh cloth for ventilation as well as to prevent escape of the insects.

Analysis of plant essential oils

Mass spectra of different essential oils extracted from plants were recorded on Bruker micrOTOF Q II Mass spectrometer. The prime and most bioactive components of essential oils especially different monoterpenes were identified by mass spectroscopy and by comparison of its mass spectra, either with known compounds or published spectra.

The following monoterpenes were used for different bioassays: linalool and alpha pinene and provided by Sigma Aldrich, India.

Fumigant toxicity of essential oils and monoterpenes against *P. interpunctella*

Vapour toxicity of essential oil and its constituent two monoterpenes against the adult insects were determined via impregnated paper assay following the method of [10] with some modifications. Different doses of 50, 100, 300 and 500 µl of essential oils and 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 0.2, 0.4, 1.2 and 2 µl/ml respectively, with respect to volume of the jars. After allowing the solvent to evaporate for 10 - 15 minutes, the filter paper was attached to the inner surface of the screw lid of the jar. At the bottom of each jar, 10 individuals of adult insect (5 - 10 day old) along with their food source were placed and exposed to the various concentrations. Insect mortalities were determined and calculated after different exposure periods to the day of complete mortality of all insects according to the formula of Abott [11].

Repellent activity of essential oils and monoterpenes against *P. interpunctella*

Repellency tests were carried out according to the experimental method described by [12]. with certain modifications. A glass Y- shaped olfactometer with a common arm, and two arms A and B, each of equal length was used in the assay. A cotton swab (1 cm diameter) was soaked separately in test solutions prepared by dissolving 100, 300 and 500 µl of plant essential oil and monoterpenes in 1 ml methanol kept in the experimental arm and cotton swab soaked in 1 ml of methanol was kept in the control arm. Twenty adult moths (5-10 day old) were introduced separately into the base of the Y-arm of olfactometer. Observations on the number of insects present in the experimental and control arm were recorded after 1, 3, 5 and 24 hrs and Percentage repellency (PR) was calculated as follows:

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

Progeny deterrency by essential oils and monoterpenes against *P. interpunctella*

A stock solution was prepared by dissolving 100 µl of essential oils and monoterpenes in 1 ml of methanol and test solutions of 10, 30 50 and 100 µl of each plant oil and monoterpene were used for bioassay. 5 g of food media for each insect species were filled in glass vials and treated separately with different test solutions. For control sets the seeds were dressed in requisite amount of methanol. The treated seeds were placed on filter paper to evaporate the solvent for 15-20 minutes. The seeds were then transferred into Petridishes and 6 pairs of insect of mixed sex were introduced in each vial separately. The mortality of insects was observed at different time intervals and dead insects were removed. Observations were made for progeny emergence. The % progeny deterrence was calculated according to the equation:

$$\%\, Deterrency = \frac{Nc - Nt}{Nc} \times 100$$

where NC is the number of adults emerged from control sand NT is the number of adults emerged from treated food media

Antifeedant activity of essential oils and monoterpenes against *P. interpunctella*

To determine antifeedant activity of essential oils and monoterpenes a no-choice test was carried out as described by [14] and [15] with some modifications. 1 ml of prepared concentrations of 100 and 300 µl of all essential oils and 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. 10 larvae (16 day old) of *P. interpunctella* were transferred to each pre-weighed food media in Petri dishes. After feeding for 72 hrs under laboratory conditions food media was re-weighed and mortality of insects was recorded. Nutritional indices and weight loss were calculated as previously described by [16].

Weight loss (%WL) = (IW - FW) × 100/ IW

Where the IW is the initial weight and FW is the final weight.

Feeding Deterrence Index was calculated by [17,18] 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

All the data concerning mortality were corrected by using Abbott's formula. Bioassays 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

Analysis of essential oils

Essential oils extracted from two plants were analysed by mass spectroscopy to elucidate the prime components especially monoterpenes of the plant oils. The mass spectrum of essential oil from *A. maritima* and *Z. armatum* and their different major components have been summarized.

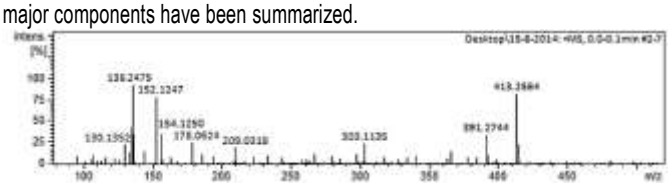

Fig-1 Mass spectrum of *A. maritima* oil

Table-1 Chemical constituents of the essential oil of *A. maritima* and parent ion peak (m/Z) values of components

Components	m/Z	
	Calculated	Observed
Alpha-pinene	136.23	136.24
Myrcene	154.24	154.12
Linalool	154.18	154.20
Camphene	136.23	136.24
Sabinene	136.19	136.24
Camphor	152.09	152.12
Methyl eugenol	178.10	178.06
1-8 cineole	154.21	154.12
Borneol	154.19	154.12
3- octanol	130.09	130.13
Thujene	136.21	136.24

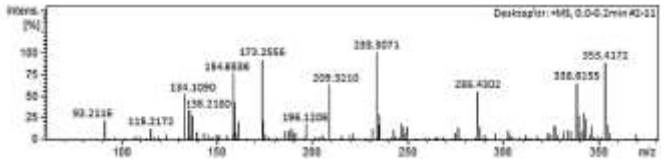

Fig-2 Mass spectrum of *Z. armatum* oil

Table-2 Chemical constituents of the essential oil of *Z. armatum* and parent ion peak (m/Z) values of components.

Components	m/Z	
	Calculated	Observed
Alpha-pinene	136.23	136.20
Myrcene	154.24	154.20
Linalool	154.23	154.20
Camphene	136.24	136.21
Sabinene	136.23	136.21
Limonene	136. 21	136.21
Geraniol	154.25	154.65
Geraniol acetate	196.15	196.12
Para-cymene	134.08	134.10
Nerol acetate	209.30	209.32

Fumigant toxicity of essential oils and monoterpenes against *P. interpunctella*

For *P. interpunctella* 100% mortality was achieved by alpha pinene, linalool, *A. maritima* and *Z. armatum* oils at all concentrations within 120 hrs. Alpha pinene at a concentration of 0.2 µl/ml gave 35.43±0.33% mortality after 24 hrs that reached

Table-3 Fumigant toxicity of essential oils and monoterpenes against *P. interpunctella*.

Doses µl/ml		% Mortality ± SE				
		24 hrs	72 hrs	96 hrs	120 hrs	168 hrs
Z. armatum	0.2	25.54±0.45^{b}	64.55±0.28^{a}	70.43±0.63^{c}	-	-
	0.4	28.43±0.55^{c}	68.23±0.40^{c}	75.55±0.39^{c}	-	-
	1.2	30.58±0.33^{c}	70.63±0.33^{c}	83.32±0.27^{d}	-	-
	2	32.62±0.20^{c}	73.54±0.55^{c}	85.66±0.39^{d}	-	-
A. maritima	0.2	28.43±0.42^{c}	67.43±0.20^{c}	73.64±0.65^{c}	-	-
	0.4	30.54±0.28^{c}	70.56±0.54^{c}	82.43±0.42^{d}	-	-
	1.2	30.32±0.39^{c}	73.53±0.33^{c}	85.32±1.12^{d}	-	-
	2	33.43±0.19^{c}	78.21±0.40^{d}	88.64±0.74^{bc}	-	-
Linalool	0.2	30.63±0.54^{c}	70.23±0.42^{c}	80.24±0.65^{d}	-	-
	0.4	30.63±0.54^{c}	72.66±0.56^{c}	85.43±0.72^{d}	-	-
	1.2	32.52±0.20^{c}	75.33±0.33^{c}	88.50±0.43^{bc}	-	-
	2	35.63±0.71^{d}	80.66±0.81^{d}	90.00±0.00^{bc}	-	-
Alpha pinene	0.2	35.43±0.33^{d}	73.34±0.44^{c}	85.36±0.72^{d}	-	-
	0.4	35.65±0.65^{d}	75.56±0.29^{c}	88.54±0.40^{d}	-	-
	1.2	38.87±0.53^{d}	78.54±0.21^{d}	90.00±0.00^{d}	-	-
	2	40.32±0.40^{bc}	82.43±1.12^{d}	92.00±0.00^{d}	-	-
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-4 Percentage repellency of essential oils and monoterpenes against *P. interpunctella* at different time intervals (Values are mean ± SE)

	Time (hrs)	Doses µl/cm^2		
		2 µl/cm^2	6 µl/cm^2	10 µl/cm^2
Alpha pinene	1	65.50±1.2^{a}	75.42±3.1^{b}	80.18±1.9^{a}
	3	70.18±3.2^{a}	87.51±1.8^{a}	88.36±1.4^{b}
	5	78.45±4.1^{b}	89.12±1.2^{a}	93.15±4.1^{b}
	24	58.60±1.4^{c}	64.40±1.6^{c}	70.38±2.2^{c}
Linalool	1	48.45±1.9^{d}	64.13±4.6^{c}	70.48±2.5^{c}
	3	58.52±3.2^{c}	76.24±1.1^{b}	80.06±1.8^{a}
	5	67.68±2.1^{a}	80.46±1.8^{d}	85.46±3.1^{b}
	24	33.54±3.6^{ab}	42.60±1.2^{ab}	59.59±1.4^{d}
Z. armatum	1	50.14±4.2^{d}	60.56±3.5^{bc}	68.50±3.6^{c}
	3	55.30±1.8^{c}	66.42±2.8^{c}	75.45±1.9^{c}
	5	67.22±2.4^{a}	72.26±1.4^{b}	80.28±2.4^{a}
	24	26.45±1.6^{ab}	35.56±1.6^{ab}	56.35±4.2^{d}
A. maritima	1	46.32±2.2^{d}	60.46±2.5^{bc}	68.37±1.5^{c}
	3	55.64±1.2^{c}	72.35±1.6^{b}	77.23±2.8^{ab}
	5	66.51±2.1^{a}	78.26±1.8^{b}	81.40±3.6^{a}
	24	30.40±1.6^{ab}	40.47±1.2^{ab}	56.46±1.2^{d}

% 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.

85.36±0.72% at an increased exposure of 96 hrs. At 0.4 µl/ml of Alpha pinene 75.56±0.29 and 88.54±0.40% mortality was produced after an interval of 72 and 96 hrs followed by linalool resulting in 72.66±0.56 and 85.43±0.72% mortality at similar concentration and time intervals respectively. *A. maritima* oil at 1.2 µl/ml caused 73.53±0.33% mortality followed by 78.21±0.40% at an increased concentration of 2 µl/ml after 72 hrs while 83.32±0.27 and 85.66±0.39% mortality was achieved by *Z. armatum* oil at a dose of 1.2 and 2 µl/ml after 96 hrs of exposure [Table-3].

Repellent activity of essential oils and monoterpenes against *P. interpunctella*

At 10µl/cm^2 alpha pinene showed the highest repellent activity of 80.18±1.9, 88.36±1.4, 93.15±4.1% after 1, 3 and 5 hrs respectively. Linalool and *Z. armatum* also showed remarkable activity at 6 µl/cm^2 against *P. interpunctella* with 76.24±1.1 and 66.42±2.8% repellence after 3 hrs followed by 80.46±1.8 and 72.26±1.4% after an interval of 5 hrs while repellent activity decreased to 42.60±1.2 and 35.56±1.6% after an increased exposure of 24 hrs respectively. At 2 µl/cm^2 *A. maritima* oil produced 66.51±2.1% repellence followed by 78.26±1.8% repellent activity at an increased concentration of 6µl/cm^2 after 5 hrs of time interval [Table-4].

Progeny deterrence of essential oils and monoterpenes against *P. interpunctella*

Alpha pinene and linalool were more potent than other producing 10.25±1.2 and 13.45±2.2 F1 progeny of *P. interpunctella* with 74.66 and 66.74% deterrent activity even at a lowest concentration of 10 µl/ml, whereas 40.45±4.8 adults emerged successfully in control. Similarly, *A. maritima* and *Z. armatum* oil also showed good activity against the insect pest. At 100 µl/ml *A. maritima* and *Z. armatum* oil resulted in 8.18±1.9 and 10.30±1.1 emergence of adults obtaining 79.77 and 74.53% progeny deterrence [Table-5].

Antifeedent activity of essential oils and monoterpenes against *P. interpunctella*

Alpha pinene at lowest concentration of 100µl/g showed 59.30±0.18% feeding deterrency with 21.32±0.13% grain damage followed by linalool producing 45.93±0.24 feeding deterrency with 23.16±0.17% grain damage as compared to

78.26±0.34% grain damage in control against *P. interpunctella*. Similarly, *Z. armatum* and *A. maritima* oil obtained 41.71±0.09 and 36.70±0.30% FDI respectively with 24.31±0.21 and 27.16±0.14% grain damage at 100 µl/g and 51.85±0.28 and 46.51±0.17% FDI and 21.27±0.35 and 22.09±0.25% grain damage was recorded at 300µl/g [Table-6].

Table-5 F1 progeny deterrence of *P. interpunctella* under variable doses of essential oils and monoterpenes

Essential oils	Doses µl/ml	Progeny deterrence (%)
Alpha pinene	10	74.66(10.25±1.2)^{b}
	30	78.86(8.55±2.3)^{b}
	50	79.55(8.27±4.1)^{b}
	100	87.09(5.22±1.8)^{a}
Linalool	10	66.74(13.45±2.2)^{c}
	30	76.51(9.50±3.4)^{b}
	50	77.33(9.17±4.8)^{b}
	100	82.15(7.22±1.6)^{d}
A. maritima	10	61.53(15.56±1.8)^{bc}
	30	74.21(10.43±3.1)^{b}
	50	74.85(10.17±2.2)^{b}
	100	79.77(8.18±1.9)^{b}
Z. armatum	10	55.12(18.15±2.4)^{cd}
	30	69.34(12.40±4.1)^{c}
	50	74.46(10.33±2.2)^{b}
	100	74.53(10.30±1.1)^{b}
Control		40.45±4.8)^{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-6 Antifeedent activity of essential oils and monoterpenes against *P. interpunctella* (Values are mean± SE).

Essential oils	Doses µl/g	Grain damage (%)	Weight loss (%)	FDI (%)
Alpha pinene	100	21.32±0.13^{a}	11.32±0.11^{b}	59.30±0.18^{a}
	300	18.42±0.09^{b}	9.41±0.21^{a}	64.97±0.14^{a}
Linalool	100	23.16±0.17^{c}	16.41±0.17^{c}	45.95±0.24^{c}
	300	20.42±0.34^{a}	13.48±0.32^{b}	53.35±0.18^{d}
Z. armatum	100	24.31±0.21^{c}	18.23±0.24^{c}	41.71±0.09^{c}
	300	21.27±0.35^{a}	14.05±0.16^{b}	51.85±0.28^{d}
A. maritima	100	27.16±0.14^{d}	20.52±0.21^{c}	36.70±0.30^{bc}
	300	22.09±0.25^{a}	16.18±0.09^{c}	46.51±0.17^{c}
Control		78.26±0.34^{ab}	44.32±0.18^{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.

Discussion

The results of fumigation tests showed considerable difference in mortality of insect pest to essential oils and monoterpenes with different concentrations and times. A number of reports are available which indicate that EO activity is the result of their inherent biologically active components and their different modes of action, either synergistic or antagonistic [19].

[20] tested eight essential oils and among them *R. rugosa* had the highest toxicity followed by *A. maritima, C. zeylanicum, Z. armatum, T. occidentalis, P. hortorum, C. oppositifolia* and *C. hystrix* against larvae of *T. castaneum and P. interpunctella*. At 0.4 µl/ml of alpha pinene 75.56±0.29 and 88.54±0.40% mortality was produced after an interval of 72 and 96 hrs followed by linalool resulting in 72.66±0.56 and 85.43±0.72% mortality at similar concentration and time intervals respectively while *A. maritima* oil was more effective among the oils and at 1.2 µl/ml caused 73.53±0.33% mortality followed by 78.21±0.40% at an increased concentration of 2 µl/ml after 72 hrs. The major components of Piper nigrum fruit extracts such as piperine, caryophyllene and limonene are reported as having insecticidal properties. Many insecticidal components of plant extracts are mainly monoterpenes, such as limonene which have been shown to be toxic to *Tribolium castaneum* [21,22]. The present study showed that 10µl/cm^2 alpha pinene showed the highest repellent activity of 80.18±1.9, 88.36±1.4, 93.15±4.1% after 1, 3 and 5 hrs respectively. Linalool and *Z. armatum* also showed remarkable activity at 6µl/cm^2 against *P. interpunctella* with 76.24±1.1 and 66.42±2.8% repellence after 3 hrs. Similar observations on the effect of plant extracts on several insect pests have been reported; essential oils of *Schyzygium aromaticum, Aegle marmelos, Corriandrum sativum* and *Citrus reticulata* showed strong repellency against *S. oryzae* and *T. castaneum* even at lowest concentration [23]. also reported that repellent behaviour of *Callosobruchus chinensis* increased with the increase in the doses of essential oils of *Callistemon lanceolatus* and *Lippia alba* or their constituents. Alpha pinene and linalool were more effective towards *P. interpunctella* with 74.66 and 66.74% progeny deterrent activity even at a lowest concentration of 10 µl/ml while 100 µl/ml of *A. maritima* and *Z. armatum* oil resulted in 79.77 and 74.53% progeny deterrence whereas 40.45±4.8 adults emerged successfully in control. The insecticidal and inhibition of progeny emergence activities of oil extracted from seeds of Jatropha curcas has been reported by earlier researchers against several insect pests [24-28]. [29] during their study reported significant reduction in F1 adults of both species of T. castaneum and Sitophilus zeamais that emerged from the media treated with the non-polar extract of dried fruits of star anise, *Illicium verum*. Feeding deterrence indices (FDI) showed that the plant essential oils and monoterpenes had antifeedant action against *P. interpunctella* at different concentrations. Highest FDI of 64.97±0.14 was obtained for alpha pinene at a dose of 300 µl/g followed by linalool with a FDI of 53.35±0.18%. Similarly, *Z. armatum* and *A. maritima* oil obtained 41.71±0.09 and 36.70±0.30% FDI respectively with 24.31±0.21 and 27.16±0.14% grain damage at a concentration of 100µl/g. The finding of the present study is also is in agreement with that of [30]. who reported good protection of cowpea seeds from *C. maculatus* damage in storage due to the use of *J. curcas* seed oil as a repellent and antifeedant. They also reported that doses of 1.0 ml/150 g grains and above gave superior mortality of the pest in cowpea. The result of present investigation are also similar to the observations of Shukla, *et al*., (2011) who reported significant deterrent effects of essential oils of *L. alba* and *C. lanceolatus* and their constituents on the feeding behaviour of *C. chinensis* and all the treatments showed significantly better results than the controls.

Application of research: The present study aimed at the extraction and screening of different plant essential oils and identification of different active components especially terpenes and terpenoids present in essential oils for studying their toxicities against major stored product insect pests.

Research Category: Agriculture Entomology

Abbreviations: %: percentage, Hrs: hours, µl: micro litre, ml: millilitre, cm: centimetre, g: gram, w: weight

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Study area / Sample Collection: Shimla, Bilaspur, Mandi, Hamirpur

Cultivar / Variety / Breed name: Basmati-370

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