Comparative Bio-efficacy and Molecular Insights of North-Western Himalayan conifers, Cedrus deodara and Juniperus macropoda Essential Oils against two storage insect Pests

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Article

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Abstract

The study, aimed to evaluate fumigation and repellent properties of essential oils (EOs) from *Juniperus macropoda* leaves and cedarwood, *Cedrus deodara* against storage pests *viz*., *Tribolium casteneum* and *Callosobruchus maculatus*. Further, the volatile compounds released from essential oils were collected through headspace extraction and analysed using Gas Chromatography–Mass Spectrometry (GC-MS). The major compounds released from *C. deodara* EO were α-Cuprenene (15.53%) and α-Himachalene (13.42%) whereas 4-terpineol (22.35%) and Limonene (14.16%) dominated the volatile composition of *J. macropoda* EO. Fumigation assay showed that *C. deodara* EO was significantly more toxic than *J. macropoda* EO against *T. casteneum* larvae (LC_{50} = 103.91 vs. 357.33 µl/l) and adults (LC_{50} = 123.10 vs. 724.66 µl/l). Similarly, *C. deodara* EO exhibited stronger fumigant activity against *C. maculatus* adult (LC_{50} = 16.13 µl/l) compared to *J. macropoda* EO (LC_{50} = 25.68 µl/l). Repellency assay revealed that *C. deodara* EO significantly repelled *C. maculatus* adults at 50 and 100 ng, whereas *J. macropoda* EO showed no significant effect. Against *T. casteneum* adults, both EOs exhibited significant repellency, except *J. macropoda* at lowest dose (10 ng). *In-silico* analysis revealed that in comparison to components from *J. macropoda*, *C. deodara* components such as *γ*-himachalene, *α*-cuprenene, *α*-himachalene, and *α*-(E)-atlantone often showed stronger and more stable binding affinities, consistent with bioassay results indicating the superior insecticidal activity of *C. deodara* essential oil. Molecular docking also revealed acetylcholinesterase as the primary target, thereby supporting its role in fumigation insecticidal activity of the essential oils.

Introduction

Post-harvest management of food grains and products is one of the major challenges owing to several factors that can aggravate the post-harvest losses. The efforts are being made to reduce these losses by following several tactics based on the prevailing factors causing losses to ensure the food security of the ever-growing global population. Besides increasing food production and productivity, Food and Agricultural Organisation (FAO) suggested an alternative approach for food security by focussing on the postharvest losses of agricultural production, which is approximately, 10% in developed countries and exceed 20.5% in developing countries[1]. There are several biotic and abiotic factors which are responsible for post-harvest losses of grains[2]. Among the biotic factors storage insect pests are one of the critical factors which can cause damage to the food grains in storage either directly or indirectly. The direct damage is mainly due to feeding on grains (either whole or broken) whereas indirect damage pertains to contamination of food grains due to presence of faeces, webbings and body parts[3].

The storage insect pests such as red flour beetle *Tribolium castaneum* (Coleoptera: Tenebrionidae) and pulse beetle *Callosobruchus* spp (Coleoptera: Bruchidae) are most important coleopteron stored grain pests generally found infesting several commodities. Red flour beetle is a cosmopolitan polyphagous pest whose adults and larvae are responsible for severe economic damage in stored products, feeding on several dried foods including flours, fruits and grains[3]. Moreover, *Tribolium* spp. are also known to produce carcinogenic compounds called quinones, which leads to allergies, dermatitis and other health disorders[5]. Pulse beetle *Callosobruchus spp*. is also a cosmopolitan field-to-store pest ranked as important post-harvest insect pest[6]. Approximately, 10–40% of the total annual production of pulses is lost annually due to damage by pulse beetles in tropical countries[7].

Traditionally fumigant like phosphine and few contact insecticides such as malathion, deltamethrin are used for management of storage pests in India. Several efforts are being made to develop alternative approaches to reduce the failure of traditionally used insecticides. The development of plant essential oil (EO) based products for storage pest management is one among these approaches. The plant essentials, basically a mixture of several volatile compounds have proved to possess several properties such as antimicrobial, antioxidant, anti-inflammatory, anticancer, antiparasitic etc. Besides, several plant essential oils are found to possess very promising insecticidal activities[8,9]. Apart for their insecticidal properties, EOs are considered to be important tool in pest management due their broad spectrum of activity and mode of action due to presence of several active compounds[9] making them ideal candidate for resistance management[11].

Himalayan pencil cedar *Juniperus macropoda* (Family: Cupressaceae) and Himalayan cedar, *Cedrus deodara* (Family: Pinaceae) are two woody conifer plants known for their diverse biological activity. There are about 75 species of *Juniperus* found in diverse geographical region right from sea level to above timberline[12]. In Himalayas, especially Northern India there is around six species of genus *Juniperus*, of which *Juniperus communis* is widespread[13]. *J. macropoda* is one of the species which is mainly found in northern western Himalayas and is already characterised for its biological activity[14,15]. *C. deodara* is a species of cedar, native to western Himalayas, widely used in Indian system of medicine due to its nutritional and pharmaceutical effects. It is known to have diverse biological activities such as an anti-inflammatory, anti-hyperglycaemic, analgesic, antiulcer, antispasmodic, antibacterial, insecticidal, molluscicidal, anticancer etc.[16]. To our knowledge there are very few reports on insecticidal potential of EO of *J. macropoda*, although insecticidal properties have been studies for other species such as *Juniperus formosana*[17], *Juniperus oxycedrus* spp *oxycedrus*[18], *Juniperus phoenisea*[19], *Juniperus recurve* and *Juniperus communis*[20] EO of *C. deodara* have been reported to possess insecticidal activity against storage pests such as *Tenebrio molitor*[21], *Callosobruchus chinensis*[22], *Sitosphilus oryzae*[23].

In present study the EOs of these two Himalayan plants were evaluated for their fumigation toxicity and repellent activity against *T. castaneum* and *C. maculatus*.

Materials and Methods

Essential oil

The essential oil of green needle and thin stem of *J. macropoda* (Fig. 1 (a)) and wood chips of Himalayan cedar, *C. deodara* (Fig. 1(b)) extracted through hydro-distillation process were procured from Dharama Ltd., Chamba, Himachal Pradesh, India.

Test insects

The test insects, *T. castaneum* and *C. maculatus*, used for the bioassay were obtained from the Storage Laboratory, Division of Entomology, ICAR-IARI, New Delhi. The mass multiplication of insects was carried out in insect culture room maintained at 28–30 ± 2 ^{o} C temperature and 75 ± 5% relative humidity. *T. castaneum* was reared on wheat flour mixed with yeast (10:1 w/w) at 12% moisture content in a glass jar (covered with muslin cloth) whereas *C. maculatus* were reared on mung bean, *Vigna radiata* seeds. To obtain all individual of same generation, 40–50 adults were released in rearing container and allowed to lay eggs for two days and then removed to obtain uniform age eggs.

Headspace collection of Volatiles of EOs

To know the real time volatiles emitted from essential oils, the dynamic headspace collection methodology[24] was followed (Fig. 2). 500 µl of essential oil was pipetted on the cotton and kept at the base of Borosil glass reagent bottle (1L). Pre-filtered air was pushed into the glass bottle at 0.5 l/min and air laden with volatiles was pulled out with vacuum pump (0.5 l/min) which goes through volatile trap (Porapak Q (150 mg, 80/100 mesh: Supelco). All connections were made with Teflon tape and silicon tubes to avoid any contamination. Collection of volatiles was done for 3 hours for each oil. Elution of the entrained volatiles was done using 300 µl dichloromethane DCM (≥ 99.7% purity; CDH (P) Ltd). The eluted samples were collected in 2ml capacity HPLC (screw-cap) vials (Borosil Glass Works Ltd, Mumbai) and stored at -20°C till further use.

Chromatographic analysis of headspace extracts using GC-MS

The qualitative and quantitative analysis of headspace extracts was carried out by using Shimadzu QP2010 Ultra gas chromatography-mass spectroscopy (GC-MS) equipped with Rtx-5 MS capillary column of 30 m length, 0.250 mm Diameter and 0.25 µm film thickness. 1µl (injection volume) samples were introduced using an autosampler with split ratio of 5:1 with an injector temperature of 230°C. Helium gas served as carrier at a constant flow rate of 1 ml/min. Initially column temperature was 40°C held for 4 minutes and then ramped at 10°C/min to 220°C and held for 1 min and finally increased at 15°C/min to 260 and held for 1 min. The GC column was then calibrated using an n-alkanes series (C_8H_{18}-$C_{21}H_{44}$), and retention indices (RIs) of the components were determined under identical operating conditions. Compound identification was performed by comparing the obtained RIs with published literature values and by matching the mass spectra with those from inbuilt NIST 14 Mass Spectral Library (2023).

Fumigation toxicity

Fumigation activity of EOs was evaluated against larvae and adult of *T. castaneum* and adult of *C maculatus*. Round bottom glass bottles (250 ml volume) with airtight lid (stopcock) were used as fumigation chambers to assay the fumigation toxicity[25]. Based on preliminary dose-range finding bioassay (to determine the dose range giving 20–80% mortality), larvae and adult of *T. castaneum* were exposed to five different concentrations of *J. macropoda* EOs (300 µl/l to 400 µl/l and 500 µl/l to 900 µl/l respectively, for larvae and adult). Similarly, for EO of *C. deodara, T. castaneum* larvae and adult of were exposed five different concentrations ranging between 60 µl/l to 140 µl/l and 40 µl/l to 200 µl/l, respectively. For bioassay against adult of *C maculatus*, the concentrations were ranged between 8 µl/l to 40 µl/l and 4 µl/l to 24 µl/l, respectively, for *J. macropoda* and *C. deodara*. The required quantity of EO in each concentration was measured with micropipette and loaded on the cotton balls, which were hung inside the fumigation bottle from stopcock. A cotton ball without essential oil (EO) treatment was used as the control in the experiment. The fumigations bottles were closed immediately to prevent the escape of insects and EO vapours. For each concentration and the control, 30 individuals of the test insect stages of *T. castaneum* and *C. maculatus* were released separately into bottles for the fumigation bioassay. Each treatment was replicated three times. After 48 hours of exposure, insect mortality was recorded by gently touching each individual with a camel-hair brush to confirm any movement, if present. The moribund insects were considered dead.

Repellent toxicity

Comparative repellent activity of the essential oil against *T. castaneum* and *C. maculatus* was evaluated using Y-tube glass olfactometer[26] with internal diameter of 1 cm and 7 cm long arm and 7 cm source and control arm at 60^{o} from each other. (Fig. 3). Air was introduced into the source and control arms of Y-tube at 500 ml/min using an air pump. All the connections were made from flexible silicon tubing. The repellency was

evaluated at three different dosages viz., 10ng, 50ng, and 100 ng. The required amount of EO aliquot (10µl) was placed on a filter paper strip (2.5cm ×0.5cm) and the strip was introduced in stimulus holding tube connected to source arm. Filter paper strip with acetone (10µl) was used as control and placed in stimulus holding tube connected with control arm. One adult of each insect was introduced at a time in the main arm of Y-tube. After introducing, insect was allowed to make choice for 15 min. If insects moved to neither arm (source and control), it was considered as no choice. After every five introductions of insects, source and control arm was interchanged after washing with acetone. The Y- tube experiment was performed under diffuse ambient light conditions at room temperature of 28–30 ± 2 ^{o} C and 75 ± 5% relative humidity. Three replications were carried out for each dose. In each replication the response was recorded from 30 insect individuals. The percent repellency (PR) of each EO at different doses was then calculated by

PR (%) = [Nc-Nt)/Nc + Nt)]×100

Where Nc is the number of insects choosing the control arm and Nt is the number of insects choosing the source arm.

In silico molecular docking

Based on the chemical profiling and characterisation of EOs, a total of eighteen major compounds *viz*., eleven compounds from *C. deodara* and seven from *J. macropoda* were assessed through *in silico* molecular docking for insecticidal and repellency potential against *T. castaneum* and *C. maculatus*. Acetylcholinesterase (AChE) enzyme, GABA receptors (GABArs), and NADH: ubiquinone oxidoreductase were chosen as targets for fumigation toxicity as literature suggests that these are most potent target for fumigation toxicity of EOs[27–30]. Similarly, odorant receptors (OR) were chosen for validating the repellent action as ORs play a crucial role in insect behaviour[29].

Receptor protein preparation

The amino acid sequences of target proteins, acetylcholinesterase (AChE), GABA receptors (GABArs), and NADH: ubiquinone oxidoreductase for *T. castaneum* and *C. maculatus* were retrieved from the UniProt database (www.uniprot.org) using their respective UniProt Ids (Table 6). The amino acid sequences of species-specific OR proteins were also retrieved from the same database in order to evaluate repellent activity. Further, three-dimensional protein structures were generated through homology modelling using SWISS-MODEL server (swissmodel.expasy.org). The resulting structural models were validated for stereochemical quality and structural reliability before proceeding to molecular docking studies.

The modelled protein structure underwent systematic preparation for molecular docking analysis. In this preparation, polar hydrogen atoms were added to accurately reflect electrostatic interactions, water molecules were removed to avoid potential interference, and Kollman charges were assigned to each atom to account for partial atomic charges. The prepared protein structure was then further converted and saved in PDBQT format, which is compatible with AutoDock Vina software generated by Trott and Olson (2010)[31].

Ligand preparation

The three-dimensional molecular structures of major essential components from each EO were retrieved from PUBCHEM database (pubchem.ncbi.nlm.nig.gov.) in SDF format. These ligand structures were processed through energy minimization and conformational optimization procedures by using Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, San Diego, CA, USA; version 21.1). The optimized ligand structure was subsequently converted to PDBQT format using AutoDockTools (ADT) to ensure compatibility with molecular docking software.

Molecular docking

To predict the binding affinities and interaction conformations between essential oil (EO) components and target proteins, molecular docking simulation was conducted using AutoDock Vina[31]. After the conversion of both proteins and possible ligands in PDBQT format, a grid box was established in AutoDock tool which guarantee comprehensive coverage of target protein, facilitating the potential binding sites and interaction modes for ligands by methodically analysing the entire surface of the protein.

Docking process was guided by a configuration file (config.txt) which specified key parameters such as the grid box centre coordinates, dimensions, exhaustiveness, number of binding models for each protein. For every protein-ligand complex, binding affinity scores were determined, and the most favourable conformations were identified based on lowest binding energy values.

The one essential oil component (ligand) from each EO showing highest -scoring complex with any of the three target enzymes (fumigation toxicity) and OR proteins of each test insect species was further visualized using Discovery Studio software (BIOVIA, Dassault Systemes, San Diego, CA, USA; version 21.1). This investigation aimed to pinpoint key amino acid residues involved in ligand binding and to characterize hydrogen bonds, hydrophobic interactions, and other non-covalent forces that contribute to the stability of the complexes.

Statistical analysis

The data on insect mortality in fumigation bioassay was recorded after 48 h of exposure. The probit analysis of mortality data was done to determine lethal concentration (LC_{50}, LC_{95}) using PoloPlus software, version 2.0 (LeOra Software, 2002). Further, LC_{50} values of both the oils were subjected to paired *t*-test analysis to determine significant difference using SPSS Statistics for Windows, version 20.0 (IBM Corp., Armonk, NY, USA). Chi-square (χ^2) test was employed to analyse repellency data, and statistical significance was determined at $p < 0.05$ (SPSS version 20.0, IBM corp., Armonk, NY, USA). Further, repellency percentage was calculated based on the proportion of insects moving toward the source and control. Mean repellency values were subjected to **two-way ANOVA** to assess the effects of essential oil type and concentration, followed by **Tukey's HSD test** for mean separation at $p < 0.05$.

Results

Phytochemical characterisation of EOs

The GCMS analysis of *J. macropoda* revealed the presence of 34 compounds that constituted 96.52 ± 0.14% of the total composition (Table 1). The oil was predominantly composed of monoterpenes (59.00 ± 0.223%) and monoterpenoids (25.04 ± 0.340%). Among the identified volatiles, the major compounds were 4-terpineol (22.35 ± 0.085%), limonene (14.16 ± 0.174%), and *γ*-terpinolene (11.53 ± 0.02%) (Fig. 4). Other significant monoterpenes included *β*-thujene (7.32 ± 0.078%), 4-carene (7.17 ± 0.03%), *α*-pinene (5.70 ± 0.039%), and *β*-myrcene (5.32 ± 0.057%). Additionally, sesquiterpenes and sesquiterpenoids were present in relatively lower concentrations at 5.59 ± 0.028% and 0.82 ± 0.021%, respectively. Notable sesquiterpenes included *δ*-cadinene (2.79 ± 0.006%) and *γ*-cadinene (0.93 ± 0.004%), while oxygenated sesquiterpene derivatives were represented by elemol (0.21 ± 0.003%) and *α*-cadinol (0.61 ± 0.016%). Other constituents, including esters and non-terpenoids, comprised 9.35 ± 0.083% of the total composition.

Table 1
Volatile composition of Himalayan pencil cedar, *Juniperus macropoda* essential oil

S. No.	Compound	Mol. formula	RI	Relative area % ± SE
1.	*α*-Thujene [c]	$C_{10}H_{16}$	928	1.43 ± 0.006
2.	*α*-Pinene [c]	$C_{10}H_{16}$	932	5.7 ± 0.039
3.	*β*-Thujene [c]	$C_{10}H_{16}$	968	7.32 ± 0.078
4.	*β*-Pinene [c]	$C_{10}H_{16}$	984	0.15 ± 0.003
5.	*β*-myrcene [c]	$C_{10}H_{16}$	993	5.32 ± 0.057
6.	*α*-phellandrene [c]	$C_{10}H_{16}$	1007	1.14 ± 0.019
7.	4-Carene [c]	$C_{10}H_{16}$	1009	7.17 ± 0.03
8.	3-Carene [c]	$C_{10}H_{16}$	1012	5.08 ± 0.139
9.	*m*-Cymene [e]	$C_{10}H_{14}$	1023	4.88 ± 0.091
10.	Limonene [c]	$C_{10}H_{16}$	1028	14.16 ± 0.174
11.	*γ*-Terpinolene [c]	$C_{10}H_{16}$	1059	11.53 ± 0.020
12.	Linalool [d]	$C_{10}H_{18}O$	1103	0.68 ± 0.009
13.	Solusterol [d]	$C_{10}H_{20}O_2$	1109	0.14 ± 0.002
14.	*cis-p*-Menth-2-ene-1-ol [d]	$C_{10}H_{18}O$	1127	0.15 ± 0.001
15.	*trans*-2-Menthenol [d]	$C_{10}H_{18}O$	1146	0.25 ± 0.001
16.	4-terpineol [d]	$C_{10}H_{18}O$	1180	22.35 ± 0.085
17.	*α*-Terpineol [d]	$C_{10}H_{18}O$	1193	1.47 ± 0.002
18.	Hexyl isovalerate [e]	$C_{11}H_{22}O_2$	1243	0.24 ± 0.004
19.	2-isopropyl-4-methylanisole [e]	$C_{11}H_{16}O$	1244	0.15 ± 0.002
20.	*(S)-(-)*-Citronellic acid, methyl ester [e]	$C_{11}H_{20}O_2$	-	0.15 ± 0.003
21.	2-Camphanyl acetate [e]	$C_{12}H_{20}O_2$	-	0.18 ± 0.004
22.	Nonyl acetate [e]	$C_{11}H_{22}O_2$	1313	0.47 ± 0.009
23.	*α*-Copaene [a]	$C_{15}H_{24}$	1377	0.14 ± 0.001
24.	*β*-Elemene [a]	$C_{15}H_{24}$	1385	0.17 ± 0.001
25.	*α*-Cedrene [a]	$C_{15}H_{24}$	1409	0.26 ± 0. 004
26.	*β*-Caryophyllene [a]	$C_{15}H_{24}$	1421	0.34 ± 0.006
27.	Cadina-1(6),4-diene [a]	$C_{15}H_{24}$	-	0.24 ± 0.005
28.	*α*-Amorphene [a]	$C_{15}H_{24}$	1482	0.20 ± 0.004
29.	*γ*-Muurolene [a]	$C_{15}H_{24}$	1491	0.38 ± 0.003
30.	Cadina-3,5-diene [a]	$C_{15}H_{24}$	-	0.14 ± 0.405
31.	*γ*-Cadinene [a]	$C_{15}H_{24}$	1514	0.93 ± 0.004
32.	*δ*-Cadinene [a]	$C_{15}H_{24}$	1528	2.79 ± 0.006

Data in the table are mean peak area (± SE) of each compound from three replicates. RI, retention index; SE, standard error.

S. No.	Compound	Mol. formula	RI	Relative area % ± SE
33.	Elemol [b]	$C_{15}H_{26}O$	1557	0.21 ± 0.003
34.	*α*-Cadinol [b]	$C_{15}H_{26}O$	1663	0.61 ± 0.016
Total				96.52 ± 0.137
Compound class				
[a] Sesquiterpenes				5.59 ± 0.028
[b] Sesquiterpenoids				0.82 ± 0.021
[c] Monoterpenes				59.00 ± 0.223
[d] Monoterpenoids				25.04 ± 0.340
[e] Others				6.07 ± 0.083
Data in the table are mean peak area (± SE) of each compound from three replicates. RI, retention index; SE, standard error.				

The volatile composition of *C. deodara* oil comprised 49 compounds, accounting for 92.14 ± 0.024% of the total content (Table 2). The oil was predominantly enriched with sesquiterpenes (41.92 ± 0.051%) and their oxygenated derivatives (sesquiterpenoids) (4.96 ± 0.006%). The major constituents were *α*-cuprenene (15.53 ± 0.304%), *α*-himachalene (13.42 ± 0.051%) and *γ*-himachalene (7.39 ± 0.006%) (Fig. 5). Other sesquiterpenes included various isomers and derivatives of himachalene and atlantone. The monoterpene fraction was relatively lower (12.36 ± 0.041%) compared to *J. macropoda* and consisted primarily 4-acetyl-1-methylcyclohexene (3.80 ± 0.068%) and limonene (2.96 ± 0.018%), with trace amounts of *p*-cymene-7-ol (0.38 ± 0.003%), 1,5,8-menthatriene (0.33 ± 0.006%), *α*-pinene (0.33 ± 0.005%), *β*-myrcene (0.17 ± 0.001%), and *β*-pinene (0.12 ± 0.002%). Besides, aromatic hydrocarbons constituted a substantial proportion of *C. deodara* EO (22.77 ± 0.016%), with 1-ethyl-3,5-dimethylbenzene (10.93 ± 0.06%) being the major component. Other notable aromatic compounds included 2-ethyltoluene (4.05 ± 0.005%), *m*-cymene (3.94 ± 0.065%), 3,5-diphenyl-1-pentene (2.90 ± 0.005%), mesitylene (2.52 ± 0.033%), pseudocumene (1.69 ± 0.025%), and several minor constituents. The remaining compounds (11.11 ± 0.019%) further contributed to the complex volatile profile of this species.

Table 2
Volatile compound emitted from Himalayan cedar, *Cedrus deodara* essential oil

S. No.	Compound	Mol. formula	RI	Relative area % ± SE
1.	Tropilidene [e]	$C_7H_6O_2$	-	0.23 ± 0.004
2.	4-Methyl-3-pentene-2-one	$C_6H_{10}O$	802	0.29 ± 0.002
3.	Furfural [e]	$C_5H_4O_2$	839	0.36 ± 0.002
4.	*α*-Furfuryl alcohol [e]	$C_5H_6O_2$	882	0.46 ± 0.008
5.	*o*-Xylene [c]	C_8H_{10}	894	1.78 ± 0.008
6.	*α*-pinene [d]	$C_{10}H_{16}$	932	0.33 ± 0.005
7.	6-Methyl-2-heptanone [e]	$C_8H_{16}O$	956	0.45 ± 0.004
8.	2-Ethyltoluene [c]	C_9H_{12}	975	4.05 ± 0.005
9.	4-Piperidinecarbonitrile [e]	$C_6H_{10}N_2$	-	1.56 ± 0.031
10.	*t*-Butyl-cumyl-peroxide [e]	$C_{13}H_{20}O_2$	-	0.94 ± 0.007
11.	*β*-pinene [d]	$C_{10}H_{16}$	984	0.12 ± 0.002
12.	Pseudocumene [c]	C_9H_{12}	991	1.69 ± 0.025
13.	*β*-myrcene [d]	$C_{10}H_{16}$	993	0.17 ± 0.001
14.	Mesitylene [c]	C_9H_{12}	995	2.52 ± 0.033
15.	3,5-Diphenyl-1-pentene [c]	$C_{17}H_{18}$	-	2.90 ± 0.005
16.	*m*-Cymene [c]	$C_{10}H_{14}$	1023	3.94 ± 0.065
17.	Limonene [d]	$C_{10}H_{16}$	1027	2.96 ± 0.018
18.	1-Ethyl-3,5-dimethylbenzene [c]	$C_{10}H_{14}$	1059	10.93 ± 0.06
19.	*α*-Chlorindane [e]	C_9H_9Cl	-	0.54 ± 0.011
20.	Isopropyl-N-p-hydroxy-phenylcarbamate [e]	$C_{10}H_{13}NO_3$	-	0.37 ± 0.005
21.	2-Propyltoluene [c]	$C_{10}H_{14}$	1063	0.44 ± 0.007
22.	*m*-Cymenene [c]	$C_{10}H_{12}$	1084	0.33 ± 0.002
23.	1-Ethyl-2,3-dimethylbenzene [c]	$C_{10}H_{14}$	1104	0.44 ± 0.009
24.	2,6-Dimethylstyrene [c]	$C_{10}H_{12}$	-	0.32 ± 0.004
25.	1,5,8-Menthatriene [c]	$C_{10}H_{14}$	1108	0.33 ± 0.006
26.	4-Acetyl-1-methylcyclohexene	$C_9H_{14}O$	1137	3.80 ± 0.068
27.	Napthalene [c]	$C_{10}H_8$	1181	0.34 ± 0.007
28.	*p*-Creosol [e]	$C_8H_{10}O_2$	1192	1.45 ± 0.003
29.	*p*-cymene-7-ol [e]	$C_{10}H_{14}O$	1288	0.38 ± 0.003
30.	Verdyl acetate [e]	$C_{12}H_{16}O_2$	-	0.54 ± 0.008
31.	*β*-Methylnaphthalene [c]	$C_{11}H_{10}$	1312	0.26 ± 0.002

Data in the table are mean peak area (± SE) of each compound from three replicates. RI, retention index; SE, standard error. RI, retention index; SE, standard error.

S. No.	Compound	Mol. formula	RI	Relative area % ± SE
32.	Nonanol acetate [e]	$C_{11}H_{22}O_2$	1313	1.02 ± 0.003
33.	*α*-Longipinene[a]	$C_{15}H_{24}$	1350	0.13 ± 0.002
34.	Himachala-2,4-diene[a]	$C_{15}H_{24}$	1429	0.44 ± 0.004
35.	*β*-Gurjenene[a]	$C_{15}H_{24}$	1432	0.17 ± 0.003
36.	*α*-Longifolene[a]	$C_{15}H_{24}$	1441	1.00 ± 0.016
37.	*α*-Himachalene[a]	$C_{15}H_{24}$	1447	13.42 ± 0.051
38.	*γ*-himachalene[a]	$C_{15}H_{24}$	1483	7.39 ± 0.006
39.	Himachalene-1,4-diene[a]	$C_{15}H_{24}$	1491	1.29 ± 0.009
40.	Valencene[a]	$C_{15}H_{24}$	1499	0.27 ± 0.001
41.	Cuparene [ac]	$C_{15}H_{22}$	1502	0.23 ± 0.001
42.	*α*-Cuprenene[a]	$C_{15}H_{24}$	1512	15.53 ± 0.304
43.	*β*-Cadinene[a]	$C_{15}H_{24}$	1515	0.39 ± 0.003
44.	*α*-Bisabolene[a]	$C_{15}H_{24}$	1547	0.67 ± 0.005
45.	Himachalol[b]	$C_{15}H_{26}O$	1656	0.88 ± 0.001
46.	*γ*-(Z)-Atlantone[b]	$C_{15}H_{22}O$	1699	0.75 ± 0.005
47.	*γ*-(E)-Atlantone[b]	$C_{15}H_{22}O$	1711	0.83 ± 0.002
48.	*α*-(Z)-Atlantone[b]	$C_{15}H_{22}O$	1722	0.45 ± 0.008
49.	*α*-(E)-Atlantone[b]	$C_{15}H_{22}O$	1785	2.05 ± 0.026
Total				92.14 ± 0.024
Compound class				
[a] Sesquiterpenes				41.92 ± 0.051
[b] Sesquiterpenoids				4.96 ± 0.006
[c] Aromatic hydrocarbons				22.77 ± 0.016
[d] Monoterpenes				12.36 ± 0.041
[e] Others				11.11 ± 0.019
Data in the table are mean peak area (± SE) of each compound from three replicates. RI, retention index; SE, standard error. RI, retention index; SE, standard error.				

Fumigation toxicity

Contact toxicity of both essential oil against test insect stages showed dose dependent-response at 48 h (Fig. S1). Significantly varied mortality was observed at different concentrations of *J. macropoda* EO against larval *T. castaneum* ($F = 32.591$; $p < 001$), adult *T. castaneum* ($F = 45.5$; $p < 0.001$), and *C. maculatus* adult ($F = 51.786$; $p < 0.001$). Similarly, significantly different mortality was observed at different concentrations of *C. deodara* EO against larval *T. castaneum* ($F = 40.833$; $p < 0.001$), adult *T. castaneum* ($F = 21.156$; $p < 0.001$), and adult *C. maculatus* ($F = 24.667$; $p < 0.001$).

The larval stage of red flour beetle *T. castaneum* was found most susceptible to *C. deodara* EO with lethal concentration LC_{50} and LC_{95} value of 103.906 µl/L and 193.514 µl/L, respectively (Table 3). Comparatively toxicity of *J. macropoda* EO was lower with LC_{50} and LC_{95} value of 357.332 µl/L and 433.517 µl/L, respectively. Similarly, the adult beetles *T. castaneum* were most susceptible to *C. deodara* EO (LC_{50} = 123.097 µl/L; LC_{95} = 379.741 µl/L) compared to *J. macropoda* EO (LC_{50} = 724.656 µl/L; LC_{95} = 1106.472 µl/L) (Table 4). The paired *t*-test analysis also revealed the significant higher LC_{50} of *C. deodara* against larvae ($t_{(4)} = 94.073$; $p < 0.001$) (Table 3) and adult *T. castaneum* ($t_{(4)} = 44.642$; $p < 0.001$) (Table 4).

The comparative fumigant toxicity among different stages shows that larval stage of *T. castaneum* was more susceptible than the adult stages to both the EOs. The fumigation toxicity against adult beetles of *C. maculatus* also revealed *C. deodara* EO as most toxic (LC_{50} = 16.125 µl/L; LC_{95} = 49.609 µl/L) than *J. macropoda* EO (LC_{50} = 25.679 µl/L; LC_{95} = 76.934 µl/L). The lethal concentrations were also found significantly different ($t_{(4)}$ = 33.458; p < 0.001) (Table 5). The overall comparison of lethal concentrations revealed that *C. deodara* EO was most effective against both the storage pest.

Table 3
Fumigation toxicity of *Juniperus macropoda* and *Cedrus deodara* essential oils against red flour beetle *Tribolium casteneum* larvae

Plant Species	LC_{50} (µl/L Air)	95% Fiducial Limits		LC_{95} (µl/L Air)	95% Fiducial Limits		Slope ± SE	χ^2(D.f)	R^2
		Lower	Upper		Upper	Lower			
J. macropoda	357.332	348.672	366.978	433.517	412.448	471.761	19.597 ± 2.789	1.039 (4)	0.260
C. deodara	103.906	95.344	114.189	193.514	162.346	267.658	6.090 ± 0.990	1.216 (4)	0.405

Note: Probit mortality of *J. macropoda* and *C. deodara* on *T. casteneum* larvae. Larvae were exposed to different concentration of EOs and mortality was recorded 48 h post treatment. Corrected mortality was subjected to probit analysis using PoloPlus software, version 2.0 (LeOra Software, 2002). LC_{50}, median lethal concentration that would kill 50% of the test insect larval population of *T. casteneum*, whereas LC_{95} (that would kill 95% of the test insect larval population). The fiducial limit is presented at the 95% confidence level (CL); Chi-square value at 95% CL; Df, degree of freedom (i.e., Df = n-2, where n is the number of concentrations administered for bioassay; statistical significance between LC_{50} of EOs were evaluated using Paired *t*- test; *p < 0.05)

Table 4
Fumigation toxicity of *Juniperus macropoda* and *Cedrus deodara* essential oils against red flour beetle, *Tribolium casteneum* adult

Plant species	LC*50* (µl/L Air)	95% Fiducial Limits		LC_{95} (µl/L Air)	95% Fiducial Limits		Slope ± SE	χ^2(D.f)	R^2
		lower	Upper		Lower	Upper			
J. macropoda	724.656	683.296	772.771	1106.472	982.578	1375.109	8.949 ± 1.437	0.788 (4)	0.26
C. deodara	123.097	105.082	145.750	379.741	276.002	693.254	3.362 ± 0.563	1.676 (4)	0.56

Note: Probit mortality of *J. macropoda* and *C. deodara* on *T. casteneum* adult. Adult beetles were exposed to different concentration of EOs and mortality was recorded 48 h post treatment. Corrected mortality was subjected to probit analysis using PoloPlus software, version 2.0 (LeOra Software, 2002). LC_{50}, median lethal concentration that would kill 50% of the test insect adult population of *T. casteneum*, whereas LC_{95} (that would kill 95% of the test insect adult population). The fiducial limit is presented at the 95% confidence level (CL); Chi-square value at 95% CL; Df, degree of freedom (i.e., Df = n-2, where n is the number of concentrations administered for bioassay; statistical significance between LC_{50} of EOs were evaluated using Paired *t*- test; *p < 0.05)

Table 5
Fumigation Toxicity of *Juniperus macropoda* and *Cedrus deodara* essential oils against pulse beetle, *Callosobruchus maculatus* adult

Plant Species	LC_{50} (µl/L Air)	95% Fiducial Limits		LC_{95} (µl/L Air)	95% Fiducial Limits		Slope ± SE	χ^2(D.f)	R^2
		Lower	Upper		Lower	Upper			
J. macropoda	25.679	22.028	30.447	76.934	56.021	140.054	3.452 ± 0.578	2.892 (4)	0.96
C. deodara	16.125	13.980	19.048	49.609	36.004	89.677	3.370 ± 0.542	1.923 (4)	0.481

Note: Probit mortality of *J. macropoda* and *C. deodara* on *Callosobruchus maculatus* adult. Adult beetles were exposed to different concentration of EOs and mortality was recorded 48 h post treatment. Corrected mortality was subjected to probit analysis using PoloPlus software, version 2.0 (LeOra Software, 2002). LC_{50}, median lethal concentration that would kill 50% of the test insect adult population of *Callosobruchus maculatus*, whereas LC_{95} (that would kill 95% of the test insect adult population). The fiducial limit is presented at the 95% confidence level (CL); Chi-square value at 95% CL; Df, degree of freedom (i.e., Df=n-2, where n is the number of concentrations administered for bioassay; statistical significance between LC_{50} of EOs were evaluated using Paired *t*- test; *p < 0.05)

Repellent activity of EOs

The orientation bioassay studies through Y-tube glass olfactometer revealed that both the essential oils possessed repellent activity against adult beetles of *T. castaneum* and *C. maculatus*. The chi-square analysis of beetle responses to essential oils revealed that the insects were influenced by the stimuli, as indicated by significant differences in their responses. Significant repellency of *T. castaneum* adults was observed for both essential oils across all tested doses, except at 10 ng of *J. macropoda* (Fig. 6). Conversely, the adult beetles of *C. maculatus* found to be less influenced by EO stimulus which evident from non-significant responses *to J. macropoda* EO at all dosage and at 10ng dose of *C. deodara* (Fig. 7). However, at 50 and 100ng dose, the *C. deodara* oil was found showing significant repellence (χ^2 = 15.385 and χ^2 = 24.143 at 50 and 100ng dose, respectively).

The two-way ANOVA analysis of percent repellency obtained for *J. macropoda* and *C. deodara* EO against *T. castaneum* and *C. maculatus* revealed that the main effects *viz*., type of essential oil and dosage had significant impact on repellency (against *T. castaneum* F = 99.528, p < 0.01; against *C. maculatus* F = 49.37, p < 0.01) (Fig. 8).

Table 6

Docking score or binding energies (kcal/mol) of essential oil components to the target enzymes

Compound	***Tribolium castaneum* (kcal/mol)**				***Callosobruchus maculatus* (kcal/mol)**			
	AChE (UniProt ID: D6W9E2)	**NADH-UO (UniProt ID: D6WVN8)**	**GABArs (Uniprot ID: A8DMT9)**	**OR (Uniprot ID: C0Z3Q0)**	**AChE (Uniprot ID: A0A653D353)**	**NADH-UO (UniProt ID: A0A343KPX7)**	**GABArs (Uniport ID: A0A653DIA8)**	**OR (Uniprot ID: A0A7G9J0X7)**
***Cedrus deodara* EO components**								
α-cuprenene	-7.0	-6.6	-6.0	-7.4	-6.8	-7.1	-5.6	-7.5
α-himachalene	-7.5	-6.6	-7.5	-8.0	-6.3	-5.6	-6.2	-7.1
1-ethyl-3,5-dimethylbenzene	-6.4	-4.8	-4.9	-5.3	-5.7	-5.2	-4.9	-6.1
γ-himachalene	-7.2	-6.1	-6.1	-7.5	-7.3	-5.6	-5.8	-7.3
2-ethyltoluene	-6.2	-4.9	-4.9	-5.1	-5.3	-4.8	-4.9	-5.9
β-cymene	-6.7	-5.0	-5.3	-5.5	-5.8	-7.1	-5.3	-6.3
4-acetyl-1-methylcyclohexene	-6.6	-4.4	-4.5	-5.4	-5.6	-6.3	-4.5	-5.7
α-(E)-atlantone	-8.4	-5.5	-6.0	-6.7	-6.8	-5.6	-6.0	-6.8
Limonene	-6.3	-4.9	-5.0	-5.5	-5.7	-6.7	-5.0	-6.1
3,5-diphenyl-1-pentene	-8.3	-6.3	-5.6	-7.2	-7.2	-6.4	-6.1	-7.7
Mesitylene	-6.3	-4.6	-4.6	-5.1	-5.4	-6.6	-4.7	-5.8
Juniperus macropoda EO components								
4-terpineol	-6.3	-5.2	-5.0	-5.6	-5.4	-5.3	-5.1	-6.2
γ-terpinolene	-6.7	-5.3	-5.1	-5.6	-6.0	-6.8	-5.2	-6.2
4-carene	-6.3	-4.9	-5.2	-5.7	-5.6	-5.5	-4.9	-6.5
α-pinene	-6.4	-4.8	-5.1	-5.6	-5.2	-5.0	-5.1	-6.2
β-thujene	-5.9	-5.1	-4.9	-5.3	-5.4	-4.9	-4.8	-6.1
β-myrcene	-5.6	-5.0	-4.4	-4.7	-5.4	-4.7	-4.2	-5.5
δ-cadinene	-8.4	-6.0	-6.0	-7.5	-7.5	-6.4	-6.0	-6.6

Note: Docking simulations were performed using AutoDock Vina (version 1.2.0) to predict the binding affinities of essential oil constituents with test insects target proteins. The values are docking scores (estimated binding free energies, in kcal/mol) obtained by molecular docking of each ligand with the indicated proteins. More negative values indicate stronger predicted binding affinity. *Tribolium casteneum* protein: Aetylcholinesterase (AChE; UniProt ID: D6W9E2), NADH: ubiquinone oxidoreductase (NADH-UO; UniProt ID: D6WVN8), GABA receptors ($GABA_{rs}$; UniProt ID: A8DMT9), Odorant Receptor (OR: UniProt ID: C0Z3Q0). *Callosobruchus maculatus* proteins: Aetylcholinesterase (AChE; UniProt ID: A0A653D353), NADH: ubiquinone oxidoreductase (NADH-UO; UniProt ID: A0A343KPX7), GABA receptors ($GABA_{rs}$; UniProt ID: A0A653DIA8), Odorant Receptor (OR; UniProt ID: A0A7G9J0X7).

A dose-dependent increase in repellency was observed for *J. macropoda* against *T. castaneum*, with values rising from 11.97% at 10 ng to 54.13% at 50 ng and 85.98% at 100 ng, with all concentrations differing significantly from one another. *C. deodara* exhibited a similar pattern against *T. castaneum*, with repellency increasing from 54.13% at 10 ng to 78.79% at 50 ng and reaching 92.95% at 100 ng, with significant differences observed across all dosage levels.

According to Tukey's HSD test, *C. deodara* at 100 ng produced the significantly higher repellency exceeding all other treatment combinations against *T. castaneum*. *J. macropoda* at 100 ng formed a distinct intermediate group. The lowest repellency was recorded for *J. macropoda* at 10 ng, which was significantly inferior to all other treatments. For *J. macropoda* against *C. maculatus*, repellency at the lowest dose of 10 ng (3.74%) was significantly inferior to that observed at 50 ng (28.21%) and 100 ng (33.17%). Importantly, no significant difference was detected between the 50 ng and 100 ng treatments, indicating a saturation effect at concentrations above 50 ng. In contrast, *C. deodara* exhibited a progressive concentration-dependent increase in repellency: 28.47% at 10 ng, 77.02% at 50 ng, and 92.79% at 100 ng, with each dosage level significantly different from the others. Tukey's HSD post-hoc comparisons indicated that *C. deodara* at 100 ng yielded the maximum repellency against *C. maculatus*, which was significantly superior to all other treatment combinations. *C. deodara* at 50 ng constituted a separate statistical group, exhibiting significantly greater efficacy than intermediate-level treatments. *J. macropoda* at both 50 and 100 ng, together with *C. deodara* at 10 ng, formed a statistically uniform intermediate group showing no significant differences among them. The lowest repellency was recorded for *J. macropoda* at 10 ng, which was significantly lower than all other treatments.

Molecular Docking studies

The potential insecticidal or repellent properties of phyto-compounds from *C. deodara* and *J. macropoda* were assessed through molecular docking using four protein targets: acetylcholinesterase (AChE), GABA receptors (GABArs), and NADH: ubiquinone oxidoreductase and Odorant Receptors (ORs) in both test insects (Table 6). Stronger interaction was indicated by more significant negative binding affinities, measured in kcal/mol. A number of *C. deodara* sesquiterpenoids including *γ*-himachalene, *α*-cuprenene, *α*-himachalene, and *α*-(E)-atlantone showed consistently strong binding affinities to all four target proteins in both insect species. Notably, *α*-(E)-atlantone demonstrated the strongest interaction with AChE in *T. castaneum* (-8.4 kcal/mol), indicating a strong potential to interfere in signal transmission in insect nervous system. Both *α*-himachalene and *α*-cuprenene showed significant interaction with a variety of proteins targets especially with ORs and acetylcholinesterase, with average binding affinities between − 6.85 and − 6.75 kcal/mol. Similarly, 3,5-diphenyl-1 pentene demonstrated consistent binding across all targets with average affinity of -6.85 kcal/mol. These interactions suggest two potential mechanisms of action of these sesquiterpenes: interference with signal transmission and sensitization of odorant receptor, a receptor responsible for detection and discrimination for odors in insects.

The most effective component of *J. macropoda* was again a sesquiterpene compound, *δ*-cadinene, which exhibited a strong binding to AChE in both *T. castaneum* (-8.4 kcal/mol) and *C. maculatus* (-7.5 kcal/mol) (Table 6). Other monoterpene component of *J macropoda*, 4-terpineol, γ-terpinolene *α*-pinene, 4-carene, *β*-thujene, and *β*-myrcene showed a significantly weaker interaction, with binding energies ranging from − 4.2 to − 6.8 kcal/mol. However, these compounds may not act as strong inhibitor individually, their presence in essential oil mixture might contribute to synergistic effects, especially when it comes to interfering with chemosensory function.

The species-specific trend of receptor-ligand interaction was also observed. In *T. castaneum*, for instance, *α*-(E)-atlantone bonded to AChE more strongly (-8.4 kcal/mol) than in *C. maculatus* (-6.8 kcal/mol). Similarly, it was found that *α*-himachalene and *δ*-cadinene bonded considerably more strongly in *T. castaneum* than in *C. maculatus*, indicating differential sensitivity (Table 6). Comparatively, compounds from *C. deodara* EO showed stronger and more stable binding affinities than that from *J. macropoda* essential oil. Besides, most often targeted protein with highest binding values was AChE ranging from − 5.2 to -8.4 kcal/mol, suggesting that it may be major site of action.

Molecular Interaction Analysis with selected ligands

For further validation, the essential oil component (ligand) from each plant (names essential oil component) that showed the highest binding affinity with target enzymes proteins was selected and visualized using Discovery Studio software. Similarly, the top-scoring ligand interacting with the odorant receptor (OR) protein of each insect was visualized to assess its potential role in repellency. Strongest binding interaction in *T. castaneum* was observed between *α*-(E)-atlantone and AChE (-8.4 kcal/mol) which can be attributed to one hydrogen bond (TYR345) and multiple hydrophobic (π–alkyl) interaction involving key residues such as TYR114, TRP342, TYR391, PHE392, and TYR395 (Fig. 9) (Table S1). Similarly, *δ*-cadinene also bound *T. castaneum* AChE with same affinity (-8.4kcal/mol), primarily through π–sigma and π–alkyl hydrophobic interactions involving TRP126, TYR391, and HIS502 (Fig. 9) (Table S1). For the *T. castaneum* Odorant receptor target, *α*-himachalene (-8.0 kcal/mol) and *δ*-cadinene (-7.5 kcal/mol) showed good affinity, supported by multiple alkyls and π–alkyl interactions (Fig. 10) (Table 7).

In *C. maculatus*, *δ*-cadinene displayed strongest affinity with AChE (-7.5 kcal/mol), stabilised by π–sigma and π–alkyl contacts with TYR102, TRP114, and PHE residues (Fig. 9) (Table S1). Similarly, *γ*-himachalene showed stronger binding with AChE (-7.3 kcal/mol) through π–sigma and π–alkyl interactions involving TRP114, TYR102, PHE326, and PHE366 residues (Fig. 10) (Table S1). For the ORs, 3,5-diphenyl-1-pentene exhibited the highest affinity (-7.7 kcal/mol), supported by π–π stacking with PHE328 an hydrophobic interaction with ILE324, VAL338, and LEU231

(Fig. 10) (Table 7). δ-Cadinene also interacted with *C. maculatus* OR (-6.6 kcal/mol) through multiple π–alkyl contacts with PHE328 (Fig. 10) (Table 7).

Discussion

The chemical composition analysis of plant essential oils is primarily undertaken to elucidate the bioactive compounds present, which may function as effective agents in various applications including biomedicine, biopesticides development, and other environmentally sustainable solutions. Extensive efforts have been carried out for exploration of *J. macropoda* and *C. deodara* bio-actives. Despite their ethnobotanical significance, the bioactive potential of these EOs remains largely underexplored, warranting comprehensive phytochemical investigations. In practical situation where fumigation is involved for insecticidal action, the volatiles emitted from essential oils brings mortality in insects, hence we analysed the headspace sample of EOs to identify such volatiles. The chemo-profiling of the volatiles from *J. macropoda* EO were characterized by a high abundance of monoterpenes and monoterpenoids wherein 4-terpineol, limonene, and γ-terpinolene, *β*-thujene, 4-carene, *α*-pinene, and *β*-myrcene constituted the major part. Essential oils from plant species belonging to Juniperus have previously been reported to contain monoterpenes and monoterpenoids, although considerable compositional variation has been observed among different species and geographical regions[32–35]. The compositional variation was also evident from the studies by Stappen *et al.*[15] from Western Himalaya and Dahmane *et al.*[36] from Algeria, who reported sabinene, cedrol, *α*-pinene and 4-terpineol as the major constituents of *J. macropoda* oil from Western Himalaya. Similar to present study, the sesquiterpenes and sesquiterpenoids in minor quantity have also been reported by Krutca et al.[33]. The contrasting chemical profiles observed across *Juniperus* species underscore the significant influence of geographical and environmental factors on terpenoid biosynthesis, with important implications for their biological activities and industrial applications. Converse, the *C. deodara* EO was found rich in sesquiterpenes (41.92 ± 0.051%), predominantly characterized by himachalane-type compounds (*α*-cuprenene, *α*-himachalene, *γ*-himachalene), consistent with previous investigations[20,37]. Kumar *et al.* reported comparable sesquiterpene profiles, including α-himachalene (13.83%), γ-himachalene (12.00%), β-himachalene (37.34%), deodaron (0.43%), α-atlantone (4.53%), γ-(Z)-atlantone (2.77%), γ-(E)-atlantone (3.34%) and, α-(E)-atlantone (10.63%)[37]. This sesquiterpene predominance represents a characteristic chemotaxonomic marker of *Cedar* species and correlates strongly with their documented bioactivities[21,39,40,41]. The monoterpene fraction in *C. deodara* was substantially lower (12.36 ± 0.041%) than that observed in *J. macropoda* oil, reflecting species-specific biosynthetic pathways and distinct ecological adaptations. Notably, the presence of aromatic hydrocarbons (22.77 ± 0.016%), including benzaldehyde (19.40%), p-cymene, and benzoic acid, corroborates earlier findings by Saab et al.[42]. The marked differences in essential oils from these two Himalayan plants highlight the need for detailed bioactivity studies to assess their potential.

There are very few reports on fumigation toxicity of EOs of *J. macropda* and *C. deodara* against storage insect pests. The present study revealed *C. deodara* oil as most effective fumigant against both test insects. The *C. deodara* EO have also been reported to possess very good fumigation toxicity against a storage pest, rice weevil *Sitophilus oryzae* with 53.33% percent mortality after 30 days of exposure[23]. The toxicity of Cedar wood oil alone was found very effective than its combination with neem oil and neem oil alone[21]. Contrast to fumigation toxicity potential of *C. deodara* oil in present study, Gupta *et al.* did not find very encouraging toxicity against *C. maculatus* (LC_{50} = 1487.29 µl/l) and *C. chinensis* (LC_{50} = 1716.80 µl/l)[20].

Our studies revealed that *J. macropoda* oil was not much effective (*T. castaneum* larvae LC_{50} = 357.332 µl/l, *T. castaneum* adult LC_{50} = 724.656 µl/l, and *C. maculatus* adult LC_{50} = 25.679 µl/l) as compared to *C. deodara* oil (*T. castaneum* larvae LC_{50} = 103.906 µl/l, *T. castaneu*m adult LC_{50} = 123.097 µl/l, and *C. maculatus* adult LC_{50} = 16.125 µl/l). But, toxicity of *J. macropoda* EO in present study is more promising than those reported by Gupta *et al.*[20] for toxicities of EOs from *J. communis* (LC_{50} = 1945.44 µl/l) and *J. recurva* (LC_{50} = 2369.76 µl/l) against *C. maculatus*, this may be attributed to differential composition of EOs. The EOs of *J communis* and *J recurva* were dominated by camphene which is a monoterpene compound.

Apart from fumigation toxicity, EO from *Juniperus* species have been studied for its repellent and contact activity against insect pests[15,17,19,43]. The repellent effect is an important characteristic in the choice of essential oil for the management of stored grain pests because high repellency can lower the infestation and the consequent reduction or absence of oviposition[44]. In the present study essential oil of *C. deodara* shows significantly higher repellent activity against *T. castaneum* adults at all doses (10ng, 50 ng, and 100ng). The antifeedant/repellent activity of this plant EO have also been reported by Buneri *et al.* against mealworm beetle (*Tenebrio molitor*) larvae[21]. Koc *et al.* also reported potential repellent effect of another species *C. libani* on brown dog tick species (*Rhipicephalus sanguineus*)[45]. The EOs of *J. macropoda* was found to be most effective repellent at concentration of 50 ng and 100 ng against *T. castaneum* but not against *C. maculatus*. Similar to this, Guo, *et al.* reported that the essential oil of *J. formosana* showed strong repellent activity against *T. castaneum* with the percentage repellency (PR) over 80% at 2 h[17]. The other species of *Juniperus* and *C. deodara* essential oil have been reported as promising repellents[20]. The variation in the repellent activity could be due to differential volatile profiles.

Molecular docking serves as a useful tool in pest management by providing predictive insights onto ligand-protein interactions, facilitating the identification of potential molecular target, enabling structure-based identification of ecofriendly insecticidal or repellent agents[46]. Literature shows that, acetylcholinesterase enzyme is reported to be most potent target for fumigation toxicity of EOs[27]. Besides this key enzyme, EOs are also reported to act on other biological targets of insects such as ion channels or respiratory system[47–49]. Further odorant receptors (ORs) on insect antennae play a crucial role in insect behaviour, sensitization of them by EOs may cause repellent effect in insects. Considering this, we performed *in silico* molecular docking of major components of both EOs against acetylcholinesterase (AchE), GABA receptors (GABArs), and NADH: ubiquinone oxidoreductase and Odorant receptors of both test insect species. *In silico* studies revealed that *C. deodara* sesquiterpenoids including *γ*-himachalene, *α*-cuprenene, *α*-himachalene, and *α*-(E)-atlantone showed consistently strong binding affinities to all four target proteins in both insect species. The toxicity and repellency observed in the present study is may be due to this binding suggesting the potential target site. The other EOs with these sesquiterpenoids as major components has also been proved good fumigants against storage pests [39,50]. A strong binding of *δ*-cadinene to AChE in both insects may be responsible for fumigation and repellent property of *J. macropoda* EO. This compound has also been proved to possess larvicidal against malaria, dengue and filariasis causing mosquitoes[51]. In comparison to compounds from *J. macropoda*, *C. deodara* compounds often showed stronger and more stable binding affinities, consistent with bioassay results indicating the superior insecticidal activity of *C. deodara* essential oil. The most often targeted protein with highest binding values was AChE, which is a vital enzyme in the insect nervous system that hydrolyses acetylcholine, a key neurotransmitter in nerve impulse transmission. Inhibition of AChE by essential Oils disrupts synaptic signalling, causing neurological dysfunction, paralysis, and ultimately insect mortality. This mechanism underlies the insecticidal (fumigation) potential of these essential oils. The present study concludes that EOs of *C. deodara* and *J. macropoda* can be a promising candidate for further in-depth studies and development of suitable formulations for management of storage pests.

Conclusion

The present investigation provides comprehensive insights into the chemical composition, bio-efficacy, and molecular interaction of *Cedrus deodara* and *Juniperus macropoda* essential oils against major storage insect pests. Distinct chemotypes were identified, with *C. deodara* oil characterized by sesquiterpene-rich derivatives and *J. macropoda* dominated by monoterpene and monoterpenoids. These compositional differences translated into markedly higher fumigation toxicity and repellency of C. deodara oil. Molecular docking analyses substantiated these finding, revealing strong and stable binding of key sesquiterpenoids (e.g., γ-himachalene, α-himachalene, α-cuprenene) to vital insect target proteins, particularly acetylcholinesterase, indicating a plausible neurotoxic mode of action. Collectively, the integrated chemical, biological and computational evidence underscores the potential of *C. deodara* essential oil as a potent and eco-compatible fumigant and repellent for sustainable post-harvest pest management.

Declarations

Competing Interests

The authors have no relevant financial and non-financial interest to disclose.

Ethical approval

The study does not contain any experiment using any animal species that require ethical approval

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Author Contribution

N. G; S. M. N: Writing Original Draft, Visualisation, Supervision, Methodology, Review and Editing, Data Curation. B. H; S. K; S. S: Writing— Review and editing, visualisation, Data curation.

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Data Availability

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

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Figures

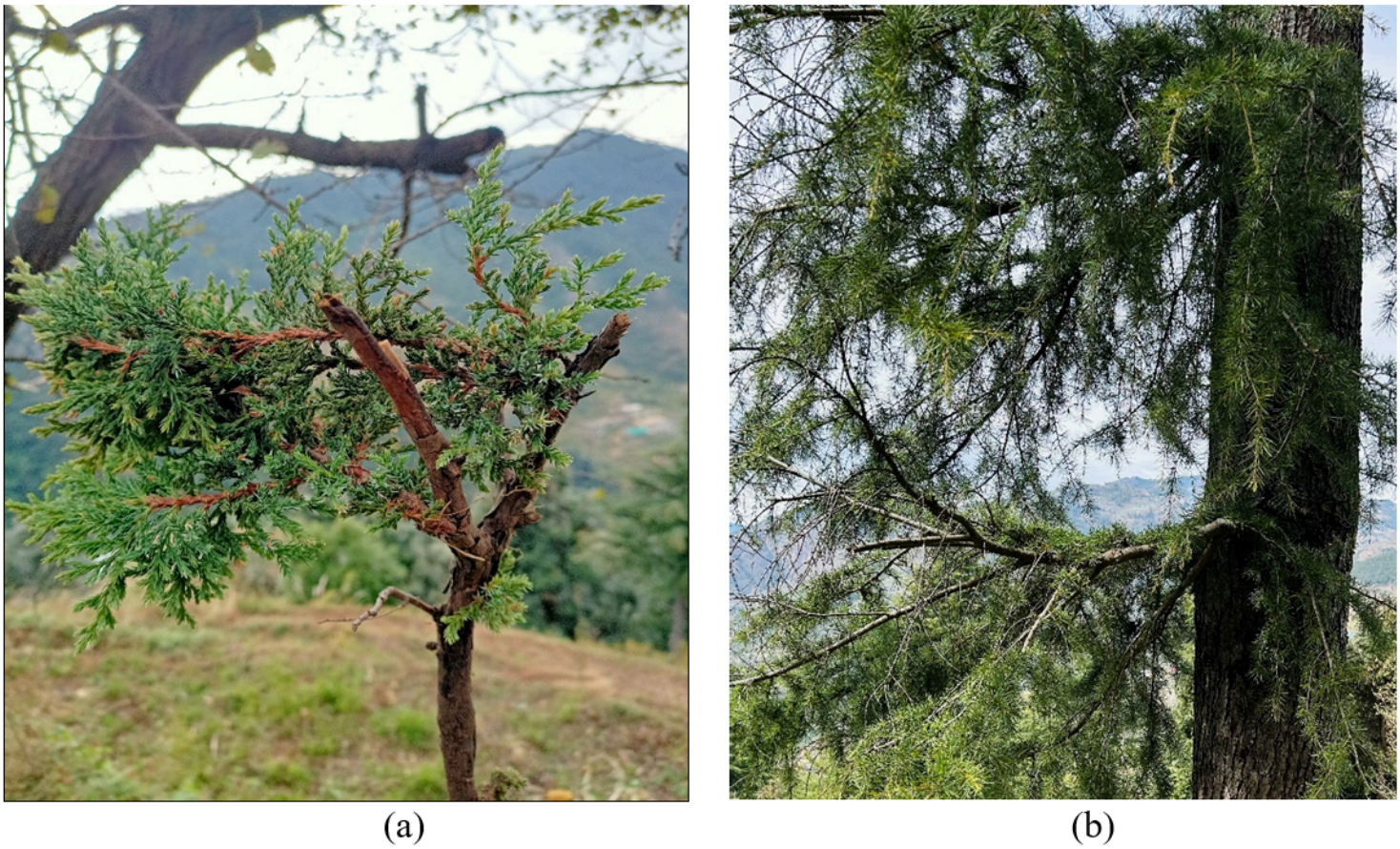

Figure 1

Plants ((a) *Juniperus macropoda* and (b) *Cedrus deodara* used for biological activity

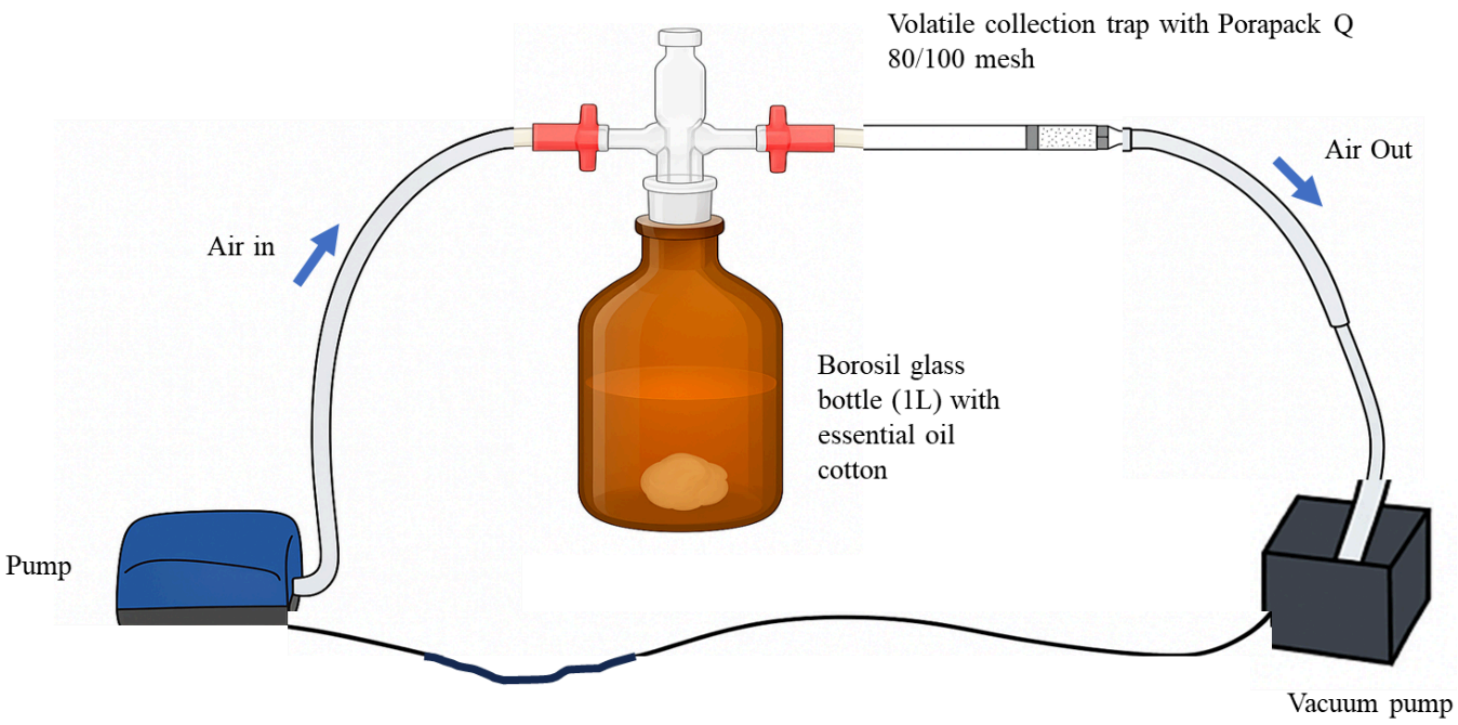

Figure 2

Headspace volatiles collection setup for collection of VOCs emitted by essential oil

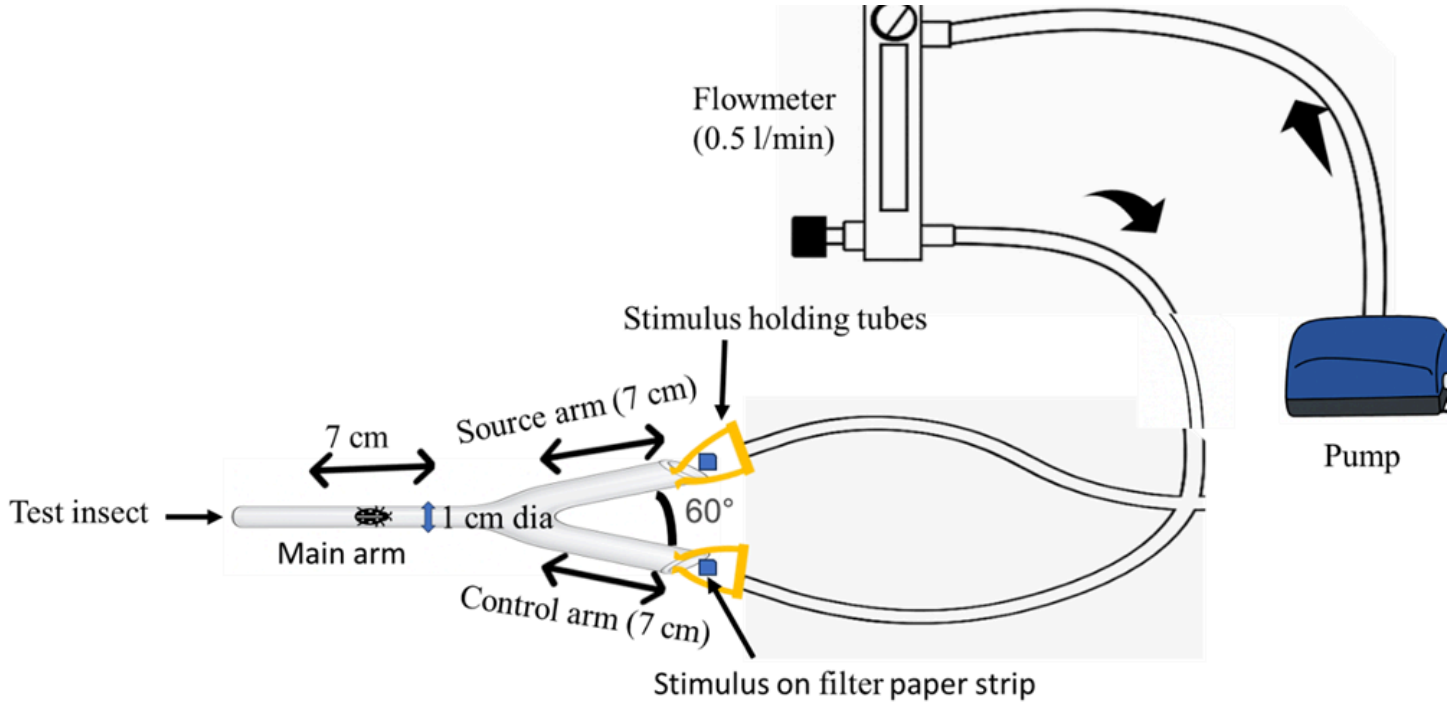

Figure 3

Y-tube olfactometer set-up for repellence bioassay

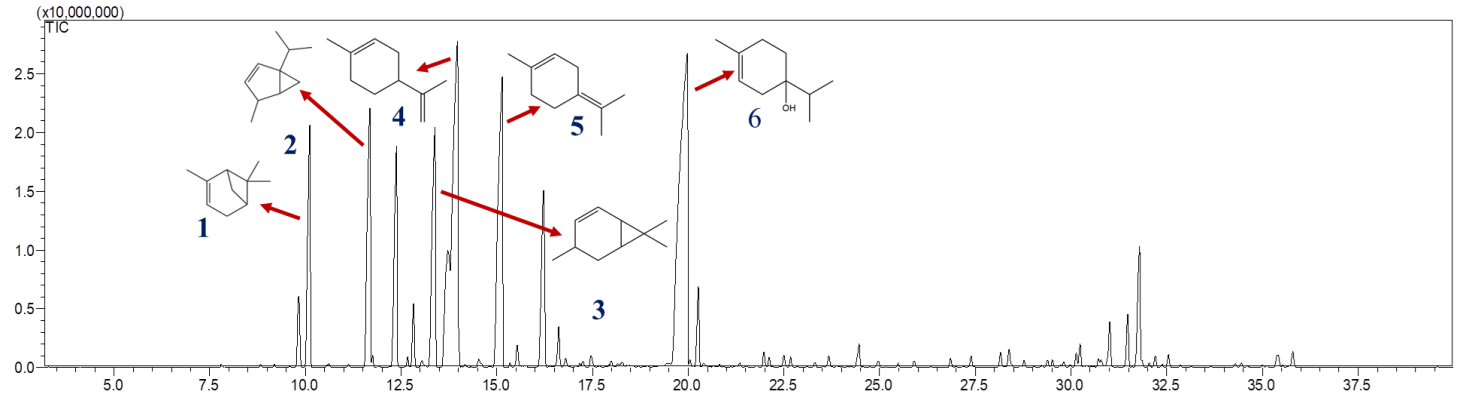

Figure 4

Gas chromatogram showing major compounds of Himalayan pencil cedar, *Juniperus macropoda.* Compound 1: α-Pinene, 2: β-Thujene, 3: 4-Carene, 4: Limonene, 5: γ-Terpinolene, 6: 4-terpineol

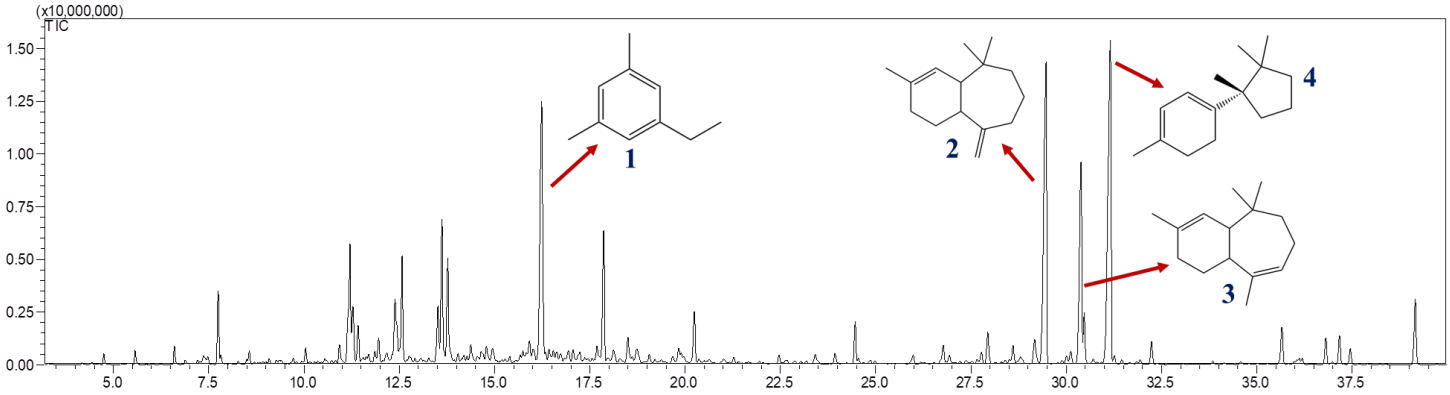

Figure 5

Gas chromatogram showing major compound of Himalayan cedar, *Cedrus deodara*. Compound 1: 1-Ethyl-3,5-dimethylbenzene, 2: α-Himachalene, 3: γ-himachalene, 4: α-Cuprenene

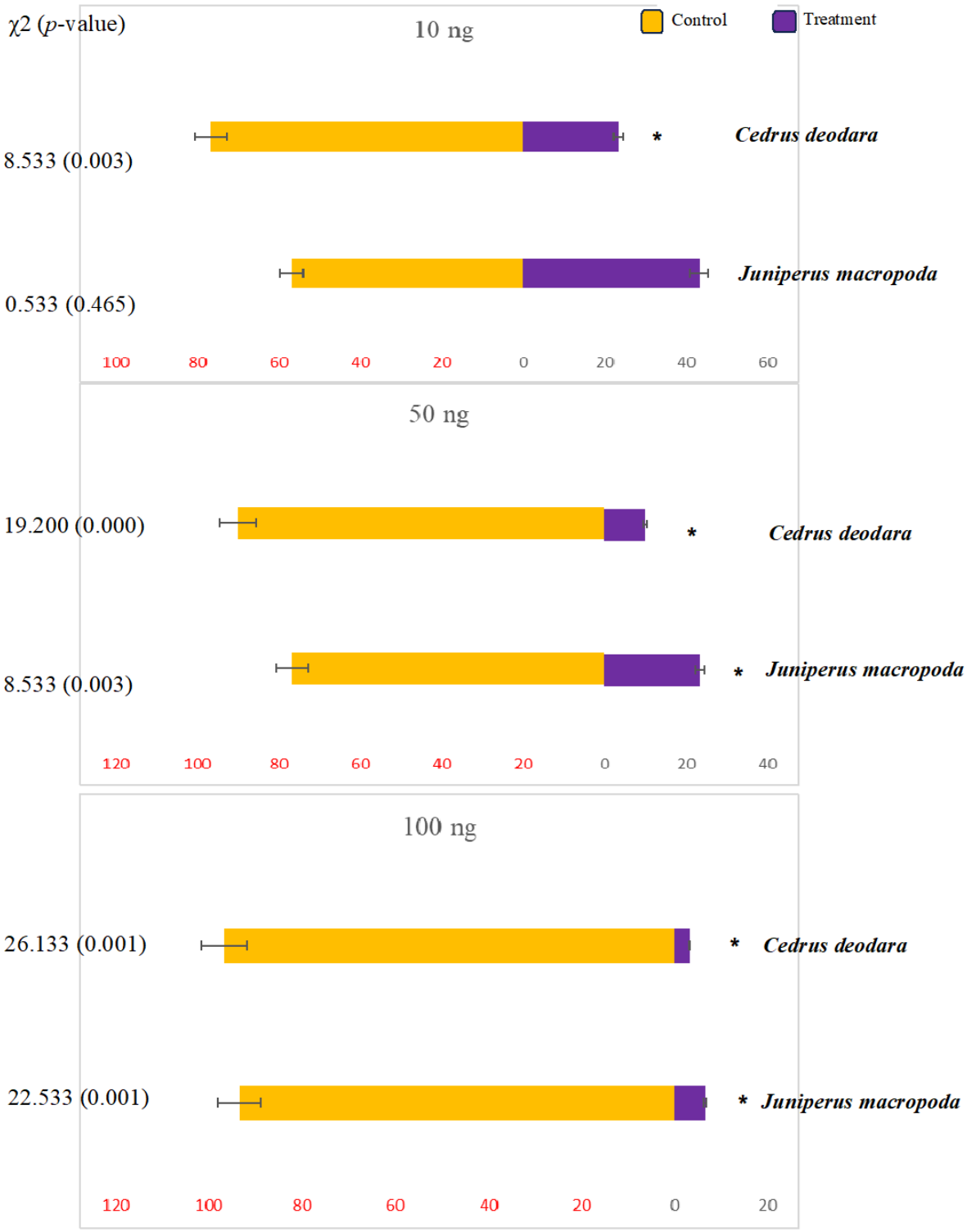

Figure 6

Repellence activity of *Juniperus macropoda* and *Cedrus deodara* essential oils against adults of red flour beetle, *Tribolium castaneum*. Repellence responses of *T. castaneum* adults were assessed at three doses (10, 50, and 100 ng) of essential oils from *Cedrus deodara* and *Juniperus macropoda*. Bars represent the mean (± SE) percentage of insects responding to control (yellow) and treated (purple) arms in a Y-tube olfactometer assay. Statistical significance between treatments and control was determined using the Chi-square (χ^2) test; corresponding χ^2 values and *p*-values are shown alongside each concentration. Asterisks (*) indicate significant differences ($p<0.05$).

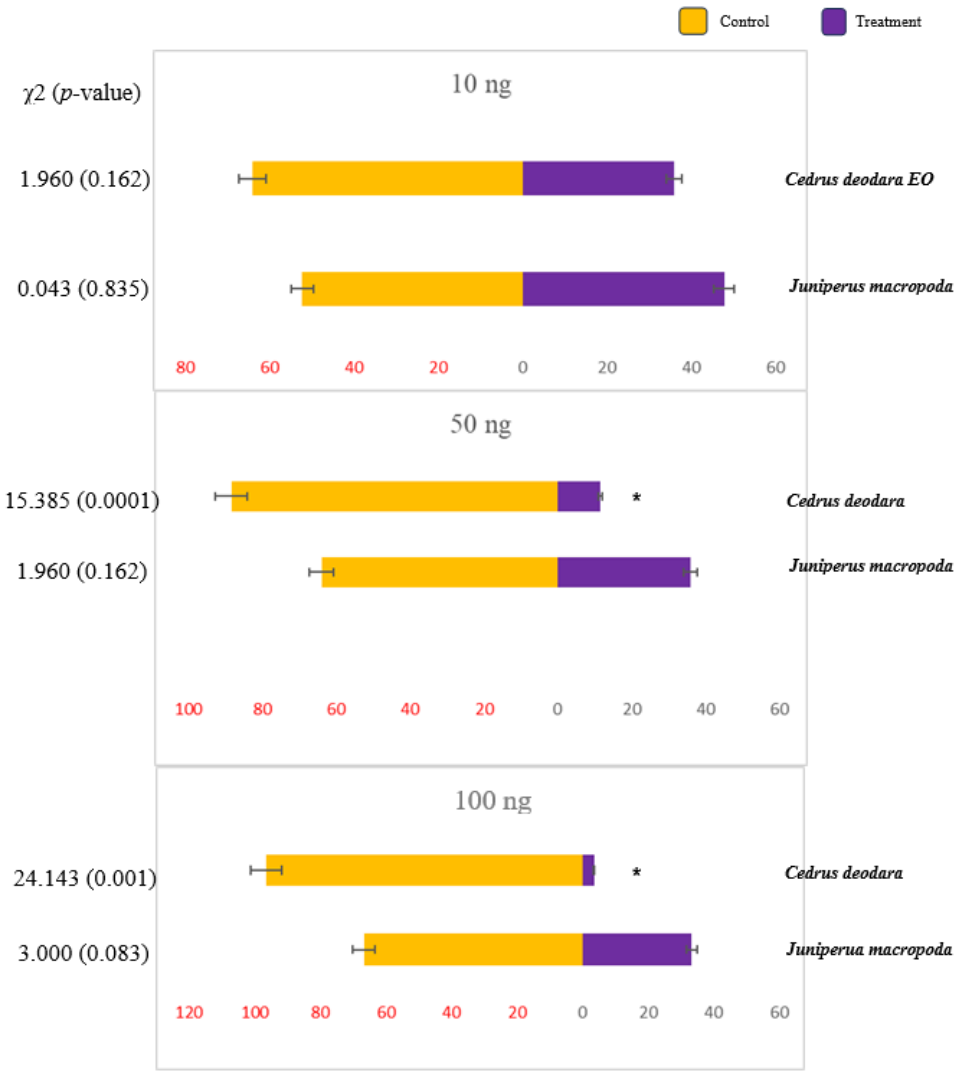

Figure 7

Repellence activity of *Juniperus macropoda* and *Cedrus deodara* essential oils against adults of pulse beetle *Callosobruchus maculatus*. Repellence responses of *C. maculatus* adults were assessed at three doses (10, 50, and 100 ng) of essential oils from *Cedrus deodara* and *Juniperus macropoda*. Bars represent the mean (± SE) percentage of insects responding to control (yellow) and treated (purple) arms in a Y-tube olfactometer assay. Statistical significance between treatments and control was determined using the Chi-square (χ^2) test; corresponding χ^2 values and *p*-values are shown alongside each concentration. Asterisks (*) indicate significant differences ($p<0.05$).

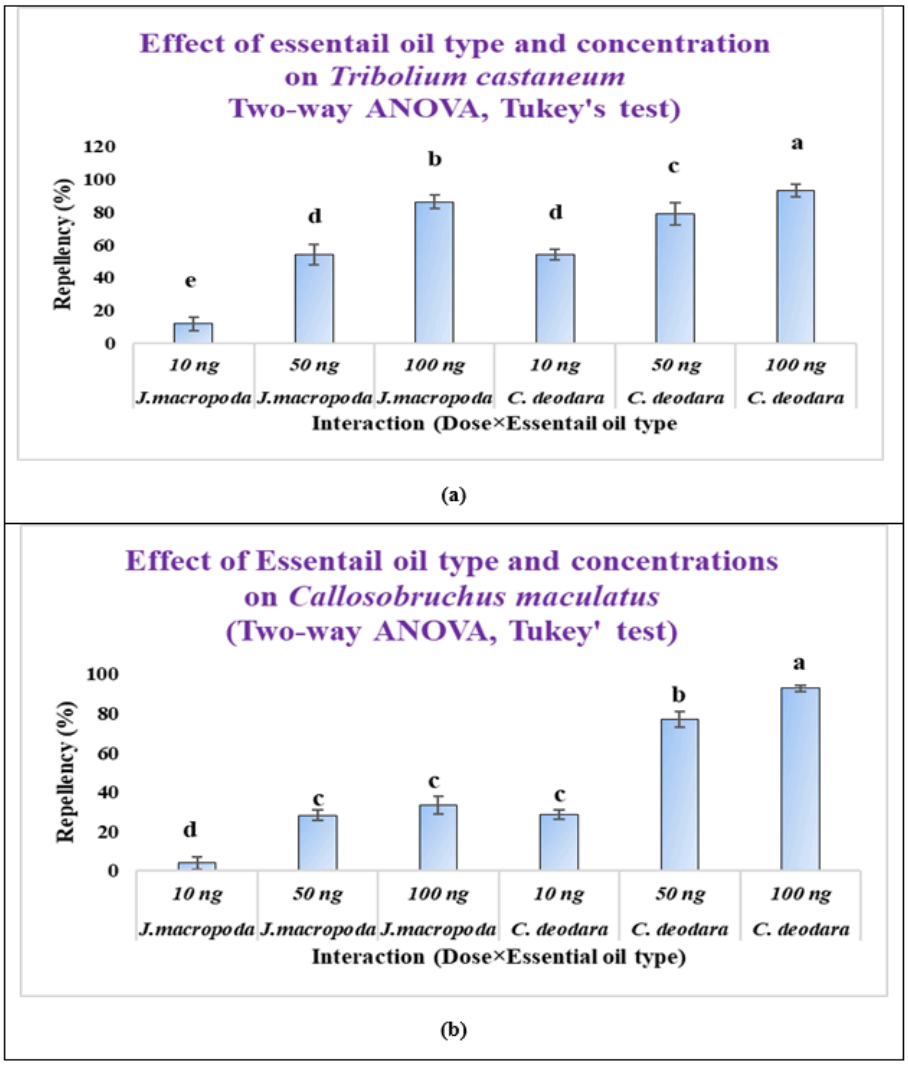

Figure 8

Effect of essential oil type and concentration on repellency against (a) *Tribolium castaneum* and (b) *Callosobruchus maculatus*. Two-way ANOVA was conducted with essential oil type (*Juniperus macropoda* and *Cedrus deodara*) and concentration (10, 50, and 100 ng) as independent factors, and percent repellency as the dependent variable. Bars represent mean percent repellency values. Different letters (a-d) above bars indicate statistically significant differences among treatments based on Tukey's HSD post-hoc test ($p < 0.05$). Error bars represent standard error of the mean. In order to homogenise the variance, mortality data was subjected to arcsine transformation before applying Tukey's HSD test

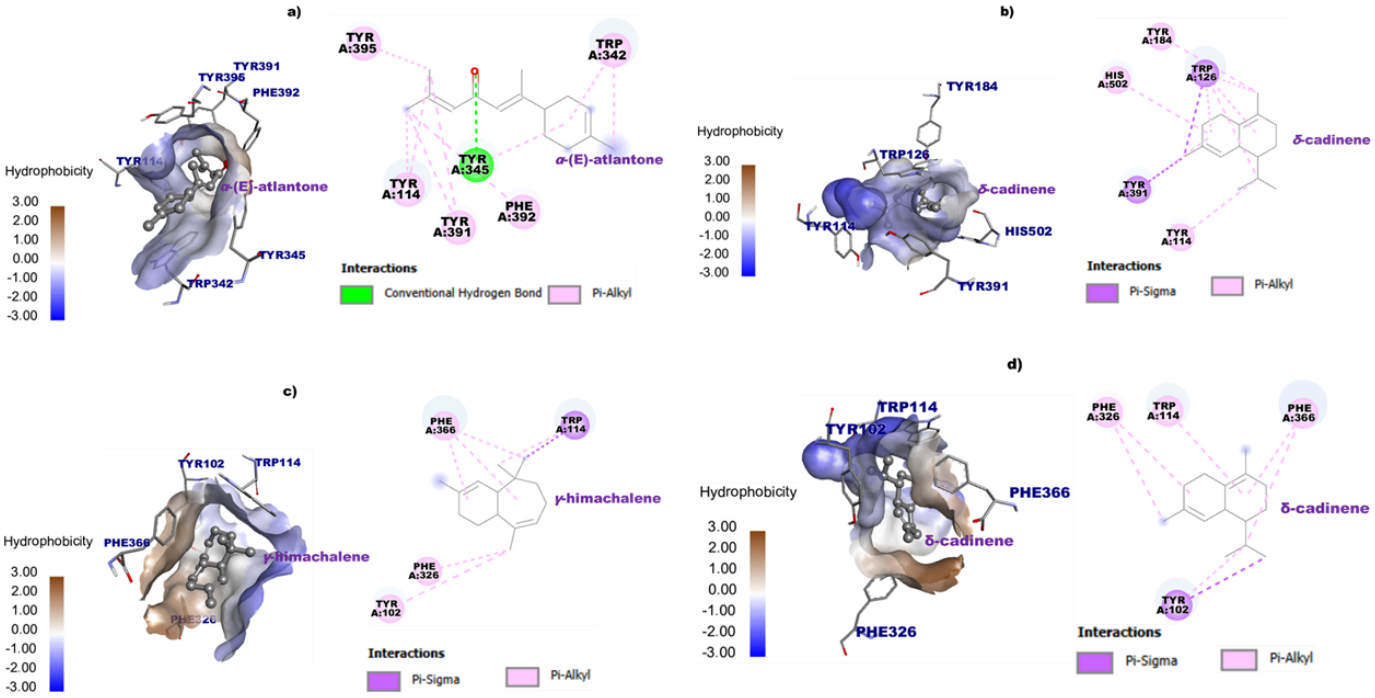

Figure 9

Major binding interaction and 2 D diagram of the receptor-ligand complexes: (a) *Tribolium casteneum* AChE with α-(E)-atlantone (b) *T. casteneum* AChE with δ-cadinene (c) *Callosobruchus maculatus* AChE with γ-himachalene (c) *Callosobruchus maculatus* AChE with δ-cadinene. Left panels display the proteins binding pocket represented as a hydrophobic surface (colour scale from blue for hydrophilic regions at -3.00 to brown for hydrophobic regions at +3.00), with key interacting amino acids residues labelled. Right panels show 2D ligand interaction diagrams depicting the spatial arrangement of amino acid residues around each ligand. Conventional hydrogen binds are shown in green, Pi-Alkyl interaction in pink, and Pi-Sigma interaction in purple.

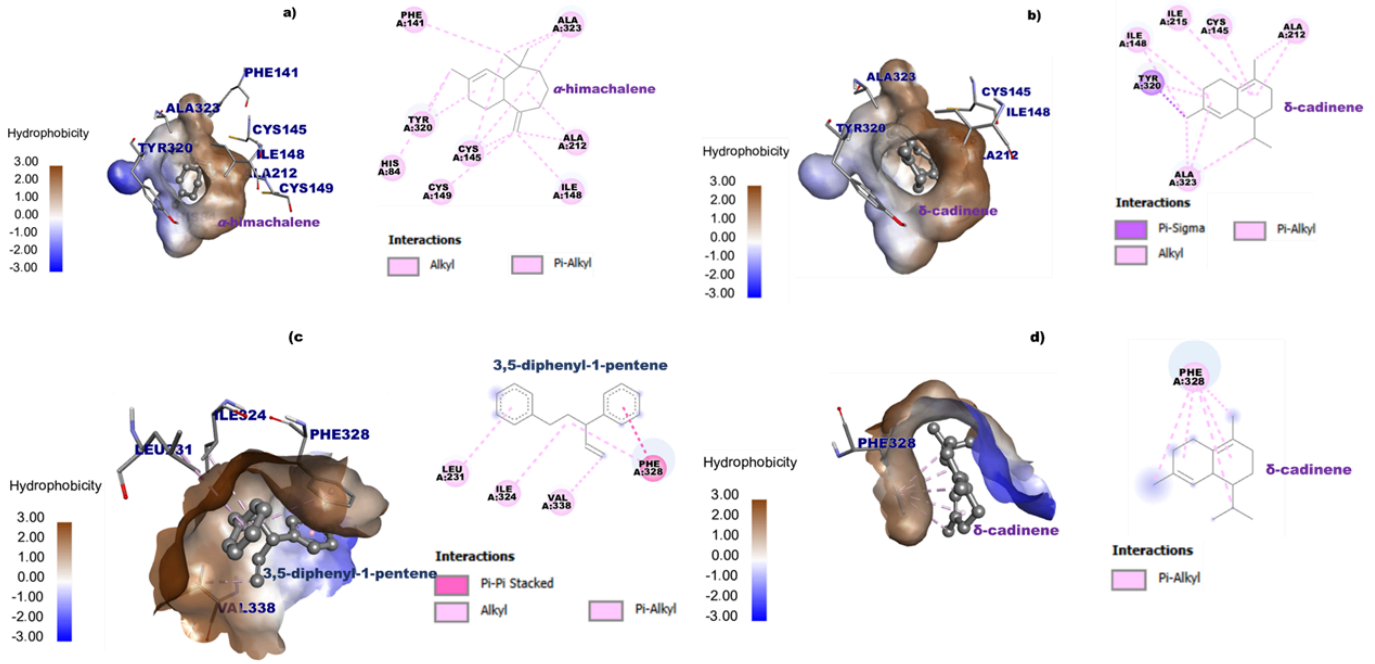

Figure 10

Major binding interaction and 2 D diagram of the receptor-ligand complexes: (a) *Tribolium casteneum* ORs with *α*-himachalene (b) *T. casteneum* ORs with *δ*-cadinene (c) *Callosobruchus maculatus* ORs with 3,5-diphenyl-1-pentene (c) *C. maculatus* ORs with *δ*-cadinene. Left panels display the proteins binding pocket represented as a hydrophobic surface (colour scale from blue for hydrophilic regions at -3.00 to brown for hydrophobic regions at +3.00), with key interacting amino acids residues labelled. Right panels show 2D ligand interaction diagrams depicting the spatial arrangement of amino acid residues around each ligand. Alkyl interaction and Pi-Alkyl interaction in pink, Pi-Sigma interactions in purple and Pi-Pi Stacked interaction in dark pink.

Supplementary Files

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