Respiratory Toxicity of *Cupressus sempervirens* Essential Oil from Two Different Habitats Against Indian Meal Moth (*Plodia interpunctella*) and Yellow Mealworm (*Tenebrio molitor*)

Somayeh Nazirzadeh [a], Vilma Bayramzadeh [b,*], Farahnaz Khalighi-Sigaroodi [c], Leila Pourhosseini [a], and Sayed Khosrow HossinAshrafi [b]

Environmental and health concerns associated with synthetic pesticides have intensified the search for effective botanical alternatives. This study investigated the insecticidal properties of essential oils derived from Mediterranean cypress (*Cupressus sempervirens* L.) leaves collected from two distinct habitats in Iran (Rudbar and Hasanabad Chalous) against the Indian meal moth (*Plodia interpunctella* Hübner) and the yellow mealworm (*Tenebrio molitor* L.). Essential oils were extracted by hydro-distillation and analyzed by GC-MS, revealing 22 and 24 compounds in the Rudbar and Hasanabad oils, respectively, with α-pinene as the dominant component in both. The respiratory toxicity was assessed through probit analysis, calculating both LC50 and LT50 values. The results demonstrated a significant correlation between oil concentration and mortality. Notably, the efficacy was both habitat-dependent and species-specific. The Rudbar essential oil was significantly more potent and faster-acting against *P. interpunctella* (LC50 = 96.65 µL/L; LT50 = 12.1 h), whereas the Hasanabad oil was more effective against *T. molitor* (LC50 = 130.90 µL/L; LT50 = 11.8 h). This differential toxicity is linked to their distinct chemical profiles. This study confirms that the habitat of *C. sempervirens* critically influences the chemical composition and resultant insecticidal activity of its essential oil, highlighting its potential for developing targeted, selective pest control strategies for specific stored-product pests.

DOI: 10.15376/biores.21.1.1013-1029

Keywords: Stored product pests; Essential oil; Cypress; Bioassay; Secondary metabolites

*Contact information: a: Department of Horticultural Science and Engineering, Ka.C., Islamic Azad University, Karaj, Iran; b: Department of Engineering of Wood Industries and Cellulose Products, Ka.C., Islamic Azad University, Karaj, Iran; c: Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran; *Corresponding Author: vbayramzadeh@gmail.com*

INTRODUCTION

The extensive use of synthetic pesticides has been linked to a range of serious issues, including the development of pest resistance, the emergence of secondary pests, and the detrimental contamination of ecosystems through bioaccumulation (Sharma *et al.* 2019). More critically, many of these chemical residues pose documented risks to human health, including potential carcinogenicity, neurotoxicity, and endocrine disruption (Kim *et al.* 2017). Furthermore, their non-selective mode of action often harms beneficial insects and natural predators, disrupting integrated pest management programs (Goulson *et al.* 2015). These compelling concerns have accelerated the search for sustainable and

eco-friendly alternatives. Therefore, in recent years, the environmental and health concerns associated with synthetic pesticides have driven a shift toward safer alternatives for pest management. Among these, essential oils derived from medicinal plants have gained attention due to their biodegradability and low environmental impact (Ziaee 2014; Ramzi *et al.* 2018; Labbafi *et al.* 2021). While the potential of essential oils as natural insecticides is promising, it is important to consider their safety profile. Generally, plant-derived essential oils are considered to have low mammalian toxicity and are readily biodegradable, contributing to a reduced environmental impact compared to persistent synthetic insecticides (Isman, 2020). However, it is acknowledged that their bioactivity is not always specific, and their effects on non-target organisms can vary depending on the oil's chemical composition and concentration. Essential oils, composed mainly of terpenoids, especially monoterpenoids and sesquiterpenoids, serve as natural defense mechanisms for plants and exhibit diverse biological activities, including insecticidal and repellent properties.

Over 17,000 plant species possess secondary metabolites that are effective against various pests, especially stored product pests (Moharramipour *et al.* 2003; Nazemi Rafih and Moharramipour 2008; Nerio *et al.* 2009; Zuzarte *et al.* 2013; Mwine *et al.* 2013). However, the insecticidal action of an essential oil cannot be reliably predicted from its individual components alone, as the complex mixture of compounds can interact synergistically or antagonistically, leading to a net effect that is unique to the whole oil.

Each year, a considerable amount of agricultural production is destroyed by stored-product pests (Bande-Borujeni *et al.* 2016). The yellow mealworm (*Tenebrio molitor* L.) and the Indian meal moth (*Plodia interpunctella* Hubner) are among the most destructive stored product pests. *T. molitor* infests starchy products, such as wheat bran, flour, biscuits, and corn grains, causing contamination through feces and larval skins, thereby reducing product quality (Tripathi *et al.* 2001; Fontenot *et al.* 2012; Nenaah 2014; Plata-Rueda *et al.* 2021). Another significant stored product pest worldwide, especially in tropical regions of Asia, Africa, Europe, and America, is the Indian meal moth (*P. interpunctella*). In Iran, *P. interpunctella* is a major pest of legumes, grains, walnuts, almonds, pistachios, dried fruits, dates, seeds, raisins, and sweets. The larvae not only damage stored products but also promote the growth of fungi and bacteria. While chemical methods remain the primary approach for controlling these pests, using safer alternatives, such as essential oils and plant extracts, is increasingly recommended. Numerous studies have demonstrated the effectiveness of plant-derived essential oils and extracts in controlling pests, fungi, and bacteria. For example, saffron extract has shown inhibitory effects on the Indian meal moth (*P. interpunctella*) (Moharramipour *et al.* 2003; Nazemi Rafih and Moharramipour 2008; Nerio *et al.* 2009; Sadeghi *et al.* 2022). Additionally, the essential oils of lavender (*Lavandula angustifolia*), asafoetida (*Ferula assa-foetida*), and oleander (*Nerium oleander*) significantly reduce the population of the red flour beetle (*Tribolium castaneum*), a common stored-product pest.

Furthermore, several scientific studies have identified secondary metabolites in coniferous trees native to Iran (Golestani *et al.* 2011; Hesabi *et al.* 2019). Among these, *C. sempervirens* essential oil at a concentration of 40 µg/mL exhibited 100% larval mortality against *Culex quinquefasciatus* due to its high monoterpene content (Almadiy and Nenaah 2022).

Similarly, other research has revealed that *C. sempervirens* essential oil effectively controlled the maize weevil (*Sitophilus zeamais*), inhibiting its reproduction (Langsi *et al.* 2018). Other studies have also investigated the insecticidal effects of

juniper (*Juniperus excelsa*), clove (*Syzygium aromaticum*), and cinnamon (*Cinnamomum verum*) essential oils in reducing the larval population of yellow mealworm (*T. molitor*) (Martínez *et al.* 2018; Gruľová *et al.* 2022; Nazirzadeh *et al.* 2024). The insecticidal properties of these essential oils are largely attributed to secondary metabolites, which vary in concentration and composition due to environmental factors such as climate, soil, and physiography. This chemotypic variation means that the efficacy of an essential oil from a given plant species can be highly habitat-dependent. Despite these findings, limited research has been carried out on the impact of environmental conditions on the phytochemical properties and bioactivity of *C. sempervirens* (Omid Beigi 1996, 2000; Moein *et al.* 2017). Therefore, simply studying individual compounds or essential oils from a single origin is insufficient. This study aimed to evaluate and compare the insecticidal properties of essential oils extracted from *C. sempervirens* leaves collected from two distinct habitats in Rudbar and Hasanabad Chalous, Iran, against *P. interpunctella* and *T. molitor* under laboratory conditions. The objective was to determine how habitat-induced chemical variations influence the efficacy of the essential oil, thereby identifying the most effective source for controlling specific stored-product pests.

EXPERIMENTAL

Material and Methods

Ethical considerations

All experimental procedures involving insects in this study were conducted in accordance with standard ethical guidelines for laboratory bioassays. The research protocol was reviewed and approved by the Institutional Research Committee at the Islamic Azad University, Karaj Branch, which oversees the ethical conduct of scientific research.

Plant Collection and Sampling

Cupressus sempervirens L. (Cupressaceae) leaves were collected from two regions: Rudbar, Gilan Province (36°65'N, 51°42'E, 1050 m elevation), and Hasanabad Chalous, Mazandaran Province (36°65'N, 51°42'E, 29 m elevation). The climatic conditions and habitats of the collection sites are summarized in Table 1. According to De Martonne's aridity index, the climates of these regions were calculated as IDM = 18.11 for Rudbar and IDM = 14.49 for Hasanabad Chalous, both classified as semi-arid.

A total of 30 healthy trees of intermediate age were randomly selected for sampling. Sampling was conducted in June during the active growth season to ensure that the plant material was at a consistent physiological stage. From each tree, terminal branches bearing leaves (needles) were carefully cut from the mid-canopy using sanitized pruning shears. This approach minimizes environmental variability, as the mid-canopy receives more uniform light exposure compared to the upper and lower canopy regions. The collected plant material consisted of living, fully expanded, green, and healthy needles. These needles were carefully separated from the branches in the laboratory. Although the initial moisture content of the leaves was not quantitatively measured at the time of collection, all samples were processed uniformly upon arrival at the laboratory to ensure consistency. The needles were then shade-dried at ambient temperature until a constant weight was achieved before essential oil extraction. This drying step was critical

to standardize the starting material for the distillation process and ensure reproducible results.

The scientific identification and verification of the plant samples were performed by experts at the Herbarium of Islamic Azad University, Karaj Branch. Each sample was assigned an herbarium code. After labeling and coding, the samples were stored in a dry, dark environment for preservation.

Table 1. Annual Climatic Conditions of the Two Study Regions

Region	Annual Mean Temperature (°C)	Annual Precipitation (mm)	De Martonne Index	Climatic Classification	Soil Type
Rudbar	16.5	480	18.11	Semi-arid	Clay-loam
Hasanabad Chalous	14.53	370	14.49	Semi-arid	Sandy-loam

Extraction and Identification

Essential oils were extracted from the dried *Cupressus sempervirens* leaves using hydro-distillation with a Clevenger apparatus. Prior to extraction, the dried plant material was coarsely ground to a uniform particle size using a mechanical grinder. For each extraction, 100 g of ground plant material was immersed in 500 mL of distilled water in a 1000 mL distillation flask

The essential oils were obtained after 3 h of hydro-distillation. The collected essential oils were dehydrated with anhydrous sodium sulfate and filtered through Whatman No. 1 filter paper to remove any residual water and solid particulates. The purified essential oils were stored in sealed, dark glass vials at 4 °C to prevent degradation and were used within two weeks of extraction (Golestani *et al.* 2011).

The identification of essential oil components was conducted using gas chromatography-mass spectrometry (GC-MS). The GC-MS system was an Agilent 6890 equipped with a BPX5 capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness). The oven temperature program was as follows: initial temperature 60 °C (held for 1 min), increased to 240 °C at 3 °C/min, then to 290 °C at 10 °C/min (held for 5 min). The essential oil sample was diluted with analytical-grade n-hexane (1:100 v/v), and 1 µL of this solution was injected in split mode (split ratio 1:50). The injector temperature was maintained at 290 °C. Helium was used as the carrier gas at a constant flow rate of 0.5 mL/min. The mass spectrometer (Agilent 5973) operated in electron impact ionization mode at 70 eV, with ion source temperature of 230 °C and quadrupole temperature of 150 °C. Mass spectra were acquired over 40-500 *m*/*z* range. Components were identified by comparing their mass spectra and retention indices with those of authentic standards available in commercial libraries (NIST 14, Wiley 9) and published data (Adams 2001; McLafferty and Stauffer 1989).

Insect Rearing

Plodia interpunctella Hubner and *Tenebrio molitor* L. were obtained from the Insect Biological Control Laboratory, University of Tehran. *P. interpunctella* larvae were reared on an artificial diet containing wheat bran, honey, and glycerol under controlled conditions (25 ± 2 °C, 55 ± 15% relative humidity (RH), and a 16:8 L:D photoperiod) (Sait *et al.* 1997). Adult females were used for bioassays due to their longer lifespans. *T. molitor* was reared on a diet of rolled oats, bran, carrot, and potato under similar

laboratory conditions (55 ± 15% RH, 25 ± 2 °C). Both male and female adult beetles were used in bioassay studies without differentiation by sex.

Bioassay Procedures

Fumigant toxicity bioassays were conducted to evaluate the respiratory toxicity of the essential oils against *P. interpunctella* and *T. molitor*. The experiments were performed in carefully sealed containers to maintain the integrity of the vapor phase. Four concentrations were tested for each species: 55, 108, 160, and 250 µL/L for *P. interpunctella*; and 30, 68, 130, and 200 µL/L for *T. molitor*, with distilled water used as a control. For *P. interpunctella*, sterile cotton was impregnated with the specified volumes of essential oil using a digital micropipette and placed inside a 750-mL airtight Plexiglas container. To prevent direct contact between the insects and the essential oil, perforated plastic containers were positioned over the cotton. Ten female moths were released into each container (Fontenot *et al.* 2012).

For *T. molitor*, the assay was conducted in 350-mL airtight Plexiglas containers. Twenty adult beetles (both sexes, undifferentiated) were introduced into each container along with a piece of carrot as a moisture source. Sterile cotton impregnated with the essential oil was placed inside, and the containers were sealed to prevent vapor leakage. It is important to note that the use of sealed containers results in the establishment of saturated vapor conditions, where the vapor concentration is determined by the vapor pressure of the essential oil components and the headspace volume of the container (Tunç *et al.* 2003).

Under such conditions, the assessment of lethal time (LT) provides a precise measure of toxicity. Mortality was recorded at 6, 10, 18, and 24 hours after exposure. The data were subjected to Probit analysis (Finney 1971) using PoloPlus software (LeOra Software, 2006). Probit analysis is a standard method for analyzing such binary (dead/alive) response data in toxicological studies (Sikder *et al.* 2013).

The LC50 (Lethal Concentration 50%) values were calculated based on mortality at 18 hours post-treatment to facilitate comparison with previous studies using the above mentioned protocols. However, in recognition of the saturated vapor conditions inherent to our bioassay system, the LT50 (Lethal Time for 50% mortality) was also calculated for each essential oil to provide a more accurate representation of toxicity dynamics and the speed of action (Robertson *et al.* 2017).

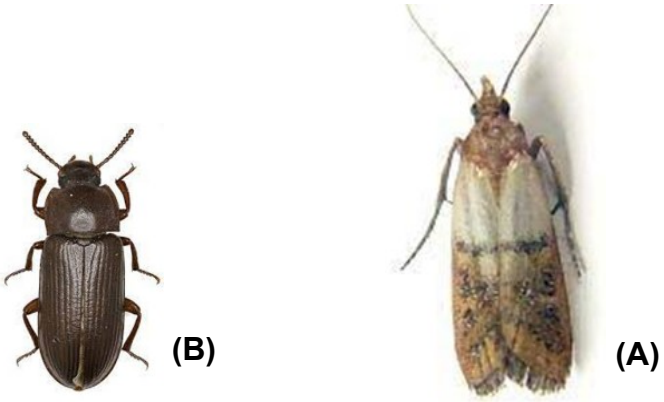

Fig. 1. Adult Indian meal moths (*Plodia interpunctella*), measuring 8 to 10 mm in length (A), and yellow mealworm (*Tenebrio molitor*), measuring 13 to 14 mm in length (B), used in the study

Data Analysis

The collected time-mortality data were statistically analyzed using PoloPlus software (LeOra Software, 2006). No mortality was observed in the control groups; therefore, no corrections to the treatment mortality data were necessary (Robertson *et al.* 2017). The response variable for the bioassays was binary (dead or alive), making Probit analysis an appropriate generalized linear model for dose-response and time-response data (Finney 1971). The concentration-mortality data at the 18-hour exposure point were analyzed to determine the LC50 (Lethal Concentration 50%) values and their corresponding 95% confidence intervals for each essential oil against both insect species. Furthermore, to precisely evaluate the speed of toxic action under the saturated vapor conditions of our bioassay, LT50 (Lethal Time 50%) values and their 95% confidence intervals were calculated from the time-mortality data for each essential oil (Tunç *et al.* 2003). Significant differences in lethal concentrations (LC50) and lethal times (LT50) between the essential oils from different habitats were determined based on the non-overlap of their 95% confidence intervals (Sakuma 1998).

RESULTS AND DISCUSSION

Chemical Composition

A comparison of Tables 2 and 3 reveals that the chemical compositions of *Cupressus sempervirens* from Rudbar and Hasanabad Chalous were largely similar for most identified compounds. However, differences in the number and type of compounds in *C. sempervirens* essential oils collected from these two habitats were also observed.

From *C. sempervirens* samples collected in Rudbar, 22 compounds were identified, accounting for 95.7% of the total composition. The chemical analysis revealed that the major compound was *α*-pinene (39.9%), followed by *δ*-3-carene (23.8%), terpinolene (4.9%), *α*-terpinyl acetate (4.4%), cedrol (4.5%), germacrene D (2.9%), and terpinen-4-ol (2.1%) (Table 2). Notably, compounds such as *α*-terpinene, *β*-phellandrene, terpinen-4-ol, *δ*-cadinene, and *α*-cadinol, were identified in the essential oils from Rudbar but were absent in those from Hasanabad Chalous.

In contrast, 24 compounds were identified in the essential oils from Hasanabad Chalous, accounting for 95.1% of the total composition. The analysis showed that *α*-pinene was also the dominant compound (58.5%), followed by *δ*-3-carene (8.0%), *α*-terpinyl acetate (5.8%), germacrene D (4.1%), cedrol (2.8%), myrcene (2.6%), and limonene (1.9%) (Table 3). Unique compounds, such as tricyclene, camphene, L-*α*-bornyl acetate, caryophyllene, *α*-humulene, *γ*-cadinene, and epimanoyl oxide, were found in the essential oils from Hasanabad Chalous but were not present in those from Rudbar.

Previous studies have also examined the phytochemical composition of *C. sempervirens* in other regions. For instance, a study conducted in Saudi Arabia analyzed the chemical composition, antimicrobial, and anti-biofilm activities of essential oils and methanolic extracts of *C. sempervirens*. That study identified 20 compounds, with *α*-pinene (48.6%), *δ*-3-carene (22.1%), limonene (4.6%), and *α*-terpinolene (4.5%) being the most abundant (Selim *et al.* 2014).

Similarly, a study in Tunisia investigated the chemical composition and biological potential of *C. sempervirens* essential oils. In Tunisia, this species is widely planted as an ornamental plant in the northern and central regions and used in traditional medicine for treating coughs, influenza, and rheumatism. The study identified 24 compounds, with *α*-

pinene (37.1%), *δ*-3-carene (19.7%), limonene (5.4%), and *α*-terpinolene (4.7%) as the major components (Boukhris *et al.* 2012).

Additionally, a study in Algeria analyzed the chemical composition and antimicrobial activity of essential oils extracted from *Juniperus phoenicea* L. and *C. sempervirens*. The study identified 35 compounds, with *α*-pinene as the main component (60.5%), followed by cedrol (8.3%) (Mazari *et al.* 2010).

In several studies, similar to the current findings, *α*-pinene has been identified as the primary component of the essential oil extracted from *C. sempervirens* leaves. However, the reported concentration of *α*-pinene in some studies is higher or lower than in the essential oils from Rudbar and Hasanabad Chalous (Mazari *et al.* 2010; Boukhris *et al.* 2012). As shown in Tables 2 and 3, the concentration of *α*-pinene, the dominant component of the essential oils, differs between Rudbar (39.9%) and Hasanabad (58.5%).

Additionally, some studies indicate that *α*-pinene is the second or third most abundant component of *C. sempervirens* essential oil. These differences in the number and type of identified compounds and the concentrations of major components may be attributed to tree age and habitat conditions (Sacchetti *et al.* 2005; Tapondjou *et al.* 2005).

Table 2. Chemical Composition of *Cupressus sempervirens* Essential Oil Collected in Rudbar

Gas Chromatography–Mass Spectrometry (GC/MS) Analysis					
No	**RT**	**%**	**Components**	**KI**	**Type**
1	11.03	0.30	*α*-Thujene	928	MH
2	**11.49**	**39.86**	***α*-Pinene**	**937**	**MH**
3	12.21	1.50	*α*-Fenchene	952	MH
4	13.47	1.60	Sabinene	977	MH
5	13.74	1.72	*β*-Pinene	982	MH
6	**14.32**	**2.57**	**Myrcene**	**994**	**MH**
7	**15.38**	**23.75**	***δ*-3-Carene**	**1014**	**MH**
8	15.79	0.32	*α*-Terpinene	1022	MH
9	**16.45**	**1.89**	**Limonene**	1035	MH
10	16.57	0.83	*β*-Phellandrene	1037	MH
11	17.98	0.52	*γ*-Terpinene	1064	MH
12	**19.37**	**4.38**	**Terpinolene**	**1091**	**MH**
13	20.20	0.40	Linalool	1108	MO
14	**24.39**	**2.11**	**Terpinen-4-ol**	**1193**	**MO**
15	31.82	0.38	*α*-Cubebene	1352	SH
16	**32.01**	**4.36**	***α*-Terpinyl acetate**	**1357**	**MO**
17	34.98	0.26	*β*-Cedrene	1425	SH
18	36.96	0.65	Bicyclosesquiphellandrene	1472	SH
19	**37.76**	**2.90**	**Germacrene D**	**1491**	**SH**
20	39.22	0.61	*δ*-Cadinene	1528	SH
21	**43.15**	**4.47**	**Cedrol**	**1629**	**SO**
22	44.80	0.37	*α*-Cadinol	1673	SO
		95.74	**Total Identified**		
Monoterpene Hydrocarbons, MH Oxygenated Monoterpenes, MO Sesquiterpene Hydrocarbons, SH Oxygenated Sesquiterpenes, SO					

Table 3. Chemical Composition of *Cupressus sempervirens* Essential Oil Collected in Hasanabad Chalous

GC/MS Analysis					
No	**RT**	**%**	**Components**	**KI**	**Type**
1	10.86	0.32	Tricyclene	925	MH
2	11.02	0.24	*α*-Thujene	928	MH
3	**11.49**	**58.53**	***α*-Pinene**	**937**	**MH**
4	12.20	0.46	*α*-Fenchene	952	MH
5	12.29	0.36	Camphene	953	MH
6	13.47	0.69	Sabinene	977	MH
7	13.73	1.77	*β*-Pinene	982	MH
8	**14.31**	**2.52**	**Myrcene**	**993**	**MH**
9	**15.32**	**8.05**	***δ*-3-Carene**	**1013**	**MH**
10	**16.44**	**2.01**	**Limonene**	**1035**	**MH**
11	17.98	0.40	*γ*-Terpinene	1064	MH
12	19.36	1.97	Terpinolene	1091	MH
13	20.20	1.42	Linalool	1108	MO
14	24.38	1.07	*L*-*α*-Bornyl acetate	1193	MO
15	31.81	0.38	*α*-Cubebene	1352	SH
16	**32.01**	**5.79**	***α*-Terpinyl acetate**	**1357**	**MO**
17	34.98	0.30	*β*-Cedrene	1425	SH
18	35.12	0.44	Caryophyllene	1428	SH
19	36.68	0.41	*α*-Humulene	1465	SH
20	36.96	0.26	Bicyclosesquiphellandrene	1472	SH
21	**37.76**	**4.07**	**Germacrene D**	**1491**	**SH**
22	39.22	0.64	*γ*-Cadinene	1528	SH
23	**43.14**	**2.82**	**Cedrol**	**1628**	**SO**
24	56.42	0.23	Epimanoyl oxide	2006	DH
		95.14	**Total Identified**		
Monoterpene Hydrocarbons, MH Oxygenated Monoterpenes, MO Sesquiterpene Hydrocarbons, SH Oxygenated Sesquiterpenes, SO Diterpene Hydrocarbons, DH					

Generally, the environmental factors of medicinal plant growth areas influence plants in three ways: (1) the total quantity of active compounds, (2) the composition of active ingredients, and (3) the dry weight of plants (Rostaefar *et al.* 2017). In this context, studies have shown that variations in the type and percentage of essential oil components can occur in different ecotypes of rosemary (*Rosmarinus officinalis*), thyme (*Thymus serpyllum*), juniper (*Juniperus communis*), and myrtle (*Myrtus communis*). These studies concluded that the quality and quantity of essential oils are affected by climatic conditions, such as differences in altitude (Mohammad-Nejad *et al.* 2006; Amir Azadi *et al.* 2013; Rostaefar *et al.* 2017).

Respiratory Toxicity

The respiratory toxicity of *Cupressus sempervirens* essential oils from Rudbar and Hasanabad habitats against adult *Plodia interpunctella* and *Tenebrio molitor* was evaluated over a 24-hour exposure period. The time-mortality relationships are presented in Figs. 2 and 3.

For *Plodia interpunctella* (Fig. 2), both essential oils exhibited clear time- and concentration-dependent mortality. The cumulative mortality increased progressively with prolonged exposure time at all tested concentrations. The essential oil from the

Rudbar habitat demonstrated a steeper increase in mortality over time compared to the Hasanabad oil (Fig. 2), particularly at the higher concentrations (160 and 250 µL/L), indicating a faster toxic action. The final mortality after 24 hours was also generally higher in the Rudbar treatments. This suggests that the unique combination of compounds in the Rudbar oil, such as α-terpinene, β-phellandrene, and terpinen-4-ol (Table 2), confers superior fumigant toxicity against the Indian meal moth.

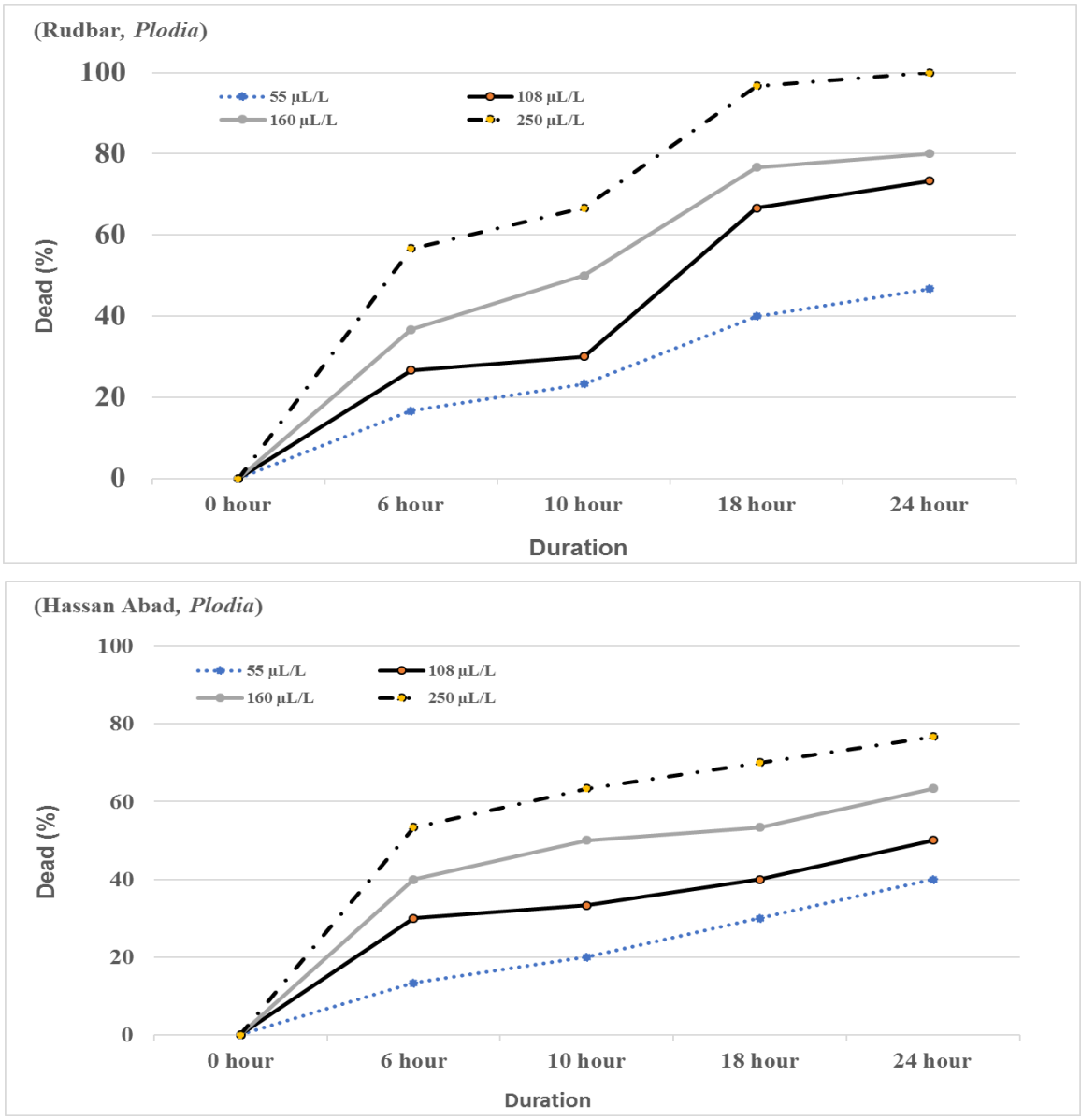

Fig. 2. Cumulative mortality (%) of *Plodia interpunctella* adults exposed to different concentrations of *Cupressus sempervirens* essential oil (µL/L) from Rudbar and Hassan Abad habitats over a 24-hour period

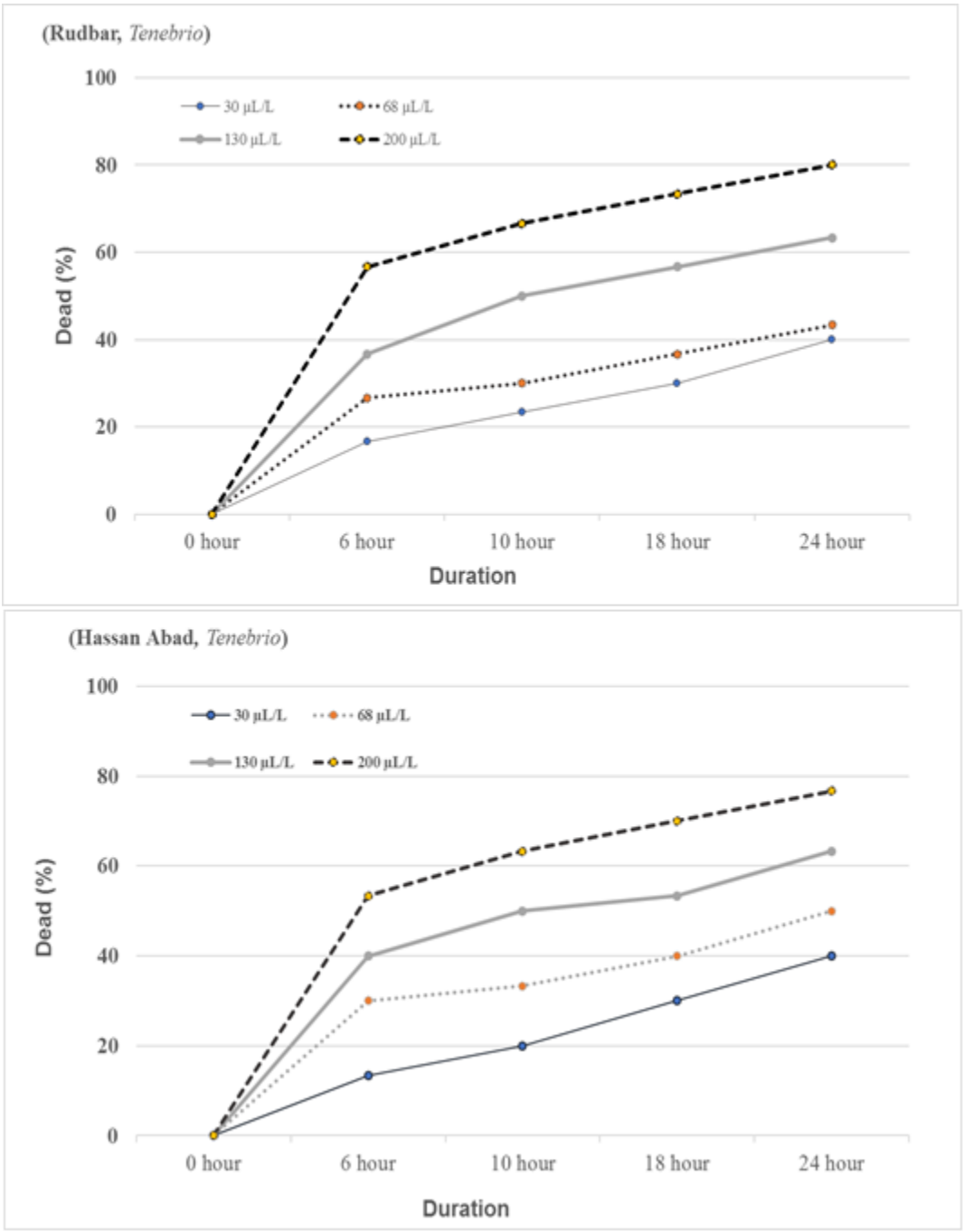

Fig. 3. Cumulative mortality (%) of *Tenebrio molitor* adults exposed to different concentrations of *Cupressus sempervirens* essential oil (µL/L) from Rudbar and Hassan Abad habitats over a 24-hour period

For *Tenebrio molitor* (Fig. 3), a different pattern of efficacy was observed. The fumigant toxicity of the essential oil from the Hasanabad habitat was markedly more pronounced than that of the Rudbar oil. This superior effect was particularly evident at the higher concentrations of 130 and 200 µL/L especially during the 18- and 24-hour exposure periods. The mortality level achieved by the Hasanabad oil was nearly equivalent to that of the Rudbar oil shorter exposure. This result clearly demonstrates that

the fumigant efficacy is both species-dependent and habitat-dependent, with the high α-pinene content (58.5%) and the specific chemical profile of the Hasanabad oil being particularly effective against the yellow mealworm.

The statistical analysis of the respiratory toxicity effects of *C. sempervirens* essential oils from different habitats against adult insects of two species, *Plodia interpunctella* and *Tenebrio molitor*, is presented in Tables 4 and 5. It is noteworthy that, due to the absence of mortality in the control group, no adjustments were made to the mortality rates in the treatments.

The probit analysis of mortality data for *Plodia interpunctella* revealed significant differences in the toxicity of essential oils from the two habitats (Table 4). The essential oil from Rudbar was significantly more toxic, requiring a much lower concentration to achieve 50% mortality after 18 hours (LC_{50} = 96.65 µL/L) compared to the oil from Hasanabad (LC_{50} = 176.90 µL/L). This nearly two-fold difference in potency was statistically significant, as indicated by non-overlapping 95% confidence intervals. Critically, the analysis of lethal time provided a deeper insight into the speed of action. The Rudbar oil also acted more rapidly, achieving 50% mortality in approximately 12.1 hours (LT_{50}), which was about 3.7 hours faster than the Hasanabad oil (LT_{50} = 15.8 hours). This combination of a lower LC_{50} and a lower LT_{50} unequivocally demonstrates the superior fumigant efficacy of the Rudbar essential oil against the Indian meal moth. The steeper slope for the Rudbar oil indicates that the insect population responded more uniformly to the treatment. This means that a small increase in concentration resulted in a predictable and sharp rise in mortality, showing a consistent susceptibility among most of the moths.

The probit analysis of fumigant toxicity against *Tenebrio molitor* revealed marked differences between the essential oils from the two habitats (Table 5). In contrast to the results observed for *P. interpunctella*, the essential oil from Hasanabad was significantly more toxic to *T. molitor*. This was evidenced by its substantially lower LC_{50} value (130.9 µL/L) compared to the Rudbar oil (253.4 µL/L). The fact that the 95% confidence intervals for these LC_{50} values do not overlap provides strong evidence that this difference in potency is statistically significant.

Analysis of the lethal time provided further insight into the speed of action. The Hasanabad oil also acted more rapidly, with an LT_{50} of 11.8 hours, achieving 50% mortality approximately 2.4 hours faster than the Rudbar oil (LT_{50} = 14.2 hours). The superior efficacy of the Hasanabad essential oil against the yellow mealworm is likely attributable to its distinct chemical profile, most notably its high concentration of α-pinene (58.5%), to which *T. molitor* appears to be particularly susceptible.

Table 4. Toxicity Parameters of *Cupressus sempervirens* Essential Oils from Two Different Habitats Against Adult *Plodia interpunctella*

Plant Essence	X^2 (df)	Sig	Heterogeneity	Slope ± SE	LT_{50} (Hours)	LC_{50} (µl/L Air)	Confidence Limits 95%	
							lower	upper
C.R[a]	1.49 (2)	0.004	0.74	2.69 ± 0.57	12.1	**96.65**	64.97	121.30
C.H[b]	0.52 (2)	0/003	0.26	1.58 ± 0.49	15.8	**176.9**	122.38	280.59

Total numbers of used insects in bioassay = 230
C.R = *Cupressus* from Roudbar, C.H = *Cupressus* from Hassanabad; a, b show a significant difference

Table 5. Toxicity Parameters of *Cupressus sempervirens* Essential Oils from Two Different Habitats Against Adult *Tenebrio molitor*

Plant Essence	X^2 (df)	Sig	Heterogeneity	Slope ± SE	LT_{50} (Hours)	LC_{50} (µl/L Air)	Confidence Limits 95%	
							lower	upper
C.R[a]	1.25 (2)	0.001	0.62	1.38 ± 0.39	14.2	**253.38**	161.29	408.28
C.H[a]	1.83 (2)	0.00	0.92	1.61 ± 0.40	11.8	**130.90**	65.7	186.7

Total numbers of used insects in bioassay = 230
C.R = *Cupressus* from Roudbar, C.H = *Cupressus* from Hassanabad; a, b show a significant difference

The results showed that the essential oils obtained from *C. sempervirens* in both Rudbar and Hasanabad habitats caused respiratory toxicity in *P. interpunctella* and *T. molitor*. Typically, the main insecticidal or deterrent effect of an essential oil is attributed to its dominant component. Based on the results, the essential oils from both regions contained high levels of *α*-pinene. Because monoterpenes, due to their lower molecular weight compared to other types of terpenes, exhibit higher fumigant properties, they effectively reduced the population of the insects (*P. interpunctella* and *T. molitor*) in this research (Boukhris *et al.* 2012; Singh and Dwivedi 2018; Labbafi *et al.* 2021).

The respiratory toxicity of pure monoterpene compounds and essential oils containing these compounds has also been reported against various pests in other scientific studies (Mahfuz and Khalequzzaman 2007). It is suggested that the blockage of the insect respiratory system by plant-derived compounds can disrupt respiration, ultimately leading to insect mortality (Ileke and Ogungbite 2014). Because this study was conducted on adult insects and the fact that plant-derived essential oils and extracts pose fewer risks to humans and the environment, *C. sempervirens* essential oil could serve as a natural alternative to chemical insecticides for the control of adult *P. interpunctella* and *T. molitor*. Emphasis on adult insects is significant, as the respiratory toxicity of monoterpenes may vary across different life stages of the insects (Rafiei-Kahroudi 2010; Bakhtiari *et al.* 2013). This research focused on adult insects, and many researchers have concluded that the deterrent effects of plant extracts and essential oils are greater on adult insects than on larvae (Labbafi *et al.* 2021; Mazdaee *et al.* 2019).

Moreover, the current study's results indicated that the fumigant toxicity of *C. sempervirens* essential oil from Rudbar was higher against *P. interpunctella* (Table 4), whereas the fumigant toxicity of *C. sempervirens* essential oil from Hasanabad was higher against *T. molitor* (Table 5). This result is likely due to differences in the chemical compositions of the essential oils (Tables 2 and 3) and the varying sensitivities of the two insect species to these compounds. As mentioned, *α*-pinene is the dominant compound in both regions; however, its concentration was 58.5% in the essential oil from Hasanabad and 39.9% in the essential oil from Rudbar. It seems that compounds, such as *α*-terpinene, *β*-phellandrene, terpinen-4-ol, *δ*-cadinene, and *α*-cadinol, identified in the essential oil from Rudbar but absent in the Hasanabad oil, contributed to the higher fumigant toxicity against adult *P. interpunctella*. In contrast, adult *T. molitor* appeared to be more sensitive to the higher concentrations of *α*-pinene, camphene, L-*α*-bornyl acetate, caryophyllene, *α*-humulene, *γ*-cadinene, and epimanoyl oxide, which were present in the Hasanabad essential oil. Additionally, differences in the chemical compositions of the

essential oils from the two habitats may be attributed to the fact that the quality and quantity of secondary metabolites in different plant organs are influenced by environmental, ecological, and genetic factors (Lei *et al.* 2010; Paolini *et al.* 2010; Motazedian *et al.* 2012).

As the chemical compositions and fumigant toxicities of the essential oils differed between the two habitats, similar studies in different habitats are recommended to investigate the relationships between qualitative indices (key chemical components) and environmental factors (*e.g.*, climate, soil).

CONCLUSIONS

1. The chemical analysis confirmed that α-pinene was the dominant component in the essential oils of *Cupressus sempervirens* from both the Rudbar and Hasanabad Chalous habitats. However, its concentration and the overall chemical profile were significantly influenced by the habitat, with the Hasanabad oil containing a higher percentage of α-pinene (58.5% *vs.* 39.9%) and a distinct suite of secondary compounds.
2. The bioassay results demonstrated that the essential oils from both habitats exhibited significant respiratory toxicity against the adult pests. Crucially, the efficacy was both habitat-dependent and species-specific. The essential oil from the Rudbar habitat was significantly more potent and faster-acting against *P. interpunctella*, whereas the oil from the Hasanabad Chalous habitat was more effective against *T. molitor*.
3. This study conclusively showed that the insecticidal activity of *C. sempervirens* essential oil is not universal but is intrinsically linked to its chemotypic variation, which is driven by geographical origin. Therefore, *C. sempervirens* essential oil holds strong potential for the development of targeted, selective, and eco-friendly strategies for managing stored-product pests, with the selection of the plant source being a critical factor for success against a specific pest.

REFERENCES CITED

Adams, R. P. (2001). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, Allured Publishing Corporation, Carol Stream, IL, USA.

Almadiy, A. A., and Nenaah, G. E. (2022). "Bioactivity and safety evaluations of *Cupressus sempervirens* essential oil, its nanoemulsion and main terpenes against *Culex quinquefasciatus* Say," *Environ. Sci. Pollut. Res. Int.* 29(9), 13417-13430. https://doi.org/10.1007/s11356-021-16634-z

Amir Azadi, Z., Pilevar, B., Meshkatsadat, M. H., Karamian, R., Alirezaii, M., and Khunsari, A. (2013). "The effect of the main ecological factors on the yield of the essential oil of the tree (*Myrtus communis* L.) in different forest habitats of Lorestan province," *Quart. Res. J. Lor. Univer. Med. Sci.* 14(3), 103-111. https://doi.org/10.1007/s11356-021-16634-z

Bande-Borujeni, S., Zandi-Sohani, N., and Ramezani, L. (2016). "Chemical composition and insecticidal effects of essential oil from *Citrus aurantium* L. leaves on three major stored product pests," *Plant. Prot. Sci. J. Agri.* 38(4), 23-32. (In Persian).

Bakhtiari, M., Rafiei Karahroudi, Z., and Sanatgar, E. (2013). "The effect of contact and respiratory repellency of four medicinal plant extract and powders on adults and last instar larvae of *Plodia interpunctella* (Hübner)," *J. Entomol. Res*. 7(1), 17-27. (In Persian).

Boukhris, M., Regane, G. H., Yangui, T. H., Sayadi, S., and Bouaziz, M. (2012). "Chemical composition and biological potential of essential oil from Tunisian *Cupressus sempervirens* L.," *J. Arid Land. Stud*. 22(1), 329-332.

Fontenot, E. A., Arthur, F. H., Nechols, J. R., and Throne, J. E. (2012). "Using a population growth model to simulate response of *Plodia interpunctella* (Hübner) to temperature and diet," *J. Pest. Sci*. 85(1), 163-167. https://doi.org/10.1007/s10340-011-0411-0

Finney, D. J. (1971). *Probit Analysis*, 3rd Ed.). Cambridge University Press.

Golestani, Z., Moravej, G. H., Azizi Arani, M., and Hatefi, S. (2011). "Fumigant toxicity of the essential oils from *Lavandula angustifolia* (Mill) and *Zataria multiflora* (Boiss) on Cowpea Weevil, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae)," *J. Plant. Prot*. 25(3), 286-295. (in Persian).

Goulson, D., Nicholls, E., Botías, C., and Rotheray, E. L. (2015). "Bee declines driven by combined stress from parasites, pesticides, and lack of flowers," *Science* 347(6229), 1255957. https://doi.org/10.1126/science.1255957

Gruľová, D., Baranová, B., Sedlák, V., De Martino, L., Zheljazkov, V. D., and Konečná, M. (2022). "*Juniperus horizontalis Moench*: Chemical composition, herbicidal and insecticidal activities of its essential oil and of its main component, sabinene," *Molecules* 27(23), article 8408. https://doi.org/10.3390/molecules27238408

Hesabi, A., Alavi, S. J., and Esmailzadeh, O. (2019). "Studying the interaction between English yew (*Taxus baccata* L.) adult trees and its regeneration in Afratakhteh Forest Reserve, Golestan province," *Iran. J. Forest*. 11(2), 165-177. (in Persian).

Ileke, K. D., and Ogungbite, O. C. (2014). "Entomocidal activity of powders and extracts of four medicinal plants against *Sitophilus oryzae* L., *Oryzaephilus mercator* (Faur) and *Ryzopertha dominica* (Fabr.)," *Jor. J. Biol. Sci.* 7(1), 57-62.

Isman, M. B. (2020). "Botanical insecticides in the twenty-first century—fulfilling their promise?" *Annu. Rev. Entomol*. 65(1), 233-249, https://doi.org/10.1146/annurev-ento-011019-025010

Kim, K. H., Kabir, E., and Jahan, S. A. (2017). "Exposure to pesticides and the associated human health effects," *Sci. Total Environ*. 575, 525-535, https://doi.org/10.1016/j.scitotenv.2016.09.009

Labbafi, M., Ahvazi, M., Khalighi-Sigaroodi, F., Khalaj, H., Ahmadian, S., and Tajabadi, F. (2021). "Essential oil bioactivity evaluation of the different populations of *Cupressus* against adult rice weevil (*Sitophilus oryzae* L.)," *J. Med. Plants* 20(77), 79-92. https://doi.org/10.29252/jmp.20.77.79

Langsi, D. J., Tofel, H. K., Fokunang, C. N., Suh, C., Eloh, K., and Caboni, P. (2018). "Insecticidal activity of essential oils of *Chenopodium ambrosioides* and *Cupressus sempervirens* and their binary combinations on *Sitophilus zeamais*," *GSC. Biol. Pharm. Sci.* 3(2), 024-034. https://doi.org/10.30574/gscbps.2018.3.2.0032

Lei, H., Wang, Y., Liang, F., Su, W., Feng, Y., and Guo, X. (2010). "Composition and variability of essential oils of *Platycladus orientalis* growing in China," *Biochem. Syst. Ecol* 38(5), 1000-1006. https://doi.org/10.1016/j.bse.2010.09.018

LeOra Software (2006). *POLO-Plus: A User's Guide to Probit or Logit Analysis*, Berkeley, CA, USA.

Mahfuz, I., and Khalequzzaman, M. (2007). "Contact and fumigant toxicity of essential oils against *Callosobruchus maculates*," *Univ. J. Zool, Rajshahi Univ.* 26, 63-66. https://doi.org/10.3329/ujzru.v26i0.701

Martínez, L. C., Plata-Rueda, A., Colares, H. C., Campos, J. M., Dos Santos, M. H., and Fernandes, F. L. (2018). "Toxic effects of two essential oils and their constituents on the mealworm beetle, Tenebrio molitor," *Bull. Entomol. Res.* 108(6), 716-725. https://doi.org/10.1017/S0007485317001262

Mazari, K., Bendimerad, N., Bekhechi, C., and Fernandez, X. (2010). "Chemical composition and antimicrobial activity of essential oils isolated from Algerian *Juniperus phoenicea* L. and *Cupressus sempervirens* L.," *J. Med. Plants Res.* 4(10), 959-964. https://doi.org/10.5897/JMPR10.169

Mazdaee, A., Hassani, M. R., and Sheibani Tezerj, Z. (2019). "Repellency effect of essential oil, plant extract and powder of seed of *Ferula assafoetida* on *Plodia interpunctella* (Lep: Pyralidae)," *J. Entomol. Res.* 11(1), 43-53. (In Persian).

McLafferty, F. W., and Stauffer, D. B. (1989). *The Wiley/NBS Registry of Mass Spectral Data*, Wiley, New York, NY, USA.

Mohammad-Nejad, S. M., Moradi, H., Ghanbari, A., and Akbarzadeh, M. (2006). "A study on the effect of height on the quality and quantity of the active ingredients of the essential oil of *Rosmarinus officinalis* L. which is cultivated in two areas of Mazandaran province," *Quart. J. Ecophytochem. Med. Plant*. 1(2), 36-42. (in Persian).

Moharramipour, S., Nazemi Rafieh, J., Morovati, M., Talebi, A. A., and Fathipour, Y. (2003). "Effectiveness of extracts of *Nerium oleander*, *Lavandula officinalis* and *Ferula assafoetida* on nutritional indices of *Tribolium castaneum* adults," *J. Entomol. Soc. Iran* 23(1), 69-89. (in Persian).

Moein, M., Hatam, G., Taghavi-Moghadam, R., and Zarshenas, M. M. (2017). "Antileishmanial activities of Greek Juniper (*Juniperus excelsa* M. Bieb.) against *Leishmania major* promastigotes," *J. Evid. Based Complementary Altern. Med*. 22(1), 31-36. https://doi.org/10.1177/2156587215623435

Motazedian, N., Ravan, S., and Bandani, A. R. (2012). "Toxicity and repellency effects of three essential oils against *Tetranychus urticae* Koch (Acari: Tetranychidae)," *J. Agri. Sci. Technol*. 14, 275-284. (in Persian).

Mwine, T. J., Van Damme, P., Hastilestari, B. R., and Papenbrock, J. (2013). "*Euphorbia triucalli* L. (Euphorbiaceae): The miracle tree: Current status of knowledge," *African Natural Plant Products, Volume II: Discoveries and Challenges in Chemistry, Health and Nutrition* 1127, 4-17. https://doi.org/10.5897/SRE10.1143

Nenaah, G. E. (2014). "Chemical composition, toxicity and growth inhibitory activities of essential oils of three Achillea species and their nano-emulsions against *Tribolium castaneum* (Herbst)," *Ind. Crops. Prod*. 53, 252-260. https://doi.org/10.1016/j.indcrop.2013.12.042

Nerio, L. S., Olivero-Verbel, J., and Stashenko, E. E. (2009). "Repellent activity of essential oils from seven aromatic plants grown in Colombia against *Sitophilus*

zeamais Motschulsky (Coleoptera)," *J. Stored. Prod. Res*. 45(3), 212-219. https://doi.org/10.1016/j.jspr.2009.01.002

Nazemi Rafih, J., and Moharramipour, S. (2008). "Repellency of *Nerium oleander* L., *Lavandula officinalis* L. and *Ferula assafoetida* L. extracts on *Tribolium castaneum* (Herbst)," *Iran. J. Med. Arom. Plants.* 23(4), 443–452. (in Persian).

Nazirzadeh, S., Bayramzadeh, V., Khalighi-Sigaroodi, F., Pourhosseini, L., and Hossinashrafi, S. (2024). "Respiratory toxicity of essential oils extracted from Greek juniper (*Juniperus excelsa* M. Bieb.) needles originated from two different habitats against Indian meal moth (*Plodia interpunctella* (Hübner)) and mealworm (*Tenebrio molitor* L.)," *J. Forest Wood. Prod*. 76(4), 313-327. (in Persian). https://doi.org/10.22059/jfwp.2024.367337.1265

Omid Beigi, R. (1996). *Producing and Processing of Medicinal Plants*, Tarahan Nashr Publication, Mashhad, Iran.

Omid Beigi, R. (2000). *The Production and Processing of Medicinal Plants*, Fekr Rooz Publication, Tehran, Iran.

Paolini, J., Barboni, T., Desjobert, J.-M., Djabou, N., Muselli, A., and Costa, J. (2010). "Chemical composition, intraspecies variation and seasonal variation in essential oils of *Calendula arvensis* L.," *Biochem. Syst. Ecol*. 38(5), 865-874. https://doi.org/10.1016/j.bse.2010.07.009

Plata-Rueda, A., Zanuncio, J. C., Serrão, J. E., and Martínez, L. C. (2021). "*Origanum vulgare* essential oil against *Tenebrio molitor* (Coleoptera: Tenebrionidae): Composition, insecticidal activity, and behavioral response," *Plants* 10(11), article 251. https://doi.org/10.3390/plants10112513

Rafiei-Kahroudi, Z. (2010). "Evaluation of insecticidal effect of essential oils and multiple extracts of medicinal plants on some biological properties of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)," *Iran. J. Med. Arom. Plant*. 25(1), 124-142. (in Persian).

Ramzi, S., Seraji, A., Azadi Gonbad, R., Kimia Mirhaghparast, S., Mojib Haghghadam, Z., and Haghighat, S. H. (2017). "Toxicity of *Artemisia annua* (Asteraceae) essential oil on the tea mealy bug, *Pseudococcus viburni Sigornet* (Hemiptera: Pseudococcidae)," *Pseudococcus viburni Sigornet* (Hemiptera: Pseudococcidae), *Arch. Phytopathol. Plant Prot.* 50(19-20), 941-956. https://doi.org/10.1080/03235408.2017.1352223

Robertson, J. L., Jones, M. M., Olguin, E., and Alberts, B. (2017). *Bioassays with Arthropods*, 3rd Ed., CRC Press.

Rostaefar, A., Hassani, A., and Sefidkon, F. (2017). "Effect of altitude on essential oil composition in different gender of *Juniperus communis* ssp. hemisphaerica growing wild in Amol," *Quart. J. Ecophytochem. Med. Plant.* 21(1), 1-10. (in Persian).

Sacchetti, G., Maietti, S., Muzzoli, M., Scaglianti, M., Manfredini, S., and Radice, M. (2005). "Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods," *Food. Chem.* 91(4), 621-632. https://doi.org/10.1016/j.foodchem.2004.06.031

Sadeghi, R., Mirzaei, M., Ebadollahi, A., Jamshidnia, A., and Javid, M. (2022). "Effect of saffron petal extract on the mortality and enzymatic changes of the larvae of Indian meal moth, *Plodia interpunctella* (Hübner)," *J. Crops. Impr*. 25(1), 197-208. (in Persian).

Sait, S. M., Begon, M., Thompson, D. J., Harvey, J. A., and Hails, R. S. (1997). "Factors affecting host selection in an insect host-parasitoid interaction," *Ecol. Entomol*. 37(2), 225-230. https://doi.org/10.1046/j.1365-2311.1997.t01-1-00051.x

Selim, S. A., Adam, M. E., Hassan, S. M., and Albalawi, A. R. (2014). "Chemical composition, antimicrobial and antibiofilm activity of the essential oil and methanol extract of the Mediterranean cypress (*Cupressus sempervirens* L.)," *BMC Complement. Altern. Med*. 14(1), article 179. https://doi.org/10.1186/1472-6882-14-179

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., Kohli, S. K., Yadaz, P., Bali, A. S., Parihar, R. D., *et al.* (2019). "Worldwide pesticide usage and its impacts on ecosystem," *SN Appl. Sci*. 1, 1446, https://doi.org/10.1007/s42452-019-1485-1

Sikder, S., Biswas, P., Hazra, P., Akhtar, S., Chattopadhyay, A., and Badigannavar, A. M. (2013). "Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS," *Indian. J. Genet. Plant Breed*. 73(4), 392-399. https://doi.org/10.5958/j.0975-6906.73.4.059

Singh, A., and Dwivedi, P. (2018). "Methyl-jasmonate and salicylic acid as potent elicitors for secondary metabolite production in medicinal plants: A review," *J. Pharmacog. Phytochem.* 7(1), 750-757.

Tapondjou, A. L., Adler, C., Fontem, D. A., Bouda, H., and Reichmuth, C. (2005). "Bioactivities of cymol and essential oils of *Cupressus sempervirens* and *Eucalyptus saligna* against *Sitophilus zeamais Motschulsky* and *Tribolium confusum* du Val," *J. Stored. Prod. Res*. 41(1), 91-102. https://doi.org/10.1016/j.jspr.2004.01.004

Tripathi, A. K., Prajapati, V., Aggarwal, K. K., and Kumar, S. (2001). "Toxicity, feeding deterrence, and effect of activity of 1,8-cineole from *Artemisia annua* on progeny production of *Tribolium castanaeum* (Coleoptera: Tenebrionidae)," *J. Econ. Entomol*. 94(4), 979-983. https://doi.org/10.1603/0022-0493-94.4.979

Tunç, I., Berger, B. M., Erler, F., and Dağlı, F. (2003). "Ovicidal activity of essential oils from five plants against two stored-product insects," *J. Stored Prod. Res*. 39(3), 245-255. https://doi.org/10.1016/S0022-474X(02)00004-4

Ziaee, M. (2014). "The effects of topical application of two essential oils against *Sitophilus granarius* (Coleoptera: Curculionidae) and *Tribolium confusum* (Coleoptera: Tenebrionidae)," *J. Crop. Prot*. 34(2), 589-595. (in Persian).

Zuzarte, M., Gonçalves, M. J., Cavaleiro, C., Cruz, M. T., Benzarti, A., and Marongiu, B. (2013). "Antifungal and anti-inflammatory potential of *Lavandula stoechas* and *Thymus herba-barona* essential oils," *Ind. Crops Prod*. 44, 97-103. https://doi.org/10.1016/j.indcrop.2012.11.002

Article submitted: May 5, 2025; Peer review completed: November 7, 2025; Revised version received: November 28, 2025; Accepted: November 30, 2025; Published: December 15, 2025.
DOI: 10.15376/biores.21.1.1013-1029