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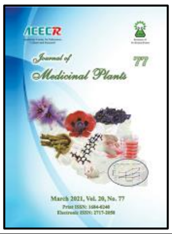

Research Article

Essential oil bioactivity evaluation of the different populations of *Cupressus* against adult rice weevil (*Sitophilus oryzae* L.)

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ABSTRACT

Background: A significant amount of crop production is destroyed annually by pests in the storage and significant damage is caused to crops. Recently, the use of essential oils as an alternative to pesticides in pest control has been considered. Due to the presence of *α*-pinene, *Cupressus* genus is a significant source of plant insecticides that can act as fumigant, repellency and inhibitory insecticides. **Objective:** The aim of this study was to investigate the insecticidal properties, repellency and nutritional parameters of essential oils of *Cupressus* species on *Sitophilus oryzae* in 2018. **Methods:** The experiment was conducted in a factorial arrangement based on completely randomized design with 4 replications. The essential oils of the different species and populations of *Cupressus* were prepared by the Clevenger apparatus using a water distillation method. The major constituents in the essential oils were analyzed by GC-MS and the biological effects of the essential oils against adult *S. oryzae* were investigated. **Results:** In the analysis of chemical compounds, *α*-pinene was identified as the main essential oils of the above species. The result showed that among the species, *C. arizonica* showed the highest Fumigant toxicity to *S. oryzae* with LC_{50} = 172.30 µl/L air. In investigating the effect of repellency on *S. oryzae*, essential oils of *C. sempervirens* L. var. *horizontalis* (France) with 80.61 % showed the highest distance. **Conclusion:** Due to the toxicity and high repellency effect of *Cupressus* essential oils on *S. oryzae*, this genus has high potential for use in storage pest control.

1. Introduction

Every year, a significant amount of agricultural production in developing countries is destroyed by storage pests and crops suffer great damage [1]. *Sitophilus oryzae* belongs to Coleoptera and they are storage pest, while feeding on storage materials and causes irreparable damage to crops [2]. In order to maintain the quantity and quality of stored cereals, it is necessary to reduce the population

Abbreviations: RGR, Relative Growth Rate; RCR, Relative Consumption Rate; ECI, Efficiency of Conversion of Ingested food

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of storeroom insects. Considering the economic importance of pests and the problems associated with the use of fumigant chemical insecticides and the prohibition on their use [3], the substitution of other suitable methods seems necessary [4]. Essential oils and plant extracts are suitable alternatives for synthetic pesticides that have the lowest risk to humans and the environment. Essential oils extracted from plants are usually decomposed fast in nature, so they are less toxic to humans and other mammals and have less detrimental effects on the environment [5]. *Cupressus* is a significant source of plant insecticides. Some studies have been proven the insecticidal and fungicidal properties of the genus of Cupressus. The *Cupressaceae* family includes 32 genera in the world which two species of them, *Cupressus arizonica* and *Cupressus sempervirens,* exist in Iran [6]. *Cupressus sempervirens* L. has two varieties called *Cupressus sempervirens* L. var. *horizontalis* (Mill.) Gord (Persian name: Zarbin), and *Cupressus sempervirens* L. var. *stricta* Aiton (Persian name: Sarv-e-Shirazi) [7].

In a review article, the researchers examined the effects of plant essential oils on storage pests and listed the most important ones [8]. In one experiment, researchers examined the biological activity of acetone, ethanol extracts of *Cupressus sempervirens* on third instar larvae of *Culex pipiens*. It was found that ethanol extract is more toxic than acetone and ethanol extract. It also causes morphological abnormalities in both stages of mosquito life (larvae and adult insects) [9]. The effect of the essential oils of *Cupressus sempervirens* on 10 pathogenic fungi examined and showed that *Cupressus sempervirens* had the fungicides property [10]. In another study, the effects of the fumigant toxicity of *Pinus eldarica* and *Cupressus sempervirens* was tested on adult moths. The results showed that *Cupressus sempervirens* had less toxic effect on flour moth [11].

The aim of this study was to evaluate the efficacy of *Cupressus* species essential oils. Another aim was to investigate the insecticidal properties, repellency and nutritional parameters of *Cupressus* essential oils in Iran and compare it with pure essential oils prepared from France for the management of adult rice weevil.

2. Materials and Methods

2.1. Plant collection and extraction of essential oils

The aerial parts of *C. sempervirens* var. *horizontalis* and *C. arizonica* were collected from Tehran province and *C. sempervirens* var. *horizontalis* and *C. sempervirens* var. *stricta* were collected from Mazandaran province (Chalus valley) (Table 1 & Fig. 1). After collection, the plants were dried in the shade. The essential oils were extracted from 100 g of every herbal samples for 3 hours using Clevenger apparatus. The essential oils were dehydrated with anhydrous sodium sulfate and stored in dark glass in the refrigerator (3-5 °C) before experiments.

2.2. Insect rearing

Colonies of rice weevil were obtained from Agricultural Entomology Research Department of Iranian Research Institute of Plant Protection (Tehran-Iran). Colonies were reared on 300 g of rice and 200 g of flour in 1-L cans in a germinator at 27 ± 1 °C with relative humidity 70 ± 5 % and 12:12 hours light-dark photoperiod. The released insects were fed on rice and flour for 3 weeks and then removed. After 7-14 days since the first insects emerged on rice and flour, they were used as the same-age insects for testing [12].

Table 1. Location, latitude, longitude and altitude of studied *Cupressus* species

Species	Persian Name	Herbarium code	Location	Latitude (N)	Longitude (E)	Altitude (m)
C. sempervirens L. var. *horizontalis* (Mill.) Gord	Zarbin	1388 (MPIH)	Tehran	35° 45' 11"	51° 16 78"	1450
C. sempervirens L. var. *horizontalis* (Mill.) Gord	Zarbin	1387 (MPIH)	Chalus	36° 31' 95"	51° 20' 79"	319
C. sempervirens L. var. *stricta* Aiton	Sarv-e-Shirazi	1386 (MPIH)	Chalus	36° 31' 95"	51° 20' 79"	319
C. arizonica Greene	Sarv-e-Simin, Sarv-e-Noghrei	1389 (MPIH)	Tehran	35° 77' 06"	51° 41' 60"	1460

C. sempervirens var. *horizontalis* (Zarbin: Chalus) | *C. sempervirens* var. *stricta* (Sarv-e-Shirazi: Chalus) | *C. arizonica* (Sarv-e-Simin: Tehran) | *C. sempervirens* var. *horizontalis* (Zarbin: Tehran)

Fig. 1. Figure of studied *Cupressus* species

2.3. Fumigant toxicity of essential oils

Fumigant toxicity measurements of essential oils were performed according to the method of Amini, et al. [12]. 15 adult rice weevil (7-14 days old) were transferred on 5 g of rice in 70-ml glass containers, then a filter paper with 2 cm in diameter inoculated with essential oils (with concentrations calculated in the 0, 71.5, 143, 214, 286, 357, 428.5 µl/L of air) was inserted into the glass container lid. The control treatment was included 15 adult rice weevil on 5 g of rice in glass containers but filter paper did not inoculate with essential oils.

In this experiment, insects that were unable to move their legs were considered to be dead. The experiment was carried out in a completely randomized design with factorial arrangement with 4 replications in Ecophysiology Laboratory, Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR in 2018. The essential oils of 5 samples including *C. sempervirens* var. *horizontalis* (Tehran & Chalus), *C. sempervirens* var. *stricta* (Chalus), *C. arizonica* (Tehran) and *C. sempervirens* (France) was performed on rice lice. Mortality rates were counted after 24, 48 and 72 hours since the experiment was started and LC_{50} was determined using Polo Plus software [13].

2.4. Repellency of the essential oils

The effect of repellency of prepared essential oils was performed on a filter paper by environmental method [14]. For this purpose, filter paper (9 cm) is divided into two halves with essential oils at concentrations of 10, 15, 20, 25 µl per 31.79 cm^2 and the other half was just stained with solvent (acetone) as a control. After 10 minutes of treatment and complete evaporation of acetone, two pieces of filter paper were bonded by adhesive tape and placed together in the bottom of the petri dishes. Then, fifteen adult insects (7-14 day old) were placed in the center of the filter paper. The number of insects in each of the two sections after 1, 2, 3, 4 and 5 hours were counted and the percentage of repellency was calculated according to following formula [15]:

$$PR = [(N_c-N_t)/(N_c+N_t)] \times 100$$

N_c: the number of insects on the untreated surface

N_t: the number of insects on the treated surface

2.5. Evaluation of nutritional indicators

In order to evaluate nutritional indices, the feeding rate of adult rice weevil from floured discs was evaluated. Flour disks were prepared according to the method of Xie et al. [16] with modifications made by Huang et al. [17]. To make flour disks into petri dishes 9 cm, a mixture of 0.2 g flour and 1 mL of water was poured to make the resulting paste into a disc. Petri dishes were then placed in a germinator at 27 ± 1 °C and 70 ± 5 % relative humidity for 48 hours to dry the discs. Afterward, discs were weighed with 0.0001 sensitive scale and doses of 0.2, 0.4, 0.8 and 1.5 µl of prepared essential oils in 150 µl acetone were added to each disc. Only 150 µl acetone was used in the control samples. In each replicate before the start of the experiment, fifteen adult insects that had been starved for 24 hours were weighed with a precision scale of 0.0001. They were added to Petri dishes containing the disc and fed for three days in the germinator with above mentioned conditions. Three days after feeding, the amount of food eaten, live insects weight and flour discs in petri dishes were weighted again and the three investigated indicators were calculated using the following formulas:

1) Relative Growth Rate (RGR) = (A - B) / (B × day)

A: Live insect weight after three days (mg/ insect)

B: Initial weight of insect (mg/ insect)

2) Relative Consumption Rate (RCR) = D / (B × day)

D: The amount of food eaten after three days (mg of food eaten/ insect)

3) Efficiency of Conversion of Ingested food (ECI) (%) = (RGR / RCR) × 100

The experiment was carried out as a factorial experiment in completely randomized design with different concentrations (0.2, 0.4, 0.8, 1.5 µl/30 µl) of essential oils of five plant species mentioned on rice lice with four replications.

2.6. Analysis and identification of the essential oils

The selected essential oils were analyzed by Agilent 6890 chromatography with 30 m column, 0.25 mm inner diameter and 0.25 mm thickness of BPX 5. To identify the constituents of the essential oils, the sample diluted by N-hexane was injected to a 1 µL gas chromatograph-mass spectrometer. In this experiment, ChemStation software was used and spectra were identified by their retention index and compared to those in reference books and articles using mass spectra of standard compounds and information contained in a computer library [18, 19].

2.7. Data analysis

Data of repellency test and nutritional indices were analyzed using procedures of SAS based on a completely randomized design. The normality of the transformed and untransformed data and also normality of residuals after analysis of variance were checked using stem-leaf and normal probability plots. Homoscedasticity was checked by observing graphical distribution plots of variance by mean (PROC PLOT). A general linear model for analysis of variance (PROC GLM) was used to compare treatments. Comparisons among treatments were made using the Tukey test where analysis of variance showed significant differences among means. In all experiments, differences between treatments were considered significant at $P < 0.05$ and mean values are given as the mean ± SD.

3. Results

3.1. Chemical composition analysis

The identified compounds in the essential oils of the studied plants are shown in Table 2. The analyzed showed that *α*-pinene and *δ*-2-carene were the two major components in all examined *Cupressus* species.

3.2. Yields of the essential oils

The yields of the essential oils were 2.25, 2.7, 0.74 and 1.30 % w/v for *C. sempervirens* var. horizontalis (Tehran), *C. sempervirens* var. *horizontalis* (Chalus), *C. sempervirens* var. *stricta* (Chalus) and *C. arizonica* (Tehran), respectively.

3.3. Fumigant toxicity of essential oils

The results showed that with increasing concentration and exposure time, the mortality rate in *S. oryzae* increases. Mortality percentages in *C. sempervirens* (France), *C. sempervirens* var. *stricta*, and *C. arizonica* after 48 hours in 286 and 428.5 µl/L air dose were 90-98 % and 100 %, respectively. The lowest percentage of mortality belonged to *C. sempervirens* (Tehran), which causes 31 % mortality at 286 µL/L air and 63 % mortality at 357 µL/L air after 48 hours on *S. oryzae* (Table 3).

3.4. Medium lethal concentration (LC_{50}) of the essential oils

LC_{50} values of the essential oils on adult *S. oryzae* showed that *C. sempervirens* (Tehran) (525.6 µl/L air) and *C. arizonica* (172.30 µl/L air) had the most and lowest LC_{50}, respectively (Table 4).

Table 2. Chemical composition of examined *Cupressus* species

No.	Compounds	KI	*C. sempervirens* var. *horizontalis* (France)	*C. sempervirens* var. *horizontalis* *(Tehran)*	*C. sempervirens* var. *horizontalis* (Chalus)	*C. sempervirens* var. *stricta* (Chalus)	*C. arizonica* (Tehran)
1	Santolina triene	906	-	0.08	-	-	-
2	Tricyclene	925	0.32	0.15	0.18	-	-
3	*α*-Thujene	929	0.64	0.15	0.24	-	0.84
4	*α*-Pinene	940	**54.98**	**34.43**	**40.28**	**45.48**	**41.42**
5	Camphene	952	-	-	-	1.15	-
6	Camphene	953	1.18	-	0.16	-	0.28

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Table 2. Chemical composition of examined *Cupressus* species (Continued)

No.	Compounds	KI	*C. sempervirens* var. *horizontalis* (France)	*C. sempervirens* var. *horizontalis* *(Tehran)*	*C. sempervirens* var. *horizontalis* (Chalus)	*C. sempervirens* var. *stricta* (Chalus)	*C. arizonica* (Tehran)
7	Thuja-2,4(10)-diene	959	0.23	-	-	-	-
8	Sabinene	978	0.95	0.74	0.79	0.38	**5.6**
9	*β*-Pinene	983	1.31	0.95	1.22	1.67	2.11
10	Myrcene	995	1.36	2.02	1.9	1.92	**5.76**
11	*δ*-2-Carene	1015	**14.03**	**23.56**	**18.27**	**22.55**	0.51
12	*α*-Terpinene	1023	0.2	0.09	0.13	-	0.65
13	*para*-Cymene	1026	-	0.06	-	-	-
14	Sylvestrene	1029	-	0.22	0.16	0.19	-
15	*o*-Cymene	1032	-	0.07	-	-	-
16	*p*-Cymene	1033	0.95	-	-	-	0.69
17	Limonene	1036	2.11	1.02	1.08	1.14	**9.87**
18	*β*-Phellandrene	1038	0.28	-	0.45	0.43	2.28
19	*E*-*β*-Ocimene	1051	-	0.06	-	-	0.16
20	*γ*-Terpinene	1065	0.41	0.19	0.25	-	1.02
21	Isoterpinolene	1086	-	-	-	0.2	-
22	Terpinolene	1092	1.4	3.12	2.76	2.88	0.93
23	*p*-Cymenene	1101	0.42	-	-	-	-
24	Linalool	1109	0.21	0.2	-	-	-
25	*trans*-Pinocarveol	1153	0.36	-	-	-	-
26	Camphor	1160	-	-	-	-	0.29
27	Karahanaenone	1167	-	0.49	-	-	-
28	Umbellulone	1183	-	-	-	-	**5.3**
29	*para*-Mentha-1,5-dien-8-ol	1184	-	0.09	-	-	-
30	Terpinene-4-ol	1193	0.84	0.4	0.47	-	2.31
31	*α*-Terpineol	1210	0.56	-	-	-	0.61
32	*γ*-Terpineol	1207	-	0.15	-	-	-
33	Citronellol	1236	-	-	-	-	0.82
34	Thymol, methyl ether	1238	-	-	-	-	0.51
35	Carvacrol, methyl ether	1247	1.05	-	0.12	-	0.21
36	Dec-9-en-1-ol	1266	-	-	-	-	0.15
37	Bornyl acetate	1294	0.27	-	0.19	-	-
38	Isobornyl acetate	1292	-	0.09	-	-	-
39	*neo*-Dihydro carveol acetate	1302	-	0.79	-	-	-
40	3-Thujyl acetate	1303	0.38	-	-	-	0.42
41	*cis*-Piperitol acetate	1341	-	-	0.72	0.7	-

Table 2. Chemical composition of examined *Cupressus* species (Continued)

No.	Compounds	KI	*C. sempervirens* var. *horizontalis* (France)	*C. sempervirens* var. *horizontalis* *(Tehran)*	*C. sempervirens* var. *horizontalis* (Chalus)	*C. sempervirens* var. *stricta* (Chalus)	*C. arizonica* (Tehran)
42	*α*-Cubebene	0.84	0.84	0.89	-	0.35	0.4
43	*α*-Terpinyl acetate	2.23	2.23	4.98	2.93	2.74	-
44	*α*-Copaene	0.35	0.35	0.2	0.24	-	-
45	*β*-Bourbonene	1390	-	-	0.09	-	-
46	*β*-Cubebene	1393	-	0.11	-	-	-
47	Longifolene	1421	0.34	-	0.17	-	0.27
48	*β*-Cedrene	1424	-	1.13	-	-	-
49	*β*-Funebrene	1425	-	-	1.7	0.41	-
50	*α*-Cedrene	1427	1.67	-	-	-	-
51	*E*-Caryophyllene	1429	0.73	0.7	0.53	1.5	0.5
52	*β*-Cedrene	1436	0.46	-	0.47	-	-
53	*β*-Gurjunene	1438	-	0.09	-	-	-
54	*cis*-Muurola-3,5-diene	1454	-	-	0.15	-	2.93
55	*α*-Humulene	1467	0.62	0.66	0.5	1.43	-
56	*cis*-Muurola-4(14),5-diene	1471	-	-	0.51	-	-
57	*epi*-Bicyclosesquiphellandrene	1472	-	1.89	-	-	**5.29**
58	*α*-Amorphene	1484	1.15	0.63	0.77	0.67	-
59	Germacrene D	1492	0.68	**7.21**	**6.25**	**8.59**	-
60	*γ*-Amorphene	1502	-	0.35	-	-	-
61	Epizonarene	1505	-	0.77	-	0.29	2.12
62	*α*-Muurolene	1507	0.75	0.32	0.43	0.25	-
63	*γ*-Cadinene	1511	0.55	0.13	0.41	0.33	-
64	7-*epi*-*α*-Salinene	1523	-	0.34	-	-	-
65	*δ*-Cadinene	1527	1.43	1.19	1.25	1.04	0.58
66	*trans*-Calamenene	1532	0.53	0.33	-	-	0.98
67	*E*-Nerolidol	1568	-	-	-	-	0.57
68	Cedrol	1628	-	2.9	-	-	-
69	*epi*-Cedrol	1631	1.62	-	**9.37**	1.26	0.48
70	*α*-Cadinol	1672	-	0.16	0.15	-	-
71	11-*nor*-Cadin-5-en-4-one, isomer B	1706	-	-	-	-	0.54
72	Manoyl oxide	2006	-	-	0.22	0.3	-
73	Isopimaradiene	2009	-	-	-	0.75	-
74	Sandaracopimarinal	2206	-	-	0.36	-	-
	Total	-	**98.39**	**94.1**	**95.87**	**98.6**	**97.4**

KI = Kovats index; Percentage > 5 % are shown in bold

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Table 3. Mean mortality percentage of rice weevil at different exposure times and concentrations from 5 essential oils compared to control

Species	Concentration (µL/L air)	Mortality percentage (Exposure time)	
		24 (hour)	48 (hour)
C. sempervirens var. *horizontalis* (Tehran)	71.5	0	0
	143	0	0
	214	0	0
	286	7	31
	357	33	63
	428.5	33	80
C. sempervirens var. *horizontalis* (Chalus)	71.5	0	0
	143	5	31
	214	11	65
	286	40	80
	357	46	91
	428.5	49	100
C. sempervirens var. *stricta* (Chalus)	71.5	0	0
	143	0	0
	214	14	41
	286	52	92
	357	66	98
	428.5	93	100
C. arizonica (Tehran)	71.5	23	35
	143	49	69
	214	62	85
	286	76	97
	357	88	100
	428.5	100	100
C. sempervirens (France)	71.5	1	4
	143	26	51
	214	35	72
	286	50	90
	357	71	93
	428.5	95	100

Table 4. LC_{50} values the essential oils for 5 species on *S. oryzae* insects after 72 h

Species	df	X^2	Heterogeneity	LC_{50} (CI) (µl/L air)
C. sempervirens var. *horizontalis* (Tehran)	4	0.545	0.136	525.666 (474.6-713.05)
C. sempervirens var. *horizontalis* (Chalus)	4	3.120	0.780	491.829 (451.75-567.08)
C. sempervirens var. *stricta* (Chalus)	4	6.37	1.59	411.099 (358.51-661.31)
C. arizonica (Tehran)	4	18.710	4.677	172.360 (142.2-143.22)
C. sempervirens (France)	4	9.77	2.44	255.069 (225.27-300.46)

LC_{50}: Medium lethal concentration; df: degree of freedom; CI: Confidence interval

3.5. The repellency effect of the essential oils

Comparison of the repellency effect of the essential oils showed that with increasing concentration, the repellency effect increased. It can also be concluded that these essential oils have a significant difference, according to Fig. 2

and standard bars. In general, the results showed that the essential oils of *C. sempervirens* (France) had the highest repellency effect (80.61 %) and even at low concentrations (10 µl/31.79 cm^2) it has the highest repellency effect than other essential oils. The essential oils of *C. sempervirens* var *stricta* and *C. arizonica* (15.24 % and 19.8 %) had the lowest repellency effect compared to other essential oils (Fig. 2).

3.6. Evaluation of nutritional indices of the essential oils

The RGR of *S. oryzae* showed that the effect of different essential oils and different concentration was significant at 1 % and the interaction between essential oils and doses was significant at 5 %. The lowest relative growth rate (0.66 mg/mg/day) of *S. oryzae* (Table 5 and 6) was observed in the 1.5 µl/30 µl concentration of *C. sempervirens* var. *horizontalis* (Tehran).

Analysis of variance of RCR for *S. oryzae* showed that the essential oils effects was significant (1 %). All of essential oils significantly reduced RCR index Except, *C. sempervirens* (Chalus) essential oils (Table 5).

The results of the ECI showed that the essential oils and different concentration were significant at 1 % level. However, *C. sempervirens* (France) and *C. Sempervirens* var. *horizontalis* (Chalus) showed the highest ECI in the *S. oryzae* with 94.30 % and 94.69 %, respectively the essential oils of *C. sempervirens* (Tehran) with 93.12 % (Table 5) caused the lowest ECI in the *S. oryzae*. The 0 µl/ 30µl concentration of the essential oils also had the lowest ECI (Table 6).

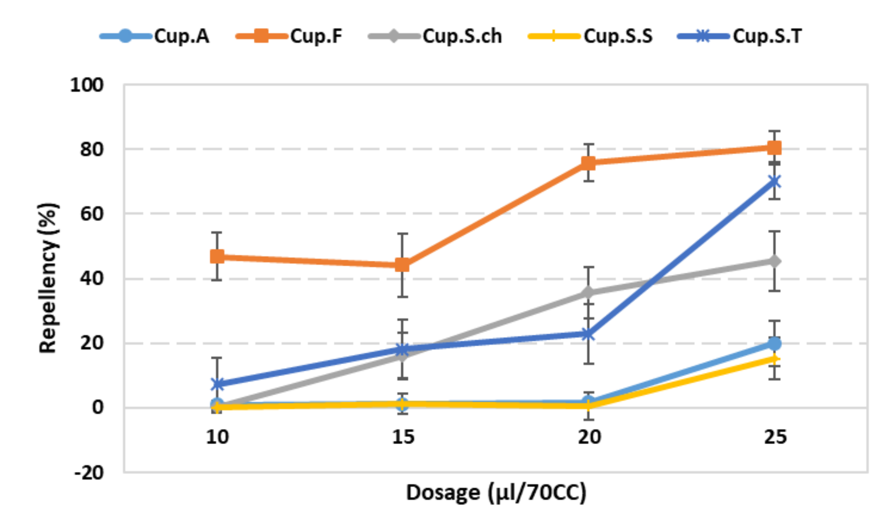

Fig. 2. Percentage repellency of the essential oils from *Cupressus* in different doses on *S. oryzae*. Cup.A: *C. arizonica*, Cup.S.ch: *C. sempervirens* var. *horizontalis* (Chalus), Cup.F: *C. sempervirens* (France), Cup.S.T: *C. sempervirens* var. *horizontalis* (Tehran), Cup.S.S: *C. sempervirens* var *stricta*.

Table 5. Effects of essential oils on nutritional indices of *S. oryzae*

Species	RGR (mg/mg/day)	RCR (mg/mg/day)	ECI (%)
C. sempervirens (Tehran)	0.662^c	0.711^b	93.122^b
C. sempervirens (Chalus)	0.672^{ab}	0.716^a	94.690^a
C. arizonica (Tehran)	0.668^b	0.709^b	93.981^{ab}
C. sempervirens (France)	0.676^a	0.711^b	94.306^a

RGR: Relative Growth Rate; RCR: Relative Consumption Rate; ECI: Efficiency of Conversion of Ingested food
^{a,b,c} Superscript lowercase letters are for significance values.

Table 6. Effects of different concentrations on nutritional indices of *S. oryzae*

Concentration (µl/30 µl)	RGR (mg/mg/day)	RCR (mg/mg/day)	ECI (%)
0	0.675a	0.713^{a}	93.033^{b}
0.2	0.673^{ab}	0.712^{a}	93.598^{ab}
0.4	0.671^{ab}	0.711^{a}	94.303^{a}
0.8	0.666^{bc}	0.712^{a}	94.623^{a}
1.5	0.664^{c}	0.711^{a}	94.566^{a}

RGR: Relative Growth Rate; RCR: Relative Consumption Rate; ECI: Efficiency of Conversion of Ingested food
^{a,b,c} Superscript lowercase letters are for significance values.

4. Discussion

In the experiments, the mortality trend showed that increasing exposure time and concentration of the essential oils increases mortality of insects. The results of this experiment were in accordance with the other results [20]. Researchers examined the insecticidal effect of *Salvia sclarea* on adult weevil and quadruped beetle insects for 7 consecutive days and found that by increasing exposure time and concentration increased casualties [20].

The LC_{50} essential oils of *C. arizonica* as bio toxicant for *S. oryzae* after 72 hours (172.30 µl/L of air) was less than the LC_{50} of four species, which indicates that it had high toxic property (Table 4). Habibi-Ghozloo *et al*. [11] examined the essential oils of *Pinus eldarica* and *Cupressus arizonica* on 5-day-old moth's insects. Their results showed that *Cupressus arizonica* with LC_{50} equivalent of 38.06 µl/L had the lower toxicity than Tehran pine oil with LC_{50} equivalent of 3.11 µl/L. Whereas, *Cupressus arizonica* had the most toxic effects in our result, this discrepancy can be attributed to the climatic conditions, the insect diversity and the experiment conditions.

According to the results of this study, essential oil of *C. arizonica* causes high respiratory toxicity and the lowest LC_{50}, but in the repellency effect, it causes low repellent effects. This result similar to Bande-Borujeni *et al.,* [1] that these two effects (respiratory toxicity and repellency) do not have a similar trend. The results of Bande-Borujeni *et al.,* [1] showed that the respiratory toxicity of *Citrus aurantium* L. essential oil on the *Rhyzopertha dominica* Fabricius and *Oryzaephilus surinamensis* L. (LC_{50} = 0.13 and 0.11 µl/ cm^2 respectively) was higher than of the *Tribolium castaneum* (Herbst) (LC_{50} = 1.84 µl/ cm^2). While the percentage of *C. aurantium* oil removal for priest *Rh. dominica, T. castaneum* and *O. surinamensis* was 48, 90 and 84 %, respectively, so despite the lower toxicity of the essential oil on the *T. castaneum*, it had high repellent effects on it. The results of another study [15] showed that the essential oils can be used against of *S. oryzae*, *Rh. dominica, T. castaneum*, *O. surinamensis* and *Callosobruchus chinensis*, they found that the *α*-pinene caused 3.47-100 % of the casualties on these insects.

Chemical analysis of the essential oils showed that *α*-pinene was the major components with tha values of 54.98, 34.43, 40.28, 45.48 and 41.42 % in *C. sempervirens* var. *horizentalis* (France), *C. sempervirens* var. *horizontalis* (Tehran), *C. sempervirens* var. *horizontalis* (Chalus), *C. sempervirens* var. *stricta* (Chalus) and *C. arizonica* (Tehran), respectively. The result showed that *C. sempervirens* var. *horizentalis* (France), *C. sempervirens* var. *stricta* and

C. arizonica (with 54.98, 45.48 and 41.42 % *α*-pinene, respectively) in 428.5 µl/L air dose after 48 hours was 100 % mortality on *S. oryzae* but the lowest mortality belonged to *C. sempervirens* var. *horizentalis* (Tehran) with 34.43 % *α*-pinene.

In fact, by identifying the compounds present in each essential oils can be found that there is a direct relationship between the constituents in each essential oils and its insecticidal properties, and they usually attribute the main effect to the major compound or index.

So far, there has been a great research into the effects of the essential oils repellency against various pests, including the potent repellency effect of the extracts of Asafetida, Lavender and *Nerium oleander* against *Tribolium castaneum* [21, 22] and a large number of storage pests [23], which confirms the high potential of these natural substances to reduce agricultural pest damage.

The present study also revealed that the essential oils of C. *sempervirens* (France), *C. sempervirens* (Tehran), *C. sempervirens* (Chalus*)* and *C. arizonica* can be used against *S. oryzae*. The results showed that the essential oils repellency depends on their concentration, which was similar with the results of some studies on the effects of essential oils repellency [24, 25]. In the mentioned experimental conditions, *C. sempervirens* (France) essential oils showed the highest and *C. sempervirens* var. *stricta* essential oils showed the lowest repellency. In this experiment, *C. arizonica* had a repellency effect of 19.89 % which was not significant compared to *C. sempervirens* var. *stricta*. In the Habibi-Ghozloo [11] study, *C. arizonica* had a low repellency effect (23.52 %), which is corresponds to the present study.

In this study, nutritional indices were used to investigate the anti-nutritional effects of *Cupressus* species essential oils. The effective factor in reducing insect weight could be related to the effectiveness of the essential oils on insect food, which was measured by the ECI index. In this study, it was found that RGR decreased in all essential oils treatments compared to control. However, the analysis of the anti-nutritional index showed that all the essential oils had anti-nutritional properties. *C. sempervirens* (France) and *C. sempervirens* (Chalus) showed the highest ECI in *S. oryzae* with 94.30 % and 94.69 %, respectively.

The effect of herbal essential oils on nutrition indices has been studied by different researchers. Reports have shown that the decline in the growth rate of weevil flour by *Evodia rutaecarpa* is mainly due to insect refusal to eat essential oils (nutritional inhibition) and may not be effective in weight loss due to its ineffective post-feeding ECI toxicity [26].

In addition, the results of research on the insecticidal properties of some *Artemisia sieberi* Besser and *A. scoparia* showed that doses of essential oils of these plants, which contain significant amounts of 1 and 8 cineole and camphor which have anti-nutritional properties and prevent insects from eating [27]. In another experiment, it was found that the extract of *Ferula asafetida* and *Artemisia aucheri* essential oils on adult weevil flours have anti-nutritional properties [22], which was in agreement with the results of this study.

5. Conclusion

Today, due to the growing trend in the use of chemical compounds in pest control and their adverse effects on the environment, researchers have focused on the production and use of safe and low-risk compounds. The use of herbicides and removing herbs is one of the ways in which herbal essential oils have a special place. Due to the toxicity and high repellency effect of

Cupressus species essential oils on *S. oryzae* and the low risk of secondary compounds of these herbs for humans and other non-target organisms. Therefore it can suggested that these herbal essential oils should be used as a potential alternative in the form of integrated pest management.

Author contributions

Project administration: MR. L.; Data analysis: MR. L.; Plant collection: M. A.; Chemical analysis: F. T.; Investigation: M. Kh. and Sh. A.; Writing original draft: H. Kh. and Sh. A.; Editing: F. KS.

Conflict of interest

The authors declare that there is no conflict of interest.

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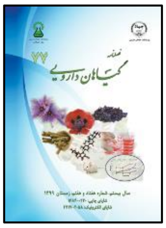

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مقاله تحقیقاتی

بررسی فعالیت زیستی اسانس جمعیت‌های مختلف گیاه زربین علیه حشره بالغ شپشه برنج

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اطلاعات مقاله

گل‌واژگان:
سمیت
دورکنندگی
شاخص‌های تغذیه‌ای
اسانس
آفات انباری

چکیده

مقدمه: سالانه مقدار قابل توجهی از تولیدات کشاورزی به وسیله‌ی آفات در انبارها از بین می‌رود و خسارت قابل توجهی به محصولات وارد می‌شود. اخیراً، استفاده از اسانس گیاهان به عنوان جایگزین سموم شیمیایی در کنترل آفات مورد توجه قرار گرفته است. جنس زربین به دلیل وجود ماده آلفا- پینن منبع قابل توجهی از حشره‌کش‌های گیاهی است که می‌توانند به عنوان حشره‌کش‌های تنفسی، دورکننده و بازدارنده تغذیه عمل نمایند. **هدف**: این مطالعه با هدف بررسی خاصیت حشره‌کشی، دورکنندگی و شاخص‌های تغذیه‌ای اسانس برخی گونه‌ها و جمعیت‌های جنس زربین روی حشرات بالغ شپشه برنج انجام شد. **روش بررسی**: این مطالعه به صورت آزمایش فاکتوریل در قالب طرح کاملاً تصادفی با ۴ تکرار انجام شد. اسانس گونه‌ها و جمعیت‌های مختلف زربین با استفاده از دستگاه کلونجر و با روش تقطیر با آب، تهیه شد. ترکیبات عمده در اسانس‌ها با استفاده از کروماتوگرافی متصل به طیف‌سنج جرمی آنالیز شد و اثرات زیستی اسانس‌ها علیه حشرات بالغ شپشه برنج بررسی شد. **نتایج**: آلفا- پینن ترکیب اصلی اسانس گونه‌های مختلف زربین بود. نتایج نشان داد از بین گونه‌های مورد بررسی، سرو سیمین با LC_{50} برابر با ۱۷۲/۳۰ میکرولیتر بر لیتر هوا، بیشترین سمیت تنفسی را روی شپشه برنج نشان داد. در بررسی اثر دورکنندگی بر شپشه‌های برنج اسانس گونه زربین فرانسه با ۸۰/۶۱ ٪ بیشترین اثر دورکنندگی را نشان داد. **نتیجه‌گیری**: با توجه به سمیت و اثر دورکنندگی بالای اسانس گیاهان جنس زربین روی حشرات بالغ شپشه برنج، این جنس پتانسیل بالایی برای استفاده در برنامه‌های کنترل این آفت در انبارها را دارد.

مخفف‌ها: RGR، نرخ رشد نسبی؛ RCR، نرخ مصرف نسبی؛ ECI، کارایی تبدیل غذای خورده شده

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