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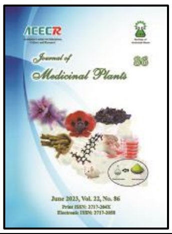

Research Article

Compositions and biological effects of different populations of *Cupressus* to control adult wheat weevil (*Tribolium castaneum* Herbst)

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ABSTRACT

Background: Chemical pesticides increasing use caused increased resistance in insects and increases the residue of pesticides in agricultural products and the environment. **Objective:** The aims of this study was to achieve a safe method and using natural essential oils of *Cupressus* against adult insects of the wheat flour (*Tribolium castaneum* Herbst). **Methods**: A factorial experiment was conducted in a completely randomized design in four replications in 2018. The *Cupressus* essential oils (EO) Clevenger by water distillation method and measured by GC-MS. Relative growth rate (RGR), repellency and nutritional indicators, and fumigant toxicity of EO on mature *Tribolium castaneum* Herbst were investigated in 27 ± 1 °C and 65 ± 5 % relative humidity. **Results:** Alpha-pinene was the major component of the EO in the studied species. The results of the fumigant toxicity test of essential oils showed that increasing concentration and duration of exposure to essential oil caused an increase in the mortality of *T. castaneum*. Among the studied species, *C. sempervirens* (France) with LC_{50} = 256.93 µL / L air had the highest fumigant toxicity (87.5%) on *T. castaneum*. **Conclusion:** Based on the insecticides, repellents and nutritional indices effect of *Cupressus* essential oils on *T. castaneum* they can be used as a biological management method for controlling storeroom pests.

1. Introduction

Storage pests of wheat weevil (*Tribolium castaneum*) have a high reproductive capacity and it is necessary to protect stored products from contamination. In the past, various chemical poisons have been used to control pests of storage products that the continued use of synthetic insecticide has many risks to the environment and consumers. Today, producing pesticides of natural origin and compatibility with the environment is the most important aim of societies. Certain plant species possess secondary compounds that can effectively combat storeroom pests. Natural products and environmentally insecticides are a good alternative to chemical pesticides [1].

Plant species with known insecticidal properties are the driving force of research to find

Abbreviations: RGR, Relative Growth Rate; RCR, Relative Consumption Rate; ECI, Efficiency of Conversion of Ingested food; FDI, Feeding deterrence percentage index

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new sources of natural insecticides. Different things such as parts of plant, extraction methods, plant phenology, harvest season, plant age, soil nature, and environmental conditions effects on the biological activity of essential oils [2, 3]. Essential oils and their compounds are carefully formulated to safeguard plants from storeroom insects, exhibiting various effects such as digestive, repellent, anti-nutritional, anti-growth, and egg-laying properties against these pests [3].

Different species of *Cupressus* are known as medicinal plants and are mainly used in traditional medicine. *Cupressus* are evergreen conifers and evergreen trees. The genus *Cupressus* in Iran has one species, three varieties and one planted species *Cupressus arizonica* [4,5]. *C. sempervirens* in Iran has two varieties *Cupressus sempervirens* L. var. *horizontalis* (Mill) (Zarbin) and *Cupressus sempervirens* L. var. *stricta* Aiton (Sarve-Shirazi). Common *cypress* (*C. sempervirens*) is traditionally used to treat colds, flu, sore throats and rheumatism, and its branches have antiseptic and antispasmodic properties [6]. The genus *Cupressus* is an essential source of herbal insecticides. Research on the genus *Cupressus* has proven its insecticidal properties.

Research shows that plants with secondary compounds are more resistant to pests and insects [7]. In the study conducted by Achiri et al. [7], it was demonstrated that the powder of cypress leaf holds potential as an effective solution against S. zeamais. As a result, they proposed that this natural formulation could be a valuable component in an integrated pest management strategy for controlling *S. zeamais* infestation in corn storage facilities. Langsi *et al*. [8] studied two hydrogenated monoterpenes (α-pinene and 3-carene) from *C. sempervirens* against *Sitophilus zeamais* in the laboratory. Their results showed that these compounds are very effective and could be exploited as novel phyto-insecticides against the maize weevil.

In the research conducted by Payandeh *et al*. [9], the results of biometric tests showed that with increasing concentration, the mortality rate increased significantly, which is consistent with the research conducted. Further investigations of Payande et al.'s research [9] show that the 50% lethality index value (LC_{50}) of mountain cypress extract on mealybugs after 72 hours is 22662 µL/L air, which is less toxic than the effect of essential oils of species Cupressus on mealybugs, even for *C. sempervirens* L. var. *stricta*, which has the highest LC_{50} value (486.328 µL/L air).

In a research conducted by Heidarizadeh et al. [10] on the compounds of *C. arizonica* extract, di-epialpha-cedrin compounds with a frequency of 45.85% followed by alpha-pinene with a frequency of 52.12% are the most volatile compounds of the extract. . In the conducted research, alpha pinene with 41.42% is the most compound in *C. arizonica* essential oil.

The purpose of this research is to investigate and compare the essential oils of different Cupressus species (*C. sempervirens* L. and *C. arizonica* Greene (Sarv-e-simin) collected from Tehran, *C. sempervirens* L. var. *horizontalis* (Mil.) Gord (Zarbin) and *C. sempervirens* L. var. *stricta* (Sarve-Shirazi) were gathered Mazandaran, Zarbin pure essential oil (France)) and identify low-risk compounds with fumigant, repellent, and anti-nutritional properties against the flour weevil (*Tribolium castaneum* Herbst).

2. Materials and methods

2.1. Plant collection

Plant samples used in this research, *C. sempervirens* L. and *C. arizonica* Greene (Sarve-simin) collected from Tehran, *C. sempervirens* L. var. *horizontalis* (Mil.) Gord (Zarbin) and *C. sempervirens* L. var. *stricta* (Sarve- Shirazi) were gathered Mazandaran, data showed in Table 1.

Table 1. Scientific name and collection site of 4 species of Cupressaceae

Species	Persian Name	Herbarium code	Collection site	Latitude (N)	Longitude (E)	Height (m)
C. sempervirens L. var. *horizontalis*	Zarbin	1388 (IMPH)	Tehran	35◦ 46' 41"	51◦ 24' 33"	1450
C. sempervirens L. var. *horizontalis*	Zarbin	1387 (IMPH)	Mazandaran (Chalus valley)	36◦ 28' 49"	51◦ 21' 00"	319
C. sempervirens L. var. *stricta*	Sarv-e-shirazi	1386 (IMPH)	Mazandaran (Chalus valley)	36◦ 28' 49"	51◦ 21' 00"	319
C. arizonica Greene	Sarv-e-simin, sarv-e-noghrei	1389 (IMPH)	Tehran	35◦ 45' 33"	51◦ 17' 39"	1460

2.2. Location of experiment and insect breeding

The present study was performed in the laboratory of the Institute of Medicinal Plants Research. Colonies of adult wheat weevil were obtained from Agricultural Entomology Research Department of Iranian Research Institute of Plant Protection (Tehran-Iran). Adult wheat weevil grew on food containing 12 parts white flour and 1 part yeast in plastic containers with lids. The containers were in the germinator (made by Grock Company) with 27 ± 1 °C temperature and 65 ± 5 % relative humidity in 12:12 light-dark conditions [11]. The adult wheat weevil was fed on wheat flour for 3 weeks for spawning and then removed. The first insects appeared on wheat flour; they were used for experiments.

2.3. Nutritional indicators evaluation

For evaluating the nutritional indicators, the feeding rate of *T. castaneum* insects was examined by flour plates. Flour plates were ready by Xie *et al*. [12] and Huang *et al*. [13] methods. To prepare flour plates 0.2 g of wheat flour and 1 ml of distilled water were mixed to dough, then petri dishes (with a 9 cm diameter) were placed in a germinator with a 27 ± 1 °C temperature and 70 ± 5 % relative humidity for 48 h for drying the flour plates. The plates were weighted with a digital scale (0.0001), then different doses of essential oils (150 µl of acetone as control, 0.2, 0.4, 0.8 and 1.5 µl of essential oils in 150 µl of acetone). Fifteen adult hungry insects were individually weighed using a scale with a precision of 0.0001 and then added to each petri dish. They were kept in a germinator for 3 days with the mentioned condition, after that, the amount of food eaten and the increase in body weight of live insects as well as the flour plates in petri dishes were re-weighed. The Relative growth rate (RGR), Relative consumption rate (RCR), Efficiency of conversion of ingested food (ECI) and Feeding deterrence percentage index (FDI) were calculated using the below formulas:

1. $RGR = (A - B) / (B \times day)$

A: Weight of live insect after three days (mg per person insect), B: Initial weight of insect (mg per person insect),

2. $RCR = D / (B \times day)$

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

3. $ECI (\%) = (RGR / RCR) \times 100$

4. $FDI (\%) = (C - T) / C \times 100$

C: Weight of food consumed in the control condition (mg per insect), T: Weight of food consumed in the treatment condition (mg per insect)

2.4. Fumigant toxicity of essential oils

Fumigant toxicity was measured based on the method of Khani *et al*. [14] in glass containers (70 ml) with lids. Filter paper (2 cm diameter) was impregnated with a Hamilton syringe to different concentrations of the essential oil (0, 71.5, 143, 214, 286, 357, and 428.5 µl per liter of air). After that 15 adult insects of *T. castaneum* (7-14 old days) were transferred to the flour with a soft brush, and to distribute the essential oil evenly, the filter paper was placed inside the lid of the glass container and the glass lid was closed. The glasses were placed in a germinator with conditions 27 ± 1 °C temperature, 70 ± 5 % relative humidity and a 12:12 light-darkness period [11]. After that, insects that had damaged legs and tentacles and were unable to move were considered dead.

2.5. Repellency effect of essential oils

The repellent effect was done on filter paper [15]. The filter paper with 9 cm diameter was divided into two parts, half of the paper was treated by 10, 15, 20, and 25 µl per 31.79 cm^2 and the other half of the paper with acetone (no treatment). After complete evaporation of the acetone, the two pieces of filter paper were attached and located in the petri dishes. After those 15 adult insects were kept in the petri dishes on the filter paper. The insects in each part were counted after 1, 2, 3, 4, and 5 hours. The repellent percentage was calculated by the formula:

$$\text{Repellent percentage} = [(Nc-Nt) / (Nc+ Nt)] \times 100$$

Nc: the number of insects on the paper with acetone (no treatment).
Nt: the number of insects on the treated surface.

2.6. The essential oils preparation

A clevenger apparatus was used to extract essential oils from 100 g of each plant sample (different *Cupressus* species) that had been dried in the shade. Anhydrous sodium sulfate was used for dehydrating the essential oils, then stored in the refrigerator (3-5 °C) by dark cover.

2.7. Essential oils analysis

The essential oils analysis was done by Gas Chromatography (GC) device of Agilent 6890 type, 30 m column length, 0.25 mm inner diameter, and 0.25 µm layer thickness of BPX5 type. To identify the essential oils, a sample diluted with N-hexane was injected into Gas Chromatography with Mass Spectrometry (GS/MS). The temperature program of the column was: 50 °C initial temperature with 5 min stopped in this situation, the temperature gradient of 3 °C per min, increase in temperature to 240 °C and then increase in temperature to 15 °C per min, increase in temperature Up to 300 °C and 3 min stop at this temperature and response time 75 min. The injection chamber temperature was 290 °C as a split 1 to 35 and helium gas was used as the carrier gas with a flow rate of 0.5 ml per min. Agilent model 5973 with an ionization voltage of 70 electron volts was used as a mass spectrometer, ionization energy method and ionization source temperature of 220 °C and copper scanning range from 40-500 was adjusted. ChemStation software was used and the spectra were identified and compared with the indices in reference books, and articles, by using the mass spectra of standard compounds [16,17].

2.8. Data analysis

The calculated data for nutritional indicators, repellency effect of essential oils and Fumigant toxicity were performed as a factorial experiment in a completely randomized design and analysis of variance by SAS software and the means were compared with Tukey test. By the Abbott [18] formula Mortality was calculated after 24, 48 and 72 hours from the starting of the treatment and LC_{50} was determined by Polo Plus software (probit or logit analysis) [19]. The data were analyzed by SAS 9.1 and the means were compared with Tukey test at 5 % level.

3. Results

3.1. Analysis of chemical compounds

The essential oils compounds of the *Cupressus* species are shown in Table 2. The result showed that *α*-Pinene and *δ*-2-Carene in the *C. sempervirens* (obtained from France), *C. sempervirens* L. *var. horizontalis* (Zarbin, Tehran), *C. sempervirens L. var. horizontalis* (Zerbin Mazandaran), *C. sempervirens* L. *var. stricta* (Sarve-Shirazi) respectively were (54.98, 14.03), (34.43, 23.56), (28.40, 18.27), (48.45, 22.55) and combinations *α*-Pinene and Limonene in the essential oil of *C. arizonica* Greene (Serv-e-Simin) are the main compounds with the amounts (41.42, 9.87) respectively.

Table 2. Chemical composition of essential oils in different *Cupressus* species

Compounds	KI*	*C. sempervirens* L. var. *horizontalis* (France)	*C. sempervirens* L. var. *horizontalis* (Tehran)	*C. sempervirens* L. var. *horizontalis* (Mazandaran)	*C. sempervirens* L. var. *stricta* (Mazandaran)	*C. arizonica* (Tehran)
Santolina triene	906	-	0.08	-	-	-
Tricyclene	925	0.32	0.15	0.18	-	-
α-Thujene	929	0.64	0.15	0.24	-	0.84
α-Pinene	940	**54.98**	**34.43**	**40.28**	**45.48**	**41.42**
Camphene	952	-	-	-	1.15	-
Camphene	953	1.18	-	0.16	-	0.28
Thuja-2,4(10)-diene	959	0.23	-	-	-	-
Sabinene	978	0.95	0.74	0.79	0.38	**5.6**
β-Pinene	983	1.31	0.95	1.22	1.67	2.11
Myrcene	995	1.36	2.02	1.9	1.92	**5.76**
δ-2-Carene	1015	**14.03**	**23.56**	**18.27**	**22.55**	0.51
α-Terpinene	1023	0.2	0.09	0.13	-	0.65
para-Cymene	1026	-	0.06	-	-	-
Sylvestrene	1029	-	0.22	0.16	0.19	-
ortho-Cymene	1032	-	0.07	-	-	-
para-Cymene	1033	0.95	-	-	-	0.69
Limonene	1036	2.11	1.02	1.08	1.14	**9.87**
β-Phellandrene	1038	0.28	-	0.45	0.43	2.28
E-*β*-Ocimene	1051	-	0.06	-	-	0.16
γ-Terpinene	1065	0.41	0.19	0.25	-	1.02
Isoterpinolene	1086	-	-	-	0.2	-
Terpinolene	1092	1.4	3.12	2.76	2.88	0.93
para-Cymenene	1101	0.42	-	-	-	-
Linalool	1109	0.21	0.2	-	-	-

Table 2. Chemical composition of essential oils in different *Cupressus* species (Continued)

Compounds	**KI***	***C. sempervirens* L. var. *horizontalis* (France)**	***C. sempervirens* L. var. *horizontalis* (Tehran)**	***C. sempervirens* L. var. *horizontalis* (Mazandaran)**	***C. sempervirens* L. var. *stricta* (Mazandaran)**	***C. arizonica* (Tehran)**
trans-Pinocarveol	1153	0.36	-	-	-	-
Camphor	1160	-	-	-	-	0.29
Karahanaenone	1167	-	0.49	-	-	-
Umbellulone	1183	-	-	-	-	**5.3**
para-Mentha-1,5-dien-8-ol	1184	-	0.09	-	-	-
Terpinene-4-ol	1193	0.84	0.4	0.47	-	2.31
α-Terpineol	1210	0.56	-	-	-	0.61
γ-Terpineol	1207	-	0.15	-	-	-
Citronellol	1236	-	-	-	-	0.82
Thymol, methyl ether	1238	-	-	-	-	0.51
Carvacrol, methyl ether	1247	1.05	-	0.12	-	0.21
Dec-9-en-1-ol	1266	-	-	-	-	0.15
Bornyl acetate	1294	0.27	-	0.19	-	-
Isobornyl acetate	1292	-	0.09	-	-	-
neo-Dihydro carveol acetate	1302	-	0.79	-	-	-
3-Thujyl acetate	1303	0.38	-	-	-	0.42
cis-Piperitol acetate	1341	-	-	0.72	0.7	-
α-Cubebene	0.84	0.84	0.89	-	0.35	0.4
α-Terpinyl acetate	2.23	2.23	4.98	2.93	2.74	-
α-Copaene	0.35	0.35	0.2	0.24	-	-
β-Bourbonene	1390	-	-	0.09	-	-
β-Cubebene	1393	-	0.11	-	-	-
Longifolene	1421	0.34	-	0.17	-	0.27
β-Cedrene	1424	-	1.13	-	-	-
β-Funebrene	1425	-	-	1.7	0.41	-
α-Cedrene	1427	1.67	-	-	-	-
E-Caryophyllene	1429	0.73	0.7	0.53	1.5	0.5
β-Cedrene	1436	0.46	-	0.47	-	-
β-Gurjunene	1438	-	0.09	-	-	-
cis-Muurola-3,5-diene	1454	-	-	0.15	-	2.93
α-Humulene	1467	0.62	0.66	0.5	1.43	-
cis-Muurola-4(14),5-diene	1471	-	-	0.51	-	-
epi-Bicyclosesquiphellandrene	1472	-	1.89	-	-	**5.29**
α-Amorphene	1484	1.15	0.63	0.77	0.67	-
Germacrene D	1492	0.68	**7.21**	**6.25**	**8.59**	**-**

Table 2. Chemical composition of essential oils in different *Cupressus* species (Continued)

Compounds	KI*	*C. sempervirens* L. var. *horizontalis* (France)	*C. sempervirens* L. var. *horizontalis* (Tehran)	*C. sempervirens* L. var. *horizontalis* (Mazandaran)	*C. sempervirens* L. var. *stricta* (Mazandaran)	*C. arizonica* (Tehran)
epi-Bicyclosesquiphellandrene	1472	-	1.89	-	-	**5.29**
α-Amorphene	1484	1.15	0.63	0.77	0.67	-
Germacrene D	1492	0.68	**7.21**	**6.25**	**8.59**	-
γ-Amorphene	1502	-	0.35	-	-	-
Epizonarene	1505	-	0.77	-	0.29	2.12
α-Muurolene	1507	0.75	0.32	0.43	0.25	-
γ-Cadinene	1511	0.55	0.13	0.41	0.33	-
7-*epi*-*α*-Salinene	1523	-	0.34	-	-	-
δ-Cadinene	1527	1.43	1.19	1.25	1.04	0.58
trans-Calamenene	1532	0.53	0.33	-	-	0.98
E-Nerolidol	1568	-	-	-	-	0.57
Cedrol	1628	-	2.9	-	-	-
epi-Cedrol	1631	1.62	-	**9.37**	1.26	0.48
α-Cadinol	1672	-	0.16	0.15	-	-
11-*nor*-Cadin-5-en-4-one, isomer B	1706	-	-	-	-	0.54
Manoyl oxide	2006	-	-	0.22	0.3	-
Isopimaradiene	2009	-	-	-	0.75	-
Sandaracopimarinal	2206	-	-	0.36	-	-
Total	-	**98.39**	**94.1**	**95.87**	**98.6**	**97.4**

*KI = Kovats Index

3.2. Essential oil percentage

In each extraction, the essential oil efficiency of species was:

For *C. sempervirens* L. var. *horizontalis* (Tehran Zarbin) 2.25 % (mass/volume), *C. sempervirens* L. var. *horizontalis* (Zarbin Mazandaran) 2.7 % (mass/volume), *C. sempervirens* L. var. *stricta* (Shirazi cypress) is 0.74 % (mass/volume) and in *C. arizonica* Greene essential oil (cypresses) is 1.30 % (mass/volume) (data showed in Table 3).

The results of mortality percentage showed that in the studied plants with increasing concentration and time, the mortality percentage on *T. castaneum* increases. High mortality percentage was related to *C. sempervirens* essential oil (France), which at a dose of 357 µL/L of air, after 24 and 48 h were 90 % and 100 %, but lowest mortality percentage was related to *C. sempervirens* var. *stricta* essential oil, which at a dose of 428.5 µL/L of air after 48 h had 59 % of mortality. Among treated species, *C. sempervirens stricta* essential oil had the lowest mortality (Table 4).

The results showed that essential oils in treated species had different lethal concentration of 50 % (LC_{50}) on *T. castaneum.* The *C. sempervirens* L. var. *stricta* (Shirazi Cypress) and *C. sempervirens* L. (France) respectively with LC_{50} = 486.328 and 256.593 µl per liter of air, had the lowest and highest toxicity on *T. castaneum* (Table 5).

Table 3. Essential oils Percentage of 4 species of *Cupressus* plants

No.	Species	Collection site	Essential oils Percentage
1	***C. sempervirens* L. var. *horizontalis***	Tehran	2.25 %
2	***C. sempervirens* L. var. *horizontalis***	Mazandaran	2.7 %
3	***C. sempervirens* L. var. *stricta***	Mazandaran	0.74 %
4	***C. arizonica* Greene**	Tehran	1.3 %

Table 4. Average mortality rate of *T. castaneum* at different concentrations and times for different species essential oils

Species	Dose (μL/L)	Mortality in hours	
		24	48
***C. sempervirens* L. var. *horizontalis* (Mazandaran)**	71.5	0	0
	143	7	34
	214	16	60
	286	42	69
	357	51	75
	428.5	69	80
***C. sempervirens* L. (France)**	71.5	33	45
	143	50	70
	214	65	82
	286	77	97
	357	90	100
	428.5	100	100
C. sempervirens* L. var. *stricta	71.5	0	0
	143	0	0
	214	9	22
	286	23	35
	357	34	52
	428.5	41	59
***C. arizonica* Greene**	71.5	7	24
	143	19	31
	214	23	43
	286	35	50
	357	42	61
	428.5	53	70
***C. sempervirens* L. var. *horizontalis* (Tehran)**	71.5	0	0
	143	۹9	15
	214	17	23
	286	25	31
	357	40	52
	428.5	52	75

Table 5. LC_{50} values calculated for 5 plant essential oils on *T. castaneum* after 72 hours

Species	df	X^2	Heterogeneity	LC_{50} (µl/ l air)	LC_{95} (µl/ l air)
C. sempervirens **L. (France)**	4	o.115	0.029	256.593 (248.28-264.78)*	309.731 (296.94-328.60)
C. sempervirens **L. var. *horizontalis* (Tehran)**	4	0.010	0.002	426.185 (416.51-439.11)	510.710 (484.43-556.37)
C. sempervirens **L. var. *horizontalis* (Mazandaran)**	4	21.918	5.476	301.8 (262.54-347.81)	503.114 (414.84-797.57)
C. sempervirens **L. var. *stricta***	4	1.372	0.343	486.328 (454.16-556.24)	699.657 (596.86-980.14)
C. arizonica **Greene**	4	3.486	0.872	358.799 (337.60-385.72)	777.385 (665.39-970.68)

* 95 % lower and upper fiducial limits are shown in parentheses

3.3. Repellent effect

Due to the low amount of *C. sempervirens stricta* essential oil, it was not possible to test the removal of this essential oil on *T. castaneum*. Comparison of the repellent effect of the other essential oils on *T. castaneum* showed that these essential oils were significantly different ($P < 0.01$). The study revealed that higher concentrations of essential oils led to an increase in the repellent effect. Specifically, the essential oil from *C. sempervirens* (France) exhibited a significantly higher repellent effect (87.5 %) on *T. castaneum* compared to the other essential oils. This essential oil at a low concentration (10 µl / 70 cm^2) showed a high repellent effect.

The essential oils of *C. sempervirens* (Tehran), *C. sempervirens* (Mazandaran) and *C. arizonica* had respectively 74.11, 73.75 and 74.5 %, repellent effect on *T. castaneum* (Figure 1).

The results of the effect of plant essential oils on the three nutritional indices of *T. castaneum* showed that they had no significant effect on the RGR. Based on Table 6, the essential oils of C. *sempervirens* (Mazandaran) with 0.7019 mg /mg/day showed the highest RGR. In addition, Table 7 shows that in all treatments (different concentrations) the RGR of insects had no significant difference with the control. However, data showed that increasing the concentration caused to decrease in the RGR compared to the control for example at the highest concentration (1.5 µl/30 µl) the RGR is lower than the control (0.6871 mg/mg/day).

There was no significant difference in the RCR of *T. castaneum* during three days (Table 6). Table 7 shows that the effect of treatments (different concentrations) and control conditions on RCR had no significant difference.

The results of the effect of plant essential oils on the efficiency feed conversion index (ECI) showed that among the species were not a significant difference (Table 6). *C. sempervirens* (Mazandaran) with no significant difference showed the highest ECI (98.64 %). In addition, different concentrations of essential oils had no significant effect on the

ECI compared to the control (Table 7). The ECI of the control condition decreases with increasing concentration and the lowest ECI corresponds to the highest concentration (1.5 µl/30 µl) 96.27 %.

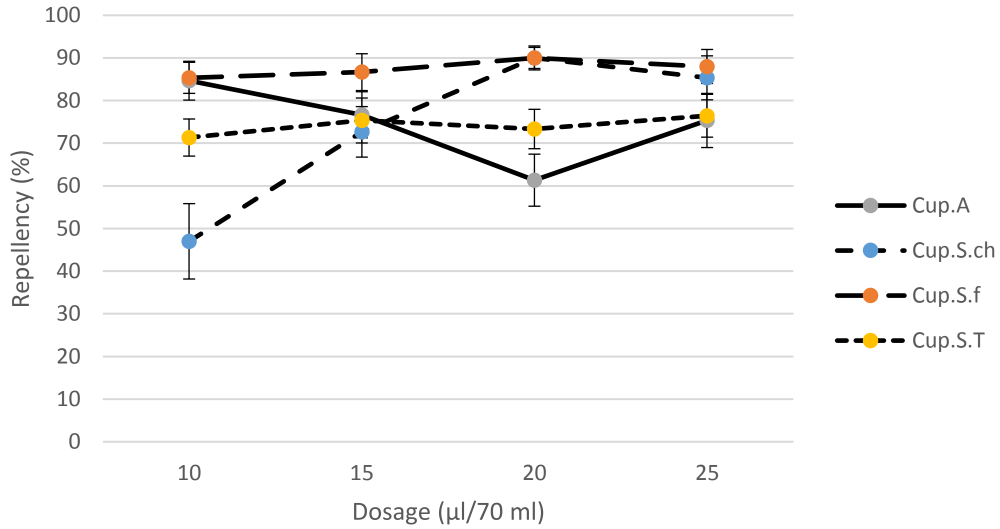

Figure 1. Percentage repellency of *Cupressaceae* essential oils from different doses against *T. castaneum* (MEAN ± SE)

Table 6. Effects of different essential oils of Cupressaceae on nutritional indices of *T. castaneum*

Essential oils	RGR mg/mg/day	RCR mg/mg/day	ECI %
C. arizonica	0.6926 ± 0.002[b]	0.7077 ± 0.0009[b]	97.8795 ± 0.43[ab]
***C. sempervirens* (Mazandaran)**	0.7019 ± 0.001[a]	0.7115 ± 0.0009[a]	98.6470 ± 0.22[a]
***C. sempervirens* (France)**	0.6928 ± 0.003[b]	0.7105 ± 0.0275[a]	97.5050 ± 2.16[b]
***C. sempervirens* (Tehran)**	0.6934 ± 0.002[b]	0.7100 ± 0.0005[a]	97.6585 ± 0.30[ab]

Means ± SE the same letter is not significantly different based on Tukey test (α = 0.05).
(RGR = Relative Growth Rate; RCR = Relative Consumption Rate; ECI = Efficiency of Conversion of Ingested food mg = Mili Gram, d = Day)

Table 7. Different concentrations effects on nutritional indices of *T. castaneum*

Concentration µl/30 µl	RGR mg/mg/day	RCR mg/mg/day	ECI %
0	0.6999 ± 0.0022[a]	0.7137 ± 0.0005[a]	99.0250 ± 0.29[a]
0.2	0.6987 ± 0.0025[a]	0.71069 ± 0.0010[ab]	98.6106 ± 0.38[a]
0.4	0.6955 ± 0.0025[ab]	0.7099 ± 0.0006[b]	97.8593 ± 0.43[a]
0.8	0.6946 ± 0.0018[ab]	0.7085 ± 0.0343[b]	97.845 ± 2.72[a]
1.5	0.6871 ± 0.0019[b]	0.7068 ± 0.0007[b]	96.2725 ± 0.31[b]

Means ± SE the same letter is not significantly different based on Tukey test (α = 0.05).
(RGR = Relative Growth Rate; RCR = Relative Consumption Rate; ECI = Efficiency of Conversion of Ingested food, mg=Mili Gram, d= Day)

4. Discussion

Essential oils (EOs) are the most important plant extracts that can be used as insecticides [20]. The essential oils effect of different plants against the storage of pests has been studied in several researches. Based on the results of various researches, Amiri [21], Wu *et al*., [22], Guo *et al*., [23] and Sriti Eljazi *et al*., [24], the essential oils were used to control the stored pests.

The efficiency of pest control depended on the type of plant and the concentration of essential oils. The result showed that *C. Sempervirens* essential oil (France) (357 µL/L of air) had the highest mortality percentage 90 % after 24 h and 100 % after 48 h.

Evaluation of the efficiency of concentration and time of consumption of plant essential oils showed that LC_{50} *C. sempervirens* L. (France) on adult *T. castaneum* insects after 25 hours (256.593 µL/L of air) was less than the other essential oils, which indicates more toxic effect. This result confirms the Ebadollahi *et al*., [25] research, which showed that increasing the dose and timing of exposure increases adult insect mortality. Fumigant toxicity of *Elettaria cardamomum* Maton essential oil against adult *Ephestia kuehniella* showed that mortality increases with increasing concentration and time. The LC_{50} obtained after 24 h was 1.57 µL/L of air, which is consistent with the results of the present study [26]. Noori Ghanbalani *et al*., [27] showed that peppermint essential oil showed the highest smoking toxicity compared to black cumin and cinnamon essential oils to the eggs and whole insects of grain weevil.

In the chemical analysis *α*-Pinene was the major constituent in five species of the *Cupressaceae* family, which is consistent with other research results [28, 29].

The repellent of essential oils depends on their concentration and after 5 hours of testing for *T. castaneum*, the essential oil of *C. sempervirens* (France) showed high repellent properties, which is consistent with the results of Hariri Moghadam *et al*., [30]. Various researchers have studied the repellent effect of plant essential oils. Negahban *et al* [31] study result showed that the essential oils of *Artemisia arborescens* had a powerful repellence effect on *Plutella xylostella* L.

In this study, the anti-nutritional effects of the essential oils of five species of the *Cupressaceae* family were examined. The main factor in the weight loss of insects was related to the ECI index. The results showed, it was found that the RGR in all essential oil treatments decreased compared to the control, but was not significant.

The results of the research by Labbafi *et al*., [32] on essential oil bioactivity evaluation of the different populations of Cupressus against adult rice weevil (*Sitophilus oryzae* L.) confirm our results. They exposed that the essential oils of *Cupressus* had anti-nutritional properties and among the treatments, *C. sempervirens* (France) and *C. sempervirens* (Chalus) showed the highest ECI in *S. oryzae* with 94.30 % and 94.69 %, respectively.

The results of the other research on the insecticide properties of several species of *Artemisia sieberi* Besser and *A. scoparia* showed that the different doses of essential oils, have anti-nutritional properties and further inhibit insect nutrition [33]. In Raisi and Aroiee [34] study, the results showed that the aqueous extract of fennel was effective against the wasp and the extracts of rosemary and black cumin were effective against the tick.

Different studies have been published on control/repellency of essential oils against insects, and none of them reported EO was be harmful. As a result, they can be considered as a suitable alternative to chemical insecticides.

5. Conclusion

The essential oils of *Cupressus* species were suitable candidates to control *Tribolium castaneum* Herbst because they had different compounds as insecticide and pesticide. Based on the properties of *Cupressus* essential oils (toxicity and repellency) and the low risk of compounds for humans, it is suggested to use these essential oils to storeroom pest management.

Author contributions

Project administration: MR. L.; Data analysis: MR. L.; Plant collection: M. A, Investigation: Sh. A and H. Kh.; Writing original draft: H. Kh. and Sh. A.

Conflict of interest

The authors declare that there is no conflict of interest.

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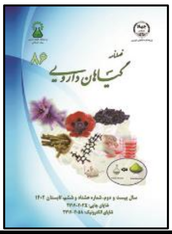

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مقاله تحقیقاتی

اثر زیستی و ترکیبات جمعیت‌های مختلف گیاه زربین بر نوعی آفت انباری شپشه آرد گندم *Tribolium castaneum* Herbst

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اطلاعات مقاله

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چکیده

مقدمه: استفاده روزافزون از حشره‌کش‌های شیمیایی باعث افزایش مقاومت در حشرات، افزایش بقایای سموم در محصولات کشاورزی و محیط زیست می‌شود. **هدف:** این تحقیق با هدف دستیابی به روشی امن و با استفاده از اسانس طبیعی گونه‌های *Cupressus* برای کنترل حشرات کامل شپشه آرد گندم *Tribolium castaneum* Herbst اجرا شد. **روش بررسی:** آزمایشی به صورت فاکتوریل در قالب طرح کاملاً تصادفی با ۴ تکرار در سال ۱۳۹۸ انجام شد. اسانس گونه‌های *Cupressus* توسط دستگاه کلونجر با استفاده از روش تقطیر آب تهیه و با GC-MS اندازه‌گیری شد. شاخص‌های نرخ رشد نسبی (RGR)، کارآیی تبدیل غذای خورده شده و شاخص بازدارندگی تغذیه علیه حشرات بالغ *Tribolium castaneum* Herbst در دمای ۱ ± ۲۷ درجه سانتی‌گراد و رطوبت ۵ ± ۶۵ درصد تعیین شد. **نتایج:** آلفا- پینن به عنوان ترکیب اصلی اسانس‌ها بود. بررسی سمیت تنفسی اسانس نشان داد که با افزایش غلظت و مدت زمان در معرض اسانس قرار گرفتن، مرگ و میر شپشه آرد به طور معنی‌داری افزایش می‌یابد. از بین گونه‌های مورد بررسی گونه (France) *C. sempervirens* با LC_{50} = 256.93 میکرولیتر بر لیتر هوا بیشترین سمیت تنفسی را روی *T. castaneum* نشان داد. در بررسی اثر دورکنندگی بر روی *T. castaneum* اسانس *C. sempervirens* (France) با۸۷/۵ درصد بیشترین خاصیت دورکنندگی را ایجاد کرد. **نتیجه‌گیری:** با توجه به سمیت و اثر دورکنندگی جنس *Cupressus* بر حشره بالغ *T. castaneum* از این جنس می‌توان برای کنترل این آفت در انبارها استفاده نمود.

مخففها: RGR، نرخ رشد نسبی؛ RCR، نرخ مصرف نسبی غذا؛ ECI، شاخص بازدهی تبدیل غذای بلعیده شده؛ FDI، شاخص درصد بازدارندگی غذایی

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