RESEARCH ARTICLE

Phytotoxicity and antifungal properties of the essential oil from the *Juniperus polycarpos* var. *turcomanica* (B. Fedsch.) R.P. Adams leaves

Leila Mehdizadeh[1] · Parisa Taheri[2] · Abdullah Ghasemi Pirbalouti[3] · Mohammad Moghaddam[1]

Abstract This study was performed to investigate the constituents, in vitro antifungal activity and phytotoxicity potential of the essential oil from *Juniperus polycarpos* var. *turcomanica* leaves. The essential oil was analyzed by GC–FID, and GC/MS, which predominantly contains α-pinene (51.21%), germacrene–B (4.80%), and Δ-cadinene (2.56%). The antifungal activity of the essential oil against some phytopathogenic fungi, including *Alternaria alternata*, *Colletotrichum trichellum, Curvularia fallax, Cytospora sacchari, Fusarium oxysporum*, and *Macrophomina phaseolina* was performed through disk diffusion and agar dilution assays. The essential oil of *J. polycarpos* var. *turcomanica* had high antifungal activity against tested phytopathogenic fungi. The most susceptible fungi to the essential oil were *C. trichellum* in agar dilution and *M. phaseolina* and *C. fallax* in disk diffusion methods, whereas, the most resistant fungus to the essential oil was obtained from *A. alternata* in both assays. Screening methods had an influence on antifungal activity of the essential oil as most of the tested fungi in this study were shown to be more resistant in disc diffusion methods. According to the phytotoxic assay results, the essential oil from *J. polycarpos* var. *turcomanica* had high phytotoxicity against three species of weeds, including *P. oleracea* L., *A. retroflexus* L., and *D. stramonium* L. The results of this research suggest that the herbicidal and antifungal activities of the essential oil from *J. polycarpos* var. *turcomanica* can be attributed to its major group of constituents, monoterpenes hydrocarbons.

Keywords Essential oil · A-Pinene · Phytopathogenic fungi · Monoterpenes hydrocarbons

Abbreviations
EO Essential oil
GC Gas chromatography
GC–MS Gas chromatography–mass spectrometry
FID Flame ionization detector
PDA Potato dextrose agar

✉ Mohammad Moghaddam
m.moghadam@um.ac.ir; moghaddam75@yahoo.com

1 Department of Horticulture Science, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91775-1163, Mashhad, Iran

2 Department of International Environmental and Agricultural Science, United Graduate School of Agricultural Science, Faculty of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Japan

3 Research Center for Medicinal Plants, Shahr-e-Qods Branch, Islamic Azad University, Shahr-e-Qods, Tehran, Iran

Introduction

Due to the diversity and abundant medicinal and physiological effects of *Juniperus*, these plants have been utilized in many different fields such as in the pharmaceutical, food, cosmetic, organic, and herbal fungicide and pesticide industries (Bakkali et al. 2008). The genus *Juniperus* from Cupressaceae family includes more than 60–70 species throughout the world and they are mostly dispersed in the northern hemisphere (Adams 2008). Five *Juniperus* species namely *J. communis* L., *J. foetidissima* Willd., *J. excelsa* M.-Bieb., *J. oxycedrus* L., *J. sabina* L. and *J. oblonga* M.-Bieb. (= *J. communis*) were listed in the Flora of Iran. *J. polycarpos* C. Koch, which is from central Asia, is a common, multi-seeded juniper (Adams 2001). One of the native juniper species in Iran is *J. polycarpos*. This species is naturally dispersed through Arassbaran, the northern

parts of Khorasan, and the southern slopes of the Alborz mountain chain. *J. polycarpos* var. *turcomanica* (B. Fedtsch.) R.P. Adams is found in the northeastern region of Iran neighboring Turkmenistan (Adams 2008). This evergreen shrub is one of the rare conifers native to Iran and that grows from northern to southern Iran (Adams and Hojjati 2012).

The *Juniperus* genus is widely used in cosmetics, folk medicine, cooking, and food production (Lesjak et al. 2011). The constituents of the essential oil from the leaves of different *Juniperus* species have been reported previously, and analysis of the leaf essential oil of *J. polycarpus* var. *turcomanica* from southern Iran showed that it consisted of α-pinene (45%), germacrene D-4-ol (8.9%) and germacrene B (2.8%) (Adams 2008; Adams and Hojjati 2013). Moreover, its essential oil from the northeast of Iran during growing season included α-pinene (41.8–75.4%), germacrene B (1–5.8%), camphor (0.7–4.5%), and α-cadinol (0.4–4.8%) (Moghaddam et al. 2018).

Competition and/or allelopathy interfere with the growth of plants. The phytotoxic characteristics of terpenoids in medicinal and aromatic plants have been examined in previous studies (Kordali et al. 2008; Mabrouk et al. 2013). According to a previous study (Langenheim 1994), volatile allelochemicals of plants can be released into the soil by aqueous filtering. Therefore, the allelochemicals in the soil and thus the level of phytotoxicity will increase. The allelochemicals reduce the seed germination and subsequent growth in many crops and weeds (Langenheim 1994).

Different testing methods are used to evaluate the in vitro antimicrobial activity of essential oils or extracts as potential antimicrobial agents. Each method has individual effects on the specific fungi. The disk-diffusion assay has many advantages in comparison to other methods as it has the ability to test very large numbers of microorganisms and antimicrobial agents, has a low cost, is simple, and its results can be explained easily (Balouiri et al. 2016). The agar dilution method, in contrast, shows greater tolerance to some fungi (Moghaddam et al. 2015).

Based on the authors' knowledge, there has been no previous research about antifungal activity of leaf essential oil of *J. polycarpos* var. *turcomanica* on the studied fungi by various methods, nor any report on its phytotoxicity effect against weeds. The objectives of this experiment were: (i) to assess the chemical constituents of essential oil from the *J. polycarpos* var. *turcomanica* leaves; (ii) to investigate antifungal activity of the *J. polycarpos* var. *turcomanica* essential oil against some phytopathogenic fungi; and (iii) to evaluate the phytotoxicity effects of the *J. polycarpos* essential oil on three main weeds: *Portulaca oleracea* L., *Amaranthus retroflexus* L. and *Datura stramonium* L.

Materials and methods

Plant materials

The *J. polycarpos* var. *turcomanica* fresh leaves were collected from plants grown in the Hezarmasjed Mountains in the north of the Khorasan province, in the northeast of Iran. The voucher specimen (Code. 24273) was deposited in the herbarium of the Research Center for Plants Sciences, Ferdowsi University of Mashhad, Mashhad, Iran.

Extraction of essential oil

Essential oil was extracted from the fresh leaves of the plants by hydrodistillation (1: 15) for 4 h using a Clevenger type apparatus. Then, the oil was collected over the water, separated and dried over anhydrous sodium sulfate. The essential oil was stored at 4 °C in dark sealed glass bottles for chemical analysis and biological testing.

Identification of the constituents of essential oil

The essential oil from *J. polycarpos* var. *turcomanica* was analyzed by gas chromatography (GC). The GC analysis was carried out on an Agilent Technologies 7890 equipped with a flame ionization detector (GC-FID). Volatile components were separated on a HP-5MS capillary column (30 m, 0.25 mm i.d.; 0.25 mm film thickness). Helium with purity of 99.999% was used as the carrier gas at a flow of 0.8 mL min^{-1}. The oven temperature was increased from 60° to 280 °C at a rate of 4 °C/minute and held isothermally at 280 °C for 10 min. The injector and detector temperatures were set at 280 °C and 300 °C, respectively. The split ratio was 1:100 such that 1 μL essential oil sample was injected manually in the split mode. GC-MS analysis was done on the Agilent Technologies 5975 Mass system. The mass spectra were recorded at 70 eV, and the mass range was from *m/z* 50–550.

The identification of the constituents was based on the comparison of their mass spectra with those of a computer library search (NIST 08 and Willey registry of mass spectral data 9th edition) and confirmed by comparing their retention indices (determined with reference to homologous series of C_5-C_{24} *n*-alkanes), either with those of authentic compounds or with data published in the literature (Adams 2007; McLafferty 2009). The peak area percentages were computed from HP–5MS column without the use of FID response factors.

Antifungal activity assays

The phytopathogenic fungi used in this study were *Alternaria alternata*, *Curvularia fallax*, *Cytospora sacchari*, *Colletotrichum trichellum*, *Fusarium oxysporum*, and *Macrophomina phaseolina*, which were obtained from the collection of the Department of Plant Diseases, Tehran University, Iran. Antifungal activity of essential oil was conducted against pathogenic fungi by two different screening methods in four replications.

Agar dilution method

Antifungal activity of the essential oil was tested against phytopathogenic fungi using a range of different concentrations (100, 200, 400, and 800 μLL^{-1}) on potato dextrose agar (PDA). The pure essential oil was dissolved in absolute ethyl alcohol and 5% Tween 20 (v/v) in a 1:1 ratio, was added to the culture medium at 40–45 °C, and was poured into Petri dishes. When the medium had solidified, the fungi were inoculated with mycelium, which was taken from the edge of seven-day-old fungal stock cultures. Disks (5 mm) of mycelial were inverted and placed in the center of each Petri dish, with the mycelium facing the medium. We prepared two controls: fresh medium with ethyl alcohol + 5% Tween 20, as well as an aqueous solution with PDA medium only. The cultures were incubated in the dark at 25 ± 2 °C. Then the colony radii were evaluated after 1, 3, and 6 days. The mycelial growth inhibition percentage was calculated from the mean values of colony diameter of treated and control groups (ethyl alcohol + 5% Tween 20).

Disc diffusion method

The inoculums for the disc diffusion assay were prepared using a suitable stock culture. 5 mm plugs of fungus were removed from each fungal colony and were placed onto a petri dish containing PDA. A single sterile (6 mm) filter paper disc, onto which 100, 200, 400 or 800 μLL^{-1} of essential oil was pipetted and placed on the inner surface of the Petri dishes. Sterile distilled water-soaked filter paper was considered as the control group. The dishes were immediately sealed with Parafilm and incubated at 25 ± 2 °C. The fungi growth (in mm) was measured at 1, 3, and 6 days. The mycelial growth inhibition percentage was calculated from the mean values of colony diameter of the treated and the control groups.

Phytotoxicity assay

The phytotoxicity potential of the *J. polycarpos* var. *turcomanica* essential oil was obtained from the leaves on the seed germination, the shoot and radicle growth of three important weeds including *P. oleracea* L., *A. retroflexus* L. and *D. stramonium* L. were investigated.

All concentrations (50, 100, 200, and 400 μLL^{-1}) of the essential oil were obtained by dispersing as an emulsion in deionized water using Tween 80 (Inouye et al. 2001). We also had two control treatments: 1) Distlled water, 2) Distlled water + Tween. All seeds of the weeds were scarified with sulfuric acid and surface sterilized with sodium hypochloride (2%). 25 seeds for each treatment, were placed in 9 cm diameter sterile petri dishes lined with double-sterile filter paper (Whatman No. 2) in four replications. 5 mL of solution was added to each Petri dish. Dishes prepared without solvent were used as a negative control. Dishes were sealed with parafilm to prevent water loss, and they were stored in a germination cabinet in the dark at 25 °C for 7 days. A seed on which the radical appeared was considered germinated. In each treatment, the germination percentage was settled as well as the measuring radicle and the shoot length after a week. Afterwards, the seedlings of *P. oleracea*, *A. retroflexus* and *D. stramonium* were collected. The shoot and radical lengths, and the fresh and dry weights were measured per petri dish to evaluate the phytotoxic activity of the *J. polycarpos* var. *turcomanica* essential oil. The inhibitory or stimulatory effects were computed using the following equation, with minor modifications from Chung et al. (2001):

$$Inhibition(-)/stimulation(+)\,\% = ((ESO - Ce)/Ce) \times 100$$

where *ESO* (essential oil effect) is the parameter measured in the presence of *J. polycarpos* var. *turcomanica* essential oil and *Ce* (control effect), the parameter measured in the presence of distilled water.

Statistical analysis

The data for inhibitory effects of *J. polycarpos* var. *turcomanica* essential oil on the mycelia of fungi, and the statistics on seedlings growth of weeds were subjected to analysis of variance (ANOVA) using Minitab 17 software. The means comparisons were made using Bonferroni multiple range tests at $p \leq 0.05$. All data are reported as mean ± standard deviation.

Results

Yield and chemical composition of the essential oil

J. polycarpos var. *turcomanica* provided a yield level of 2 (mL per 100 g fresh matter) essential oil. The results obtained by GC–FID, and GC/MS analysis of *J. polycarpos*

var. *turcomanica* oil are presented in Table 1. The major essential oil compounds were α-pinene (51.21%) and germacrene–B (4.80%). Our findings have shown that the major groups of essential oil compounds in *J. polycarpos* var. *turcomanica* were monoterpene hydrocarbons (60.67%) and sesquiterpene hydrocarbons (18.56%), which were followed by oxygenated sesquiterpenes.

Antifungal activity of essential oil

The antifungal activities of the essential oil from the *J. polycarpos* var. *turcomanica* leaves against phytopathogenic fungi was tested at different concentrations by two different antifungal screening assays, which are depicted in Table 2. All of the tested essential oil concentrations inhibited the growth of fungi and showed moderate to high antifungal activity in comparison with the control. The resistance variability of each fungus against the tested fungi was observed by the percentage of mycelia growth inhibition that related to the essential oil concentrations and times.

Based on the variance analysis, a significant effect ($p \leq 0.05$) was observed which was connected to the concentration, time, fungi screening techniques, and corresponding interactions. The variation of the tolerances versus the essential oil is a result of the proportions inhibition of mycelia growth of different fungi.

Agar dilution method

The results of inhibition percent of mycelia growth showed that different concentrations had a different impact on each fungus (Table 2). In the agar dilution method, the antifungal activity of the essential oil at 100, 200, 400, and 800 μLL^{-1} concentrations against all of the examined fungi was observed as a result of the concentrations and times from 27.7% to 100% mean values. The most susceptible fungus to the essential oil was *C. trichellum* at all the tested concentrations and days (at 1, 3, and 6 day). Furthermore, *A. alternata* and *C. fallax* with 27.7 ± 6.66 were the most resistant fungi at the lowest concentration (100 μLL^{-1}) of the essential oil after 6 days (Table 2).

Disk diffusion method

The percent inhibition of mycelia growth in this method was shown in Table 2. A broad differentiation was observed among different fungi. Four concentrations of the essential oil had control on all of the tested fungi. The antifungal activity of the essential oils at 100, 200, 400, and 800 (μLL^{-1}) concentrations was observed against the tested fungi with inhibition of mycelia growth percentage from 27.5% to 100% at different concentrations and times.

Our findings showed that, after six-days, *C. fallax* and *M. phaseolina* were the most susceptible fungi (with 86.0 and 85.0 ± 3.79 inhibition percent, respectively) at the highest concentration (800 μLL^{-1}). Also, at the highest concentration (800 μLL^{-1}) after six-days, *A. alternata* was the most resistant fungus with a 49.4 ± 5.25 percent inhibition of mycelia growth (Table 2).

On the basis of the applied methods, the susceptibility and resistance of the tested fungi were different. At the highest concentration (800 μLL^{-1}) of the essential oil after six-days, a comparison between the two methods showed that the agar dilution was the better method for inhibiting the growth of *C. sacchar, F. oxysporum* and *C. trichellum* fungi whereas disc diffusion was the better method for *M. phaseolina* and *C. fallax* fungi. On the other hand, in the case of *A. alternata*, there was no significant difference between two methods (Fig. 1).

The results indicated that *J. polycarpos* var. *turcomanica* essential oil exhibited different antifungal activities against various plant pathogenic fungi. In addition, the different concentrations showed numerous results on each fungus. Therefore, increasing the oil concentration causes an enhancement of the zones of growth inhibition in all cases. Moreover, the resistances of all of the tested fungi were increased as the time passed. In order to develop new antifungal agents to control important fungal diseases of plants, it is recommended to investigate the effect of essential oil of *J. polycarpos* var. *turcomanica* against other serious fungi. Our investigation showed that different antifungal activity methods had various effects on the percent inhibition of mycelia growth. Therefore, the most resistant and susceptible fungi were different depending on the method used.

Phytotoxicity potential

The phytotoxic effects of the essential oil of *J. polycarpos* var. *turcomanica* at four concentrations are summarized in Table 3.

In accordance with the phytotoxic assay results, the essential oil of *J. polycarpos* var. *turcomanica* indicated high phytotoxic activity against *P. oleracea* with 33.3% seed germination at 100 μLL^{-1}, whereas *A. retroflexus* and *D. stramonium* with 22.7% and 33.33% seed germination at the highest concentration (400 μLL^{-1}), respectively. By increasing the essential oil concentration, the radicle and shoot length, as well as the fresh and dry weight of the seedlings were significantly stunted. Concentrations higher than 100 μLL^{-1} completely inhibited seed germination, and radicle and shoot growth of *P. oleracea*. However, the essential oil from the leaves of *J. polycarpos* var. *turcomanica* displayed different phytotoxic effects on *P. oleracea, A. retroflexus* and *D. stramonium* seeds. Its toxicity in

Table 1 Chemical compositions of the essential oil from the *Juniperus polycarpos* var. *turcomanica* leaf

No	Component	RT[a]	RI[b]	RI[c]	Percentage	Methods of identification[d]
Monoterpene hydrocarbons					**60.67**	
1	α-Thujene	3.96	931	929.20	0.42	MS, LRI
2	α-Pinene	4.20	939	941.20	**51.21**	MS, LRI
3	Camphene	4.37	953	949.92	0.65	MS, LRI
4	Verbenene	4.47	967	955.67	0.23	MS, LRI
5	Sabinene	4.84	976	974.17	0.10	MS, LRI
6	β-Pinene	4.92	980	978.25	1.10	MS, LRI
7	β-Myrcene	5.12	991	988.36	1.82	MS, LRI, S
8	α-Phellandrene	5.51	1005	1006.09	0.12	MS, LRI
9	Δ-3-Carene	5.65	1011	1011.39	0.02	MS, LRI
10	α-Terpinene	5.98	1018	1016.31	0.1	MS, LRI
11	*p*-Cymene	6.10	1026	1028.46	0.92	MS, LRI
12	Limonene	6.20	1031	1032.34	1.93	MS, LRI, S
13	(E)-β-Ocimene	6.30	1040	1039.21	0.12	MS, LRI
14	γ-Terpinene	6.84	1062	1056.43	0.74	MS, LRI
15	α-Terpinolene	7.51	1088	1081.68	1.19	MS, LRI
Oxygenated monoterpenes					**5.00**	MS, LRI
16	1,8-Cineole	6.15	1033	1030.43	0.05	MS, LRI
17	Camphor	9.27	1143	1141.54	2.32	MS, LRI, S
18	Borneol	9.90	1165	1161.74	0.20	MS, LRI
19	Terpinen-4-ol	10.23	1177	1172.70	0.23	MS, LRI
20	α-Terpineol	10.65	1189	1186.25	0.24	MS, LRI
21	Myrtenol	10.72	1194	1194.12	0.32	MS, LRI
22	Verbenone	10.93	1204	1209.23	1.24	MS, LRI
23	Bornyl acetate	13.75	1285	1286.43	0.30	MS, LRI
24	Thymol	13.89	1290	1287.81	0.05	MS, LRI, S
25	Carvacrol	14.19	1298	1297.04	0.05	MS, LRI
Sesquiterpene hydrocarbons					**18.56**	
26	Δ-Elemene	15.24	1339	1330.94	0.78	MS, LRI
27	α-Cubebene	16.41	1351	1368.83	0.11	MS, LRI
28	β-Elemene	16.94	1375	1385.82	0.69	MS, LRI
29	α-Gurjunene	17.30	1409	1429.35	0.41	MS, LRI
30	β-Caryophyllene	17.76	1418	1412.24	1.70	MS, LRI, S
31	γ-Elemene 18.20		1433	1426.68	1.24	MS, LRI
32	α-Humulene 18.77		1447	1444.96	0.24	MS, LRI
33	Germacrene D 19.63		1480	1474.23	1.66	MS, LRI
34	β-Selinene	19.77	1485	1478.79	0.26	MS, LRI
35	α-Selinene	20.04	1494	1487.66	0.47	MS, LRI
36	Δ-Selinene	20.13	1495	1484.06	0.26	MS, LRI
37	α-Muurolene	20.24	1499	1501.63	0.40	MS, LRI
38	Germacrene A	20.32	1503	1505.43	0.34	MS, LRI
39	γ-Cadinene	20.61	1513	1506.53	1.73	MS, LRI
40	Δ-Cadinene	20.92	1524	1516.73	2.56	MS, LRI
41	Elemicin	21.31	1554	1553.54	0.91	MS, LRI
42	Germacrene B	21.81	1560	1561.05	4.80	MS, LRI
Oxygenated sesquiterpenes					**6**	
43	Elemol	21.69	1547	1542.40	1.32	MS, LRI
44	Caryophyllene oxide	22.60	1581	1574.86	0.14	MS, LRI

Table 1 continued

No	Component	RT[a]	RI[b]	RI[c]	Percentage	Methods of identification[d]
45	t-Muurolol	23.97	1642	1642.45	1.72	MS, LRI
46	α-Cadinol	24.27	1653	1650.00	1.23	MS, LRI
47	t-Cadinol	24.63	1661	1666.00	1.59	MS, LRI
Total					**90.23**	
Essential oil yield					**2%(v/w)**	

[a]Retention time (min)

[b]Linear retention indices measured on a HP-5 MS capillary column

[c]Linear retention indices reported in the NIST Chemistry WebBook Database and citations therein

[d]MS, identification performed by GC–MS spectrometry library, AMDIS software; LRI, a book by Dr. Robert P. Adams and NIST Chemistry Webbook; S, authentic standards

P. oleracea appeared at a concentration higher than $100 \ \mu LL^{-1}$. Therefore, the phytotoxic influence on *P. oleracea*, *A. retroflexus* and *D. stramonium* germination, and the seedling growth had a significant difference based on the essential oil concentration and plant species.

Our results revealed that the constituents separated from *J. polycarpos* var. *turcomanica* essential oil have significantly delayed the seed germination of the four weeds, and *P. oleracea*, *A. retroflexus* and *D. stramonium* seedlings growth. These inhibitory activities appeared, mainly, because of the toxic compounds present in them.

The germination rate was reduced with an increase in essential oil concentration (Figs. 2, 3, 4). The highest rate of germination was obtained in the control group. The lowest germination rate was observed at an essential oil concentration of $400 \ \mu LL^{-1}$ for all weed seeds.

Discussion

Chemical composition of the essential oil

Due to the major and minor components of essential oil of *Juniperus* species from different regions of Iran, there are significant variations between the chemical constituents of the essential oils (Mazloomifar et al. 2004). In conformity to our results, the leaf essential oil of *J. navicularis* mainly consisted of monoterpene hydrocarbons (67.1–88.0%) (Cavaleiro et al. 2003). In agreement with our findings, the main compound of the leaf essential oils from *J. excelsa* subsp. *polycarpos* (Moein et al. 2010), *J. phoenicea* L. (Derwich et al. 2010; Ait-Ouazzou et al. 2012) and *J. excelsa* (Ehsani et al. 2012) was α-pinene, which was present in different percentages. By comparing the essential oil components of *Juniperus* species from different studies qualitatively (Derwich et al. 2010; Salehi Shanjani et al. 2010; Ait-Ouazzou et al. 2012), significant variation

in different compositions were observed. While some constituents have been determined as the main compounds in previous studies, they were not present in our specimen. These variations could be caused by the collection of plants from different regions, the harvesting period, and the fresh or dried herbage (Salehi Shanjani et al. 2010). Results of previous investigations showed that many factors can explain the essential oil constituent variability. The essential oil constituents are influenced by the species and genotype, the environmental and ecological conditions, the growth regulators, the age of the plants, the growth stage, the method of drying, and the extraction methods (Moghaddam et al. 2007; Ghasemi Pirbalouti et al. 2013; Memarzadeh et al. 2015; Bajalan et al. 2018).

Antifungal activity of essential oil

To better control plant diseases, synthetic fungicides are widely used. However, these chemicals can cause environmental pollution due to their slow biodegradation and pose a risk to the development of resistant microorganisms (Kordali et al. 2008). The antimicrobial and antifungal activities of the plant essential oils from *J. excelas* (Lesjak et al. 2011; Tumen et al. 2012; Khan et al. 2012) and *J. macrocarpa* Sibth. (Lesjak et al. 2014) were evaluated before.

In a previous study on anti-phytopathogenic activity of peppermint (*Mentha piperita* L.) essential oil, it was shown that *M. phaseolina* had a greater tolerance among the chosen concentration range and at the highest concentration. Moreover, *Fusarium oxysporum* f.sp. ciceris had a complete inhibitory effect (Moghaddam et al. 2013). The antimicrobial activity of the essential oils is related to their constituents and their concentrations. Two or three main components at high concentration (up to 80%) usually described the essential oil properties (Bakkali et al. 2008). Moreover, some types of synergism between the minor

Table 2 Antifungal activity of *Juniperus polycarpos* var. *turcomanica* essential oil by two different methods

Fungi	Concentration (μL L⁻¹)	Percent inhibition of mycelia growth[a]					
		Method					
		Agar dilution			Disc diffusion		
		Day			Day		
		1	3	6	1	3	6
Alternaria alternata	100	74.4 ± 2.72 b[b]	49.6 ± 8.76 d	27.7 ± 6.66 f	58.8 ± 2.50 b-e	43.5 ± 9.56 fg	27.5 ± 7.11 h
	200	100.0 ± 0.00 a	50.0 ± 4.22 d	39.4 ± 3.79 e	67.1 ± 6.14a-c	46.9 ± 3.56 ef	28.6 ± 6.18 h
	400	100.0 ± 0.00 a	75.9 ± 4.07 b	51.4 ± 2.41 d	71.8 ± 3.60 ab	57.7 ± 8.31c-e	31.1 ± 7.03gh
	800	100.0 ± 0.00 a	77.6 ± 3.54 b	65.6 ± 0.91 c	77.0 ± 6.09 a	61.1 ± 6.51b-d	49.4 ± 5.25 d-f
	Control (tween + alcohol)	3.7 ± 1.1 g	2.3 ± 1.2 g	0.0 ± 0.0 g	–	–	–
	Control	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 i
Macrophomina phaseolina	100	71.5 ± 4.03 d	56.9 ± 2.07 e	44.7 ± 4.29 g	100.0 ± 0.00 a	76.9 ± 7.88 de	65.0 ± 4.67 f
	200	87.0 ± 9.45 b	69.4 ± 5.77 cd	53.3 ± 2.41 f	100.0 ± 0.00 a	89.9 ± 1.04b	73.1 ± 3.67e
	400	100.0 ± 0.0 a	77.3 ± 4.73 c	62.8 ± 2.65 d	100.0 ± 0.00 a	100.0 ± 0.00 a	81.4 ± 2.46 cd
	800	100.0 ± 0.0 a	85.8 ± 4.47 b	69.2 ± 1.38 cd	100.0 ± 0.00 a	100.0 ± 0.00 a	85.0 ± 3.79 bc
	Control (tween + alcohol)	3.8 ± 1.2 h	2.2 ± 1.4 h	0.8 ± 0.5 h	–	–	–
	Control	0.0 ± 0.0 h	0.0 ± 0.0 h	0.0 ± 0.0 h	0.0 ± 0.0 i	0.0 ± 0.0 i	0.0 ± 0.0 g
Curvularia fallax	100	73.7 ± 2.48 bc	49.3 ± 11.19 d	27.7 ± 6.66 f	100.0 ± 0.00 a	76.9 ± 7.88 de	65.0 ± 4.67 f
	200	100.0 ± 0.00 a	50.0 ± 4.22 d	39.4 ± 3.79 e	100.0 ± 0.00 a	89.9 ± 1.04 b	73.1 ± 3.67 e
	400	100.0 ± 0.00 a	73.4 ± 6.04 bc	53.9 ± 5.94 d	100.0 ± 0.00 a	100.0 ± 0.00 a	81.4 ± 2.46 cd
	800	100.0 ± 0.00 a	77.6 ± 3.54 b	66.6 ± 0.91c	100.0 ± 0.00 a	100.0 ± 0.00 a	85.0 ± 3.79 bc
	Control (tween + alcohol)	3.5 ± 1.4 g	2.2 ± 1.1 g	0.0 ± 0.0 g	–	–	–
	Control	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 g
Cytospora sacchari	100	100.0 ± 0.00 a	75.0 ± 4.07 c	65.6 ± 5.51d	72.8 ± 4.20 cd	63.5 ± 3.79 ef	48.7 ± 8.07 g
	200	100.0 ± 0.00 a	100.0 ± 0.00 a	75.6 ± 2.59 c	100.0 ± 0.00 a	71.5 ± 2.96 cd	59.2 ± 2.46 f
	400	100.0 ± 0.00 a	100.0 ± 0.00 a	83.6 ± 1.05 b	100.0 ± 0.00 a	75.3 ± 2.75 c	67.0 ± 1.06 de
	800	100.0 ± 0.00 a	100.0 ± 0.00 a	87.8 ± 2.38 b	100.0 ± 0.00 a	90.6 ± 1.92 b	75.8 ± 1.90 c
	Control (tween + alcohol)	4.1 ± 1.6 e	3.7 ± 1.3 ef	2.5 ± 1.2 ef	–	–	–
	Control	0.0 ± 0.0 f	0.0 ± 0.0 f	0.0 ± 0.0f	0.0 ± 0.0 h	0.0 ± 0.0 h	0.0 ± 0.0 h
Fusarium oxysporum	100	100.0 ± 0.00 a	70.0 ± 2.37 b	61.1 ± 0.89 b	100.0 ± 0.00 a	68.0 ± 3.33d	51.7 ± 2.67 f
	200	100.0 ± 0.00 a	77.9 ± 4.30 ab	70.0 ± 3.24 b	100.0 ± 0.00 a	76.6 ± 8.61c	59.2 ± 2.24 e
	400	100.0 ± 0.00 a	100.0 ± 0.00 a	80.6 ± 1.42 ab	100.0 ± 0.00 a	86.4 ± 2.91b	71.1 ± 2.84 cd
	800	100.0 ± 0.00 a	100.0 ± 0.00 a	65.6 ± 2.42 b	100.0 ± 0.00 a	90.4 ± 1.47 b	76.4 ± 1.43 d
	Control (tween + alcohol)	3.9 ± 1.3 c	3.6 ± 1.4 c	2.2 ± 1.1c	–	–	–
	Control	0.0 ± 0.0c	0.0 ± 0.0 c	0.0 ± 0.0 c	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 g

Table 2 continued

Fungi	Concentration (μLL^{-1})	Percent inhibition of mycelia growth[a]					
		Method					
		Agar dilution			Disc diffusion		
		Day			Day		
		1	3	6	1	3	6
Colletotricbum trichellum	100	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	66.1 ± 6.93 bc	50.6 ± 4.16 ef	41.7 ± 7.98 f
	200	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	71.3 ± 6.29 bc	62.9 ± 4.56 cd	54.8 ± 3.93de
	400	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	74.6 ± 4.17 b	63.3 ± 6.11 cd	61.4 ± 1.39 cd
	800	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	100.0 ± 0.00 a	66.2 ± 7.41 bc	67.2 ± 1.43bc
	Control (tween + alcohol)	4.5 ± 1.3 b	3.8 ± 1.2 c	2.7 ± 1.2 d	–	–	–
	Control	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 e	0.0 ± 0.0 g	0.0 ± 0.0 g	0.0 ± 0.0 g

[a]Data are mean ± SD (standard deviation). (n = 4)

[b]Means comparison were done between different concentrations of *Juniperus* oil in various days for each fungus. Means with different letters about each fungus in each method are statistically significant at 5% level probability

compounds and the other active compounds might exist (Deba et al. 2008). Results of previous studies (Kordali et al. 2008; Diánez et al. 2018; Wang et al. 2018) indicated that the inhibitory effects of the essential oils, which were isolated from *different* plant species, contain mainly aromatic monoterpenes on the mycelia growth of different phytopathogenic fungal species. These variations are dependent of different compounds, such as their antifungal constituents, their structural formation, and their functional groups. Likewise, there might be synergistic interactions between the different constituents (Bajpai et al. 2013a).

According to the distinctive concentrations, different results were obtained because of the configuration of hydrogen bonds between the hydroxyl groups of essential oil phenolics (Kim et al. 2008). Furthermore, monoterpenes in the essential oils increased the concentration of lipidic peroxides, like hydroxyl, alkoxyl and alkoperoxyl radicals, and they bring about cell death (Lucini et al. 2006). Recently, an investigation has reported that the major biological agents of essential oils with therapeutic activity are monoterpene and sesquiterpene hydrocarbons, and their oxygenated derivatives, which exhibit characteristics like strong microbial pathogens growth and toxicity against plant pathogenic fungi.

Terpenes with aromatic rings and phenolic hydroxyl groups are capable of forming hydrogen bonds with the active sites of the target enzymes. They may describe the antimicrobial activity as well as the monoterpene hydrocarbons (Khalighi-Sigaroodi et al. 2005). The mechanisms of antifungal activity of monoterpenes are not fully understood. However, several reports concluded that essential oils, such as lipophilic agents, perform their action at the level of the membrane and the membrane-embedded enzymes. The lipophilic characteristics of the hydrocarbon skeleton of essential oils and the hydrophilic characteristics of their functional groups are very important (Böhme et al. 2014). Essential oils mainly act against the cell cytoplasmic membrane of microorganisms and monoterpenes to destroy the cellular integrity by inhibiting the respiration process in the microbial cell and the inactivation and/or the synthesis inhibition of intracellular and extracellular enzymes. The hydrophobicity of essential oils cause cell membrane permeability to increase by accumulating in cell membranes and thereby disturbing the cell membrane structures (Bajpai et al. 2013b). Therefore, the oxygenate monoterpenes, monoterpene hydrocarbons, and sesquiterpenes have an important role in antifungal properties of *J. polycarpus* var. *turcomanica*. The decrease or structural changes in the major constituents and possible functional synergistic interactions between the compounds have an important role in antifungal activity (Kim et al. 2008). In addition, monoterpenes disrupt the microbial cytoplasmic membranes; therefore, high impermeability of

Fig. 1 Effect of different methods of antifungal activity assay on percentage of growth inhibition in the studied fungi after 6 days at the highest concentration (800 μL.L^{-1}). (n = 4)
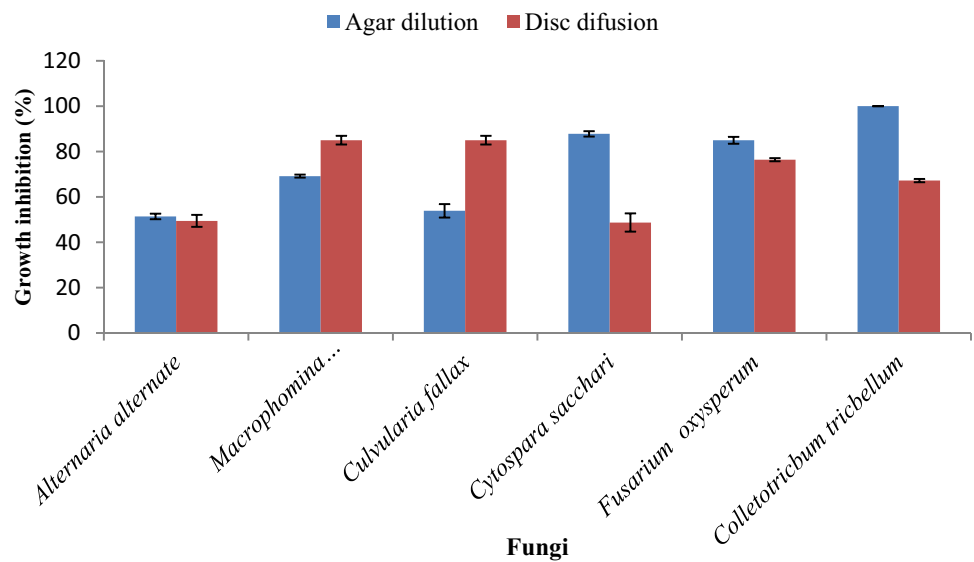

Table 3 Allelopathic effect of the leaf essential oil of *Juniperus polycarpos* var. *turcomanica* on germination characteristics of *Portulaca oleracea*, *Amaranthus retroflexus* and *Datura stramonium*

Concentration (μLL^{-1})	Seed germination (%)	Shoot length (mm)	Radicle length (mm)	Root length/stem length	Fresh weight (g)	Dry weight (g)
Portulaca oleracea						
Control (Distilled water)	85.33 ± 6.1 a[b]	3.5 ± 0.3 a	2.8 ± 0.3 a	1.52 ± 0.09 a	0.076 ± 0.005 a	0.004±0.0 a
Control (Distilled water + Tween)	74.7[a] ± 2.3 b	2.8 ± 0.4 a	2.3 ± 0.3 a	1.4 ± 0.45 a	0.060 ± 0.002 b	0.0034 ± 0 ab
50	44.0 ± 4.0 c	1.9 b	1.2 ± 0.3 b	0.4 ± 0.04 b	0.043 ± 0.003 c	0.0026 ± 0 bc
100	33.3 ± 2.3 d	1.7 ± 0.5 b	0.8 ± 0.3 b	0.3 ± 0.06 b	0.400 ± 0.001 c	0.0020 ± 0 c
200	0 e	0 c	0 c	0 c	0 d	0 d
400	0 e	0 c	0 c	0 c	0 d	0 d
Amaranthus retroflexus						
Control (Distilled water)	85.33 ± 6.11 a	2.9 ± 0.6 a	2.8 ± 0.3 a	1.52 ± 0.06 a	0.076 ± 0.005 a	0.004 ± 0 a
Control (Distilled water + Tween)	74.7 ± 2.3 ab	2.4 ± 0.1 ab	2.3 ± 0.3 ab	1.4 ± 0.45 a	0.060 ± 0.002 b	0.0080 ± 0.1 a
50	61.3 ± 6.1 bc	2.0 ± 0.2 b	1.7 ± 0.2 bc	0.7 ± 0.11 b	0.056 ± 0.0046 bc	0.0036 ± 0 a
100	50.7 ± 4.6 cd	1.97 ± 0.2 b	1.6 ± 0.3 bc	0.7 ± 0.08 b	0.050 ± 0.002 bd	0.0034 ± 0 a
200	40.0 ± 4.0 d	1.87 ± 0.2 b	1.5 ± 0.3 c	0.6 ± 0.08 b	0.047 ± 0.001 cd	0.0027 ± 0 a
400	22.7 ± 6.1 e	1.7 ± 0.5 b	1.2 ± 0.2 c	0.6 ± 0.06 b	0.040 ± 0.003 d	0.0010 ± 0 a
Datura stramonium						
Control (Distilled water)	86.67 ± 4.6 a	3.3 ± 0.2 a	2.8 ± 0.3 a	1.52 ± 0.09 a	0.077 ± 0.005 a	0.004 ± 0 a
Control (Distilled water + Tween)	73.3 ± 2.3 ab	3.0 ± 0.2 ab	2.6 ± 0.8 ab	1.4 ± 0.45 ab	0.063 ± 0.002 b	0.0034 ± 0 ab
50	70.7 ± 6.1 ab	2.7 ± 0.1 ab	2.3 ± 0.2 ab	1.1 ± 0.12 ab	0.060 ± 0.001 bc	0.0031 ± 0 bc
100	66.7 ± 4.6 bc	2.3 ± 0.1 bc	2.3 ± 0.3 ab	0.9 ± 0.02 bc	0.060 ± 0.00 bc	0.0025 ± 0 cd
200	52.0 ± 6.9 b	1.9 ± 0.2 c	2.3 ± 0.32 ab	0.7 ± 0.09 c	0.050 ± 0.001 cd	0.0021 ± 0 de
400	33.3 ± 6.1 d	1.7 ± 0.5 c	2.1 ± 0.1 bb	0.6 ± 0.03 c	0.046 ± 0.003 d	0.0015 ± 0 e

[a] Data are mean ± SD (standard deviation). (n = 4)

[b] Means with different letters in a column are statistically significant at 5% level probability

Fig. 2 Germination rate of *Portulaca oleracea* influenced by the essential oil concentrations (0, 50, 100, 200 and 400 μLL^{-1}) of *Juniperus polycarpos* var. *turcomanica* leaves. (n = 4)

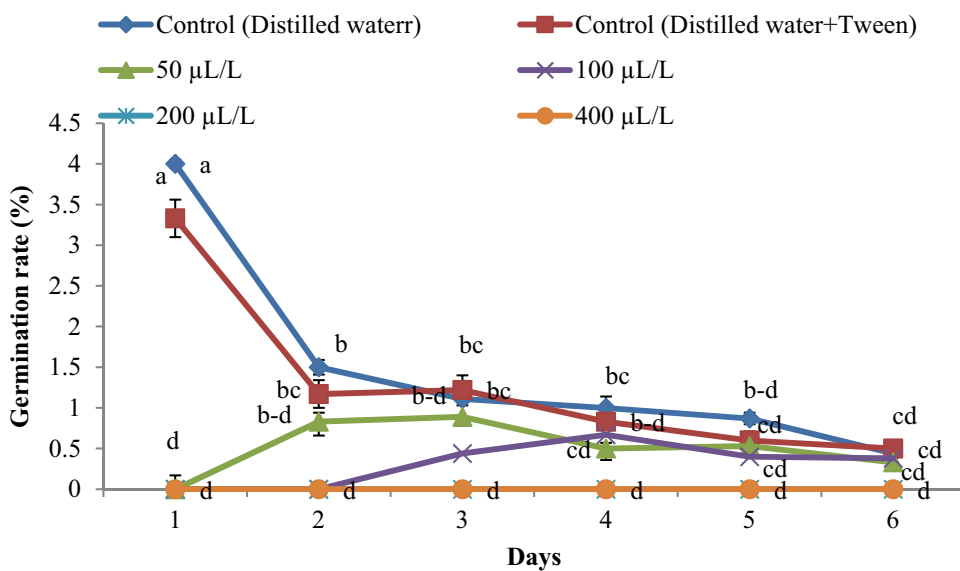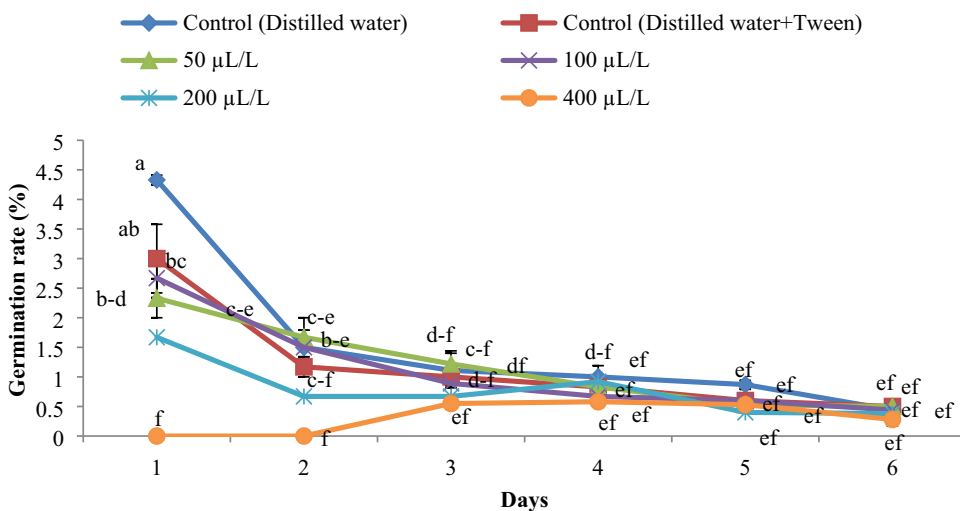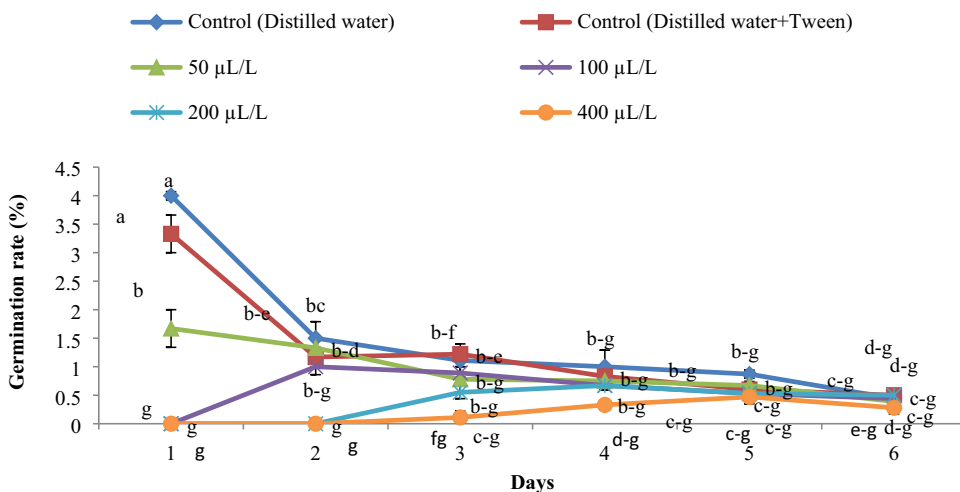**Fig. 3** Germination rate of *Datura stramonium* influenced by the essential oil concentrations (0, 50, 100, 200 and 400 μLL^{-1}) of *Juniperus polycarpos* var. *turcomanica* leaves. (n = 4)

Fig. 4 Germination rate of *Amaranthus retroflexus* influenced by the essential oil concentrations (0, 50, 100, 200 and 400 μLL^{-1}) of *Juniperus polycarpos* var. *turcomanica* leaves. (n = 4)

the membrane against protons and larger ions are lost (Lopes-Lutz et al. 2008). Furthermore, the method which is used to examine the antifungal activity of the essential oil, can influence the results obtained. In previous research, two assays were performed to examine the antifungal activity of *Echinophora platyloba* DC. essential oil. The experiments demonstrated that the consequences were different, and that each method had a special effect on the particular fungi (Moghaddam et al. 2015). The variations between the most susceptible and resistant fungi between each method was caused by the differences between the constituents of the essential oil and their interactions with each fungus. In addition, the morphological and physiological characteristics of the fungal hyphae cause an exhibition of different sensitivities to various pathogens. Generally, the dose, the fungus species and the day of observation can affect the percentages of inhibition. The agar dilution and disk diffusion methods seemed to give an indication of the susceptibility of particular fungi. Therefore, both methods can be useful in the initial screening of the antifungal activity of essential oils (Moghaddam et al. 2015).

Agar dilution assay has been used to report antifungal activity of essential oils. It was studied that by changing the concentration of Tween 80, it was possible to influence the susceptibility of the fungi to the different essential oils. For instance, it has been suggested that Tween 80 may increase fungi growth or modify cell membrane permeability. Also, it may act antagonistically against the active essential oil constituents or it may allow better spreading of components through the agar. The major factors in testing procedures are the variation in mycelia growth, the time of exposure of fungi to the essential oil, and the application and quantity of an emulsifier. All these factors considerably influence the outcome produced (Grayer and Kokubun 2001).

The differentiation between the most resistant fungi in each method is caused by the production of more enzymes by the fungi which catalyze the oxidation, and therefore the inactivation of the added essential oil. Moreover, growth inhibition of the fungal pathogens may be caused by the emulsion that destroys the cell wall and the cell membrane to different degrees due to various capacities to penetrate essential oil into the chitin based cell walls of fungal hyphae (Grayer and Kokubun 2001). Thus, as reported in this research, each method influenced the individual fungi differently.

Phytotoxicity potential

Chemical herbicides intensively contaminate the soil and the groundwater, as well as the development of weed resistance (Duke et al. 2000), and the risk of toxic residues in crops, which are increased at high concentrations. Applications of synthetic herbicides have a negative influence on the environment, so the need for natural herbicides has increased (Duke et al. 2000). In fact, the reduction in seed germination and shoot length may be connected with the reduced rate of cell division and cell elongation due to the presence of the allelochemicals (Uniyal and Chhetri 2010). In conformity with our results, there were a few reports related to toxic compounds of essential oils (e.g. caryophyllene oxide, limonene, spathulenol, etc.), as well as phytotoxic properties (Chung et al. 2001; Mabrouk et al. 2013). Some chemical compounds could be responsible for high phytotoxic potential of essential oils. Hence, low abundance control of *J. polycarpos* var. *turcomanica* in its habitat and poor diversity of other species surrounding it, is caused by its allochemicals.

Conclusion

The sensitization of the importance of fungal infections and some troubles in their treatment, cause an increase in interest of finding new fungicides to replace the chemical ones. Some chemical composition could be responsible for high phytotoxic potential of essential oils. Thus, from to the findings of this experiment, it can be concluded that the essential oil of *J. polycarpos* var. *turcomanica* leaves is a suitable alternative component to synthetic fungicides for application in agro industries. Moreover, the control of many phytopathogens has caused serious destruction in crops; therefore, novel types of selective and natural fungicides are screened and developed. Finally, the phytotoxic activity of *J. polycarpos* var. *turcomanica* essential oil on different plants can help to produce new herbicide for weed control.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Adams RP (2001) Geographic variation in leaf essential oils and RAPDs of *Juniperus polycarpos* K. Koch in central Asia. Biochem Syst Ecol 29:609–619

Adams RP (2007) Identification of essential oil components by gas chromatography/mass spectroscopy. Allured Publishing Corporation, Carol Stream

Adams RP (2008) The *Junipers* of the world: the genus *Juniperus*, 2nd edn. Trafford Publications, Victoria

Adams RP, Hojjati F (2012) Taxonomy of *Juniperus* in Iran: Insight from DNA

Adams RP, Hojjati F (2013) Taxonomy of Juniperus in Iran: Insight from DNA sequencing. Phytologia 94:219–227

Ait-Ouazzou A, Loran S, Arakrak A, Laglaoui A, Herrera A, Pagan R, Conchello P, Rota C (2012) Evaluation of the chemical composition and antimicrobial activity of *Mentha pulegium*, *Juniperus phoenicea*, and *Cyperus longus* essential oils from Morocco. Food Res Int 45:313–319

Bajalan I, Rouzbahani R, Ghasemi Pirbalouti A, Maggi F (2018) Quali-quantitative variation of essential oil from Iranian rosemary (*Rosmarinus officinalis* L.) accessions according to environmental factors. J Essent Oil Res 30:16–24

Bajpai VK, Sharma A, Baek KH (2013) Antibacterial mode of action of *Cudrania tricuspidata* fruit essential oil, affecting membrane permeability and surface characteristics of food-borne pathogens. Food Control 32:582–590

Bakkali F, Averbeck S, Averbeck D, Idaomar M (2008) Biological effects of essential oils—a review. Food Chem Toxicol 46:446–475

Balouiri M, Sadiki M, Ibnsouda SK (2016) Methods for in vitro evaluating antimicrobial activity: a review. J Pharm 6:71–79

Böhme K, Barros-Velázquez J, Calo-Mata P, Aubourg SP (2014) Antibacterial, antiviral and antifungal activity of essential oils: mechanisms and applications. Antimicrobial compounds. Springer, Berlin, pp 51–81

Cavaleiro C, Salgueiro LR, da Cunha AP, Figueiredo AC, Barroso JG, Bighelli A, Casanova J (2003) Composition and variability of the essential oils of the leaves and berries from *Juniperus navicularis*. Biochem Syst Ecol 31:193–201

Chung IM, Ahn JK, Yun SJ (2001) Assessment of allelopathic potential of barnyard grass (*Echinochloa crus-galli*) on rice (*Oryza sativa* L.) cultivars. Crop Protect 20:921–928

Deba F, Xuan TD, Yasuda M, Tawata S (2008) Chemical composition and antioxidant, antibacterial and antifungal activities of the essential oils from *Bidens pilosa* Linn. var. *radiata*. Food Control 19:346–352

Derwich E, Benziane Z, Boukir A (2010) Chemical composition of leaf essential oil of *Juniperus phoenicea* and evaluation of its antibacterial activity. Int J Agric Biol 12:199–204

Diánez F, Santos M, Parra C, Navarro MJ, Blanco R, Gea FJ (2018) Screening of antifungal activity of 12 essential oils against eight pathogenic fungi of vegetables and mushroom. Lett Appl Microbiol 67:400–410

Duke SO, Dayan FE, Romagni JG, Rimando AM (2000) Natural products as sources of herbicides: current status and future trends. Weed Res 40:99–111

Ehsani E, Akbari K, Teimouri M, Khadem A (2012) Chemical composition and antibacterial activity of two *Juniperus* species essential oils. Afr J Microbiol Res 6:6704–6710

Ghasemi Pirbalouti A, Hashemi M, Taherian Ghahfarokhi F (2013) Essential oil and chemical compositions of wild and cultivated *Thymus daenensis* Celak and *Thymus vulgaris* L. Ind Crop Prod 48:43–48

Grayer RJ, Kokubun T (2001) Plant–fungal interactions: the search for phytoalexins and other antifungal compounds from higher plants. Phytochemistry 56:253–263

Inouye S, Tsuruoka T, Uchida K, Yamaguchi H (2001) Effect of sealing and Tween 80 on the antifungal susceptibility testing of essential oils. Microbiol Immunol 45(3):201–208

Khalighi-Sigaroodi F, Hadjiakhoondi A, Shahverdi HR, Mozaffarian VA, Shafiee A (2005) Chemical composition and antimicrobial activity of the essential oil of *Ferulago bernardii* Tomk. and M. Pimen. DARU J Pharm Sci 13:100–104

Khan M, Khan AU, Rehman NU, Gilani AH (2012) Pharmacological explanation for the medicinal use of *Juniperus excelsa* in hyperactive gastrointestinal and respiratory disorders. J Nat Med 66:292–301

Kim J, Lee Y, Lee S, Shin S, Park I (2008) Fumigant antifungal activity of plant essential oils and components from West Indian bay (*Pimenta racemosa*) and thyme (*Thymus vulgaris*) oils against two phytopathogenic fungi. Flavour Frag J 23:272–277

Kordali S, Cakir A, Ozer H, Cakmakci R, Kesdek M, Mete E (2008) Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and *p*-cymene. Bioresour Technol 99:8788–8795

Langenheim JH (1994) Higher plant terpenoids: a phytocentric overview of their ecological roles. J Chem Ecol 20:1223–1280

Lesjak M, Beara I, Orcic D, Anackov G, Balog K, Franciskovic M, Mimica-Dukic N (2011) *Juniperus sibirica* Burgsdorf. as a novel source of antioxidant and antiinflammatory agents. Food Chem 124:850–856

Lesjak MM, Beara IN, Orčic DZ, Petar KN, Simin ND, Emilija SD, Mimica-Dukic NM (2014) Phytochemical composition and antioxidant, anti-inflammatory and antimicrobial activities of *Juniperus macrocarpa* Sibth. et Sm. J Funct Foods 7:257–268

Lopes-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP (2008) Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. Photochem 69:1732–1738

Lucini EI, Zunino MP, Lopez ML, Zygadlo JA (2006) Effect of mono-terpenes on lipid composition and sclerotial development of *Sclerotium cepivorum* Berk. J Phytopathol 154:441–446

Mabrouk S, Bel Hadj Salah K, Elaissi A, Jlaiel L, Ben Jannet H, Aouni M, Harzallah-Skhiri F (2013) Chemical composition and antimicrobial and allelopathic activity of Tunisian *Conyza sumatrensis* (Retz.) E. Walker essential oils. Chem Biodivers 10:209–223

Mazloomifar H, Saber-Tehrani M, Rustaiyan A, Masoudi S (2004) Constituents of the essential oil of *Echinophora platyloba* DC. growing wild in Iran. J Essent Oil Res 16:284–285

McLafferty FW (2009) Wiley registry of mass spectral data, 9th edn. Wiley, Hoboken, p 66

Memarzadeh SM, Ghasemi Pirbalouti A, AdibNejad M (2015) Chemical composition and yield of essential oils from Bakhtiari savory (*Satureja bachtiarica* Bunge.) under different extraction methods. Ind Crop Prod 76:809–816

Moein MR, Ghasemi Y, Moein S, Nejati M (2010) Analysis of antimicrobial, antifungal and antioxidant activities of *Juniperus excelsa* M. B subsp. *polycarpos* (K. Koch) Takhtajan essential oil. Pharmacogn Res 2:128–131

Moghaddam M, Omidbaigi R, Sefidkon F (2007) Changes in content and chemical composition of *Tagetes minuta* oil at various harvest times. J Essent Oil Res 19:18–20

Moghaddam M, Pourbaige M, Kourosh Tabar H, Farhadi N, Ahmadi Hosseini SM (2013) Composition and antifungal activity of peppermint (*Mentha piperita*) essential oil from Iran. J Essent Oil Bear Plant 16:506–512

Moghaddam M, Taheri P, Ghasemi Pirbalouti A, Mehdizadeh L (2015) Chemical composition and antifungal activity of essential oil from the seed of *Echinophora platyloba* DC. against phytopathogens fungi by two different screening methods. LWT- Food Sci Technol 61:536–542

Moghaddam M, Ghasemi Pirbalouti A, Farhadi N (2018) Seasonal variation in *Juniperus polycarpos* var. *turcomanica* essential oil from northeast of Iran. J EO Oil Res 30:225–231

Salehi Shanjani P, Mirza M, Calagari M, Adams RP (2010) Effects drying and harvest season on the essential oil composition from foliage and berries of *Juniperus excelsa*. Ind Crop Prod 32:83–87

Tumen I, Süntar I, Keles H, Akkol EK (2012) A therapeutic approach for wound healing by using essential oils of *Cupressus* and

Juniperus species growing in Turkey. Evid Based Complement Altern Med 2012:1–7

Uniyal AK, Chhetri S (2010) An assessment of phytotoxic potential of promising agro forestry trees on germination and growth pattern of traditional field crops of Sikkim Himalaya, India. Am Euras J Agric Environ Sci 9:70–78

Wang K, Jiang S, Pu T, Fan L, Su F, Ye M (2018) Antifungal activity of phenolic monoterpenes and structure-related compounds against plant pathogenic fungi. Nat Prod Res 33:1423–1430