

# Antifungal activity of *Zataria multiflora* Boiss. essential oils and changes in volatile compound composition under abiotic stress conditions

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## ABSTRACT

There is an increasing need for natural compounds for pest control and food preservation in agriculture, food and dairy industries. To satisfy this need, essential oils (EOs) from aromatic plants can serve as flavors, food preservatives and ecofriendly pesticides. This study investigated the potential of different EOs from field-collected leaves of fourteen *Zataria multiflora* Boiss. populations representing three different chemotypes (carvacrol, thymol and linalool) to inhibit a broad spectrum of fungal pathogens important in food industry and agriculture and the relationship between total leaf elements concentration and EOs compounds. Furthermore, a greenhouse experiment was performed to elucidate the effects of heat stress (33 °C vs. 20 °C), drought stress (50 % reduced irrigation), and ultraviolet light intensity (3, 6 and 9 W m<sup>-2</sup> UV-A radiation) on the relative content of specific volatile compounds. The results indicated that low concentrations of carvacrol and thymol, but not of linalool chemotype EOs inhibit significantly the growth of pre- and postharvest pathogens *Colletotrichum lindemuthianum*, *Fusarium sambucinum*, *Fusarium culmorum*, *Alternaria dauci* and *Botrytis cinerea* (thymol/carvacrol EOs: 0.8–1 µL, linalool EOs: 4 µL). The analyses revealed further significant correlations between the concentrations of mineral elements in *Z. multiflora* leaves and relative amounts of EO compounds and antifungal activity. Abiotic stresses, particularly heat and the interaction of drought and heat, induced changes in plants of the linalool chemotype resulting in higher relative amounts of carvacrol (22.7 % and 32.9 % vs. 1.5 %), while drought stress alone did not influence the relative amount of the main volatile compounds of *Z. multiflora* (carvacrol 1.7 %). Furthermore, the relative amount of linalool was slightly reduced in the linalool chemotype, when plants were subjected to high intensities of UV-A radiation (33.9 % vs. 44.6 %), whilst the relative amount of carvacrol was slightly increased (20.1 % vs. 9%). Moreover, the main volatile compounds of plants from the carvacrol chemotype did not change in response to abiotic stresses. Understanding the effect of environmental conditions on aromatic plant populations and chemotype development helps agriculture and food industry fully exploiting the potential of aromatic plants as a source of natural sustainable fungicides or insecticides.

## 1. Introduction

Recently, biobased crop protection and food preservation have become important issues along with the demand for an environmentally friendly agriculture and an improved consumer health (Ogunnupebi et al., 2020). The use of natural compounds from medicinal and aromatic plants for disease and pest control and food preservation increased strongly in agriculture, food and dairy industries. Many studies revealed the potential of natural compounds such as monoterpenes, phenolic compounds, flavonoids or saponines as biobased herbicides, fungicides,

and insecticides (Muñoz et al., 2020; Walia et al., 2017; Stević et al., 2014). Utilizing the large biodiversity of aromatic and medicinal plants reveals the potential of many natural compounds, whose efficiencies are comparable to synthesized fungicides, to function as biopesticides defeating various fungal pre- and postharvest pathogens.

Fungal pathogens of the genera *Colletotrichum*, *Fusarium*, *Alternaria* and *Botrytis* negatively affect the growth, development, and health status of a wide variety of cultural plants and cause considerable post-harvest losses of fruits and vegetables. For example, *Colletotrichum* species cause severe diseases like anthracnose on fruits (Scariot et al.,

**Abbreviations:** EO, essential oil; GIR, growth inhibition rate; CCA, canonical correlation analysis.

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2020), while *Alternaria dauci* causes blights on carrot (Pane et al., 2013). Moreover, phytopathogenic fungi that contaminate crops and stored products can cause potential risk to human and animal health due to mycotoxins (Rai et al., 2020). Therefore, it is eligible to be able to identify medical or aromatic plants with specific compounds or chemotypes as suitable sources for biobased plant protection agents against fungal diseases as well as for food preservation. Moreover, to fully harness this diversity it is necessary identifying a) plant populations or chemotypes with high concentrations of specific compounds with high activity against pathogens, and b) abiotic factors that affect the production of these compounds in specific chemotypes. Chemotypes are subspecies of plant populations that contain different secondary metabolites or the same secondary metabolites in different quantities (Keefover-Ring et al., 2009).

Interactions with the environment result in the adaptation of plants to specific biotic and abiotic stress conditions. The stress conditions also select for certain chemotypes and determine the variability of secondary metabolites (Verma and Shukla, 2015), because specific compounds help these plants to cope with an adverse environment (Mahajan et al., 2020). Environmental metabolomics is a promising approach to identify and select specific plant resources (populations and/or chemotypes) rich in specific compounds based on environmental variables as source, e.g. for plant protection agents, pharmacologicals or food ingredients (Karimi et al., 2020a; Thompson et al., 2007). An important factor for the formation of secondary metabolite compounds is the internal composition of mineral and trace elements, which play vital functions in plant metabolism. For example, manganese cations ( $Mn^{2+}$ ) and magnesium cations ( $Mg^{2+}$ ) are required for terpene synthases activity and the varying concentrations of these divalent cations can change the monoterpene profile of lavender (Landmann et al., 2007). Various factors like bioavailability of trace elements in the soil, plant species and genotype with their specific uptake ability and metabolism determine mineral and trace element uptake from the soil (Kabata-Pendias, 2010). Plants take up the elements from their immediate environment during their growth phase leading to differences in leaf elements concentration and altered plant biochemical processes, which are in turn reflected in the composition of plant chemical compounds (Kabata-Pendias, 2010).

Environmental stresses may cause damage on plants and induce the synthesis of specific secondary metabolites playing an important role in the adaptation and/or protection against various environmental stresses such as herbivores, pathogens and adverse climatic conditions (Mahajan et al., 2020). For example, phenolic compounds are precursors of lignin, a cell-wall component of plants involved in plant defense mechanisms (Chalker-Scott and Fuchigami, 2018). Specific monoterpene emissions can help plants to avoid permanent damage to the photosynthetic apparatus under stressful heat conditions, e.g., by maintaining the stability of thylakoid membranes (Haberstroh et al., 2018). Environmental factors such as ultraviolet A (UV-A) irradiation, drought and heat stresses affect plant growth, accumulation of bioactive compounds, antioxidant capacity and EO chemical composition, e.g. in *Mentha piperita* (Maffei et al., 1999), *Origanum vulgare* L. (Morshedloo et al., 2017) and *Melissa officinalis* L. (Pistelli et al., 2019).

*Zataria multiflora* Boiss., commonly known by the Persian name of Avishan Shirazi, is a "thyme-like" plant, endemic to the Middle East (Iran, Afghanistan, Pakistan), and belongs to the Lamiaceae family (Sajed et al., 2013). *Z. multiflora* is used frequently in traditional folk remedies because of its anti-inflammatory, antiseptic, analgesic, carminative, anthelmintic, antimicrobial and antidiarrheal properties (Khazdair et al., 2020; Sajed et al., 2013). Furthermore, it is used as food preservative and as aromatic flavoring spice in a wide variety of foods and food products (Sajed et al., 2013). The main essential oil (EO) compounds of *Z. multiflora* are carvacrol, thymol, linalool,  $\gamma$ -terpinene and *p*-cymene representing different chemotypes meaning chemically distinct entities, with differences in their composition (Karimi et al., 2020b; Sadeghi et al., 2015; Saei-Dehkordi et al., 2010). In a previous study, three different *Z. multiflora* chemotypes were found with linalool

(up to 55 %), thymol (up to 48 %) and carvacrol (up to 73 %) as the main components of the respective chemotype. Based on the main compounds of EOs, the 14 studied populations were assigned to one of these chemotypes each (Karimi et al., 2020b). Based on the presence and the known medicinal or biological activity of phenolic EO compounds, it was expected that EOs from populations of a chemotype with a high amount of specific phenolic compounds exhibit effective inhibitory effects on harmful pathogens and can thus be used as a suitable source of natural antimicrobial agents. Several studies have described the antibacterial and antifungal activity of *Z. multiflora* EOs predominantly against clinical pathogens and rarely in agricultural or postharvest pathogens (Sajed et al., 2013). The phenolic monoterpenes thymol and carvacrol, main antimicrobial and antifungal EO compounds, are derived from  $\gamma$ -terpinene, which is synthesized via two alternative pathways: (i) the methylerythritol 4-phosphate (MEP) pathway in the plastidic and/or (ii) in the cytosolic mevalonic acid (MVA) pathway (Fig. 1) (Zebec et al., 2016; Lima et al., 2013).

In a previous study we used an environmental metabolomics approach to characterize 14 *Zataria multiflora* populations from Iran, where (I) the linalool chemotype of *Z. multiflora* grows in areas characterized by lower temperature (14–20 °C), high altitude range and higher iron and potassium soil content; (II) the growth habitat of the carvacrol chemotype is characterized by high temperature (21–29 °C), low altitude range and higher calcium soil content; (III) the thymol chemotype grows in habitats characterized by mid temperature (20–24 °C) and approximately high altitude range (Karimi et al., 2020b). Based on these findings, it was hypothesized that carvacrol and thymol chemotypes of *Z. multiflora* can cope with high temperatures and drought conditions, whereas the linalool chemotype is adapted to lower temperatures. Despite several studies on *Z. multiflora* reporting the chemical diversity of its EO profile (including the presence of over fifty mono- and sesquiterpenes) and human antimicrobial properties, there is a lack of information on the effect of environmental stress conditions on terpene biosynthesis and EO composition in this species. Therefore, the present study aims to understand the differentiation of EO chemical composition in relation to environmental stresses and leaf mineral and trace elements to be able to enhance the quantity of specific bioactive EO compounds.

To utilize the chemical diversity in *Z. multiflora* plants for defeating various pre- and postharvest fungal pathogens, wild-collected leaf material were employed to investigate (i) the antifungal activity of EOs from 14 different *Z. multiflora* populations against five different fungi; and (ii) the relationship between *Z. multiflora* total leaf elemental concentration and EO composition. Furthermore, a greenhouse experiment was performed to examine (iii) the volatile compound profile responses of two main *Z. multiflora* chemotypes to the abiotic stresses heat and drought and to UV-A radiation intensity.

## 2. Materials and methods

Two experiments were performed. Experiment 1 determined the antifungal activity of the EOs isolated by hydrodistillation from plants of 14 populations of *Z. multiflora* and the elements concentration in *Z. multiflora* leaves. Experiment 2 determined the effect of the abiotic stresses temperature, drought and UV-radiation on volatile compounds in two chemotypes of *Z. multiflora* that were extracted by isooctane.

### 2.1. Chemicals and reagents

Pure standard substances including carvacrol, myrcene, linalool, *p*-cymene and  $\gamma$ -terpinene from Sigma Aldrich Fluka (Germany), and thymol and  $\alpha$ -pinene from Roth (Germany) were purchased as analytical standards. Isooctane (> 99 %) for solvent extraction of volatiles and 6-methyl-5-penten-2-one as internal standard were obtained from Roth (Germany).

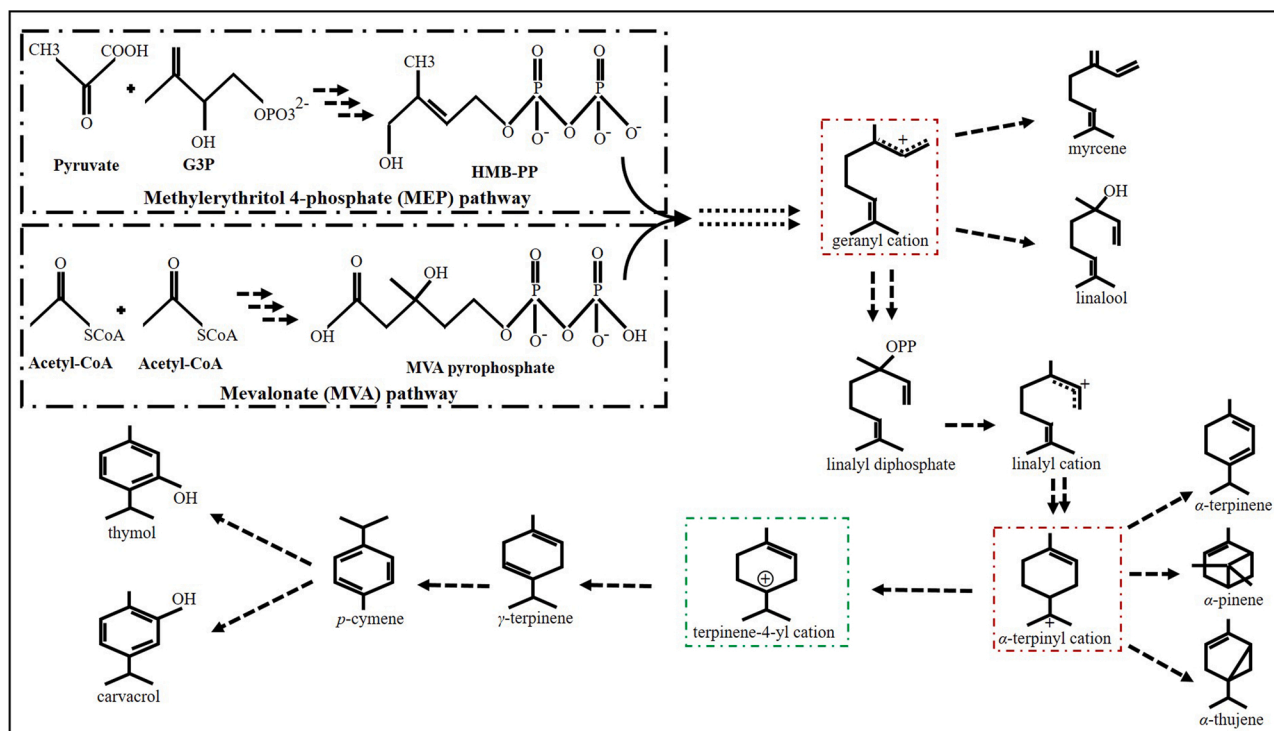


Fig. 1. Overview of the biosynthetic pathway of linalool, thymol and carvacrol (adapted from Zebec et al., 2016; Lima et al., 2013).

## 2.2. Experiment 1

### 2.2.1. Plant material and extraction of essential oils

To investigate the relationships between *Z. multiflora* leaf elements concentration and EO compounds, 123 *Z. multiflora* samples were collected at flowering stage from 14 natural habitats in Iran, as previously described (Karimi et al., 2020b).

Voucher specimens (No. MPH-1799) were deposited in the Herbarium of Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Tehran, Iran. The extraction of the EOs from leaves was performed according to previous work (Karimi et al., 2020b). In brief, 10 g of air-dried leaf material was subjected to hydrodistillation using a Clevenger type apparatus. The EO content ranged from 2.75 % (population Siriz) to 5.89 % in dry matter (population Konar Siah) (for detailed information on EO content see Karimi et al., 2020b). The extracted EOs were collected and kept at 4 °C in sealed glass vials before GC-FID and GC-MS analysis.

### 2.2.2. Determination of antifungal activity

Antifungal assays were performed with *Colletotrichum lindemuthianum*, *Fusarium sambucinum*, *Fusarium culmorum*, *Alternaria dauci*, and *Botrytis cinerea* (from the mycological culture collection of the BBA/JKI, Berlin, Germany) using the filter paper disc diffusion method according to Soyulu et al. (2006) with minor modifications. Mycelial discs (6 mm diameter) of the 9–14 days old fungus grown on synthetic nutrient poor agar were placed at the center of Petri dishes (9 cm) containing potato dextrose agar culture medium and incubated at 20 °C in darkness. Ten  $\mu$ L of the diluted EOs (30  $\mu$ L/mL in 0.05 % Tween 80) were applied on a sterilized filter paper disc (10 mm diameter), and placed on the inner surface of the inverted lid of Petri dishes. The plates were incubated for 7 days at 20 °C. Nine EO samples of each population were tested per fungus. To determine the growth inhibition rate (GIR), colony diameter was measured daily and the GIR was calculated using the formula:  $GIR = ((dc - dt) / dc) \times 100$ , where dc represents mycelial growth diameter of the fungal colony in the control and dt represents the mycelial growth diameter in the treatment. To determine the minimum

inhibitory concentration of the EOs of *Z. multiflora* chemotypes, fumigation bioassays were performed according to the method used by Feng et al. (2011). For the carvacrol chemotype EOs of Konar Siah, Jandaq and Daarbast plants were pooled, for the thymol chemotype that of Darab and Fasa plants, and for the linalool chemotype that of Siriz and Haneskh plants. Different volumes (0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 4  $\mu$ L) of the pure EOs of the different chemotypes were added to the sterilized filter paper and the plates were incubated for 5–7 days at 20 °C. Fungal growth was measured using ImageJ software (an open platform for scientific image analysis). Each concentration was tested in triplicate. Tween 80 and untreated inoculum served as controls.

### 2.2.3. Determination of leaf elements concentration

The concentrations of 11 elements [boron (B), calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), phosphor (P), sulfur (S) and zinc (Zn)] in *Z. multiflora* leaf samples from different regions were determined after drying, grinding to a uniform powder, digestion and pressure dissolution with supra pure nitric acid (Rotipuran® Supra, 69 %) by inductively coupled plasma optical emission spectrometry ICP-AES (iCAP™ 7600 Duo, Thermo Fischer Scientific) as described previously (Karimi et al., 2020a).

## 2.3. Experiment 2

### 2.3.1. Pot experiments and growth conditions

In order to investigate the effect of heat, drought and UV-A radiation on *Z. multiflora* chemotypes, seeds from two chemotypes including the carvacrol chemotype (seeds collected in Gezeh and Konar Siah) and the linalool chemotype (seeds collected in Siriz) were collected. For pre-growth eighty seeds of each of the three selected *Z. multiflora* populations were sown in 96-cell trays filled with potting substrate (containing 120 ppm N, P, Ca, Mg and 170 ppm K as main nutrients) in a greenhouse at the Julius Kuehn Institute in Berlin (52 ° 27' 32" N, 13 ° 17' 52" E) under controlled conditions (humidity: 60 ± 10 %; day: 22 °C 14 h; night: 18 °C 10 h). After eight weeks, the seedlings were transplanted into 9 cm diameter pots containing substrate with an elevated

nutrient composition (180 ppm N, P, Ca; 130 ppm Mg and 260 ppm K) and kept under controlled environmental conditions. After four weeks of adaptation, seedlings were randomly selected and placed for six weeks in controlled growth chambers and treated according to the experiment. In each of the two factorial experiments - UV-A light radiation experiment and heat and drought stress experiment - a randomized block design with three populations of *Z. multiflora* (one linalool and two carvacrol chemotypes) and different stress conditions was accomplished ( $n = 10$  plants per treatment).

**2.3.1.1. Heat and drought stress.** Acclimatized plants were subjected to three stress treatments along with a control treatment in four separate growth chambers (10 pots of each population per condition). For control (C), plants were grown at normal temperature ( $20 \pm 2$  °C) and normal irrigation (50 mL per day to maintain high soil moisture). In heat stress conditions (H), plants were grown at high temperature ( $33 \pm 2$  °C) and normal irrigation. Drought stress (D) was carried out by reducing the water supply. Plants were grown at normal temperature and daily irrigation with 25 mL of water as compared with normal irrigation of 50 mL. In the heat and drought stress condition (H + D), plants were grown at high temperature as well as under drought stress conditions. Lighting (400–700 nm) was applied for 14 h per day (as described in Section 2.3.1.2) and humidity was set at  $60 \pm 10$  % within chambers.

**2.3.1.2. Effect of UV-A radiation.** For UV-A treatments, fan-cooled light emitting diodes (LED) (SUNtec Technology /LUMITronics P1–1000, FUTURELED®, Berlin, Germany) were mounted horizontally 140 cm above growth chamber benches to provide photosynthetically active radiation (PAR) (400–700 nm) and UV-A (315–400 nm). To assess the effect of the intensity of UV-A radiation, the plants were subjected to the following treatments: C), control (PAR + no UV-A radiation); LI), PAR + low intensity UV-A radiation ( $3 \text{ W m}^{-2}$ ); II), PAR + intermediate intensity UV-A radiation ( $6 \text{ W m}^{-2}$ ); HI), PAR + high intensity UV-A radiation ( $9 \text{ W m}^{-2}$ ). Light intensity, spectral composition and photon distribution were measured and recorded under experimental conditions using a spectral PAR meter (PG200 N, UPRtek, Aachen, Germany). The software package of the spectrometer (uSpectrum PC laboratory software) was used to calculate all electromagnetic parameters including photon flux density (PFD in  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) between 315 and 400 nm, photosynthetic photon flux density (PPFD in  $\mu\text{mol m}^{-2} \text{ s}^{-1}$ ) between 400 and 700 nm and total radiation (in  $\text{W m}^{-2}$ ). Temperature was set at  $20 \pm 2$  °C and humidity at  $60 \pm 10$  % within chambers. Each treatment was accomplished in two chambers that were set at the same conditions (10 pots of each population per treatment).

### 2.3.2. Extraction and analysis of volatile compounds

For the measurement of volatile compounds, fresh leaves were harvested and immediately lyophilized in liquid nitrogen in an Eppendorf tube (50 mL) and stored at  $-80$  °C. The nitrogen-frozen leaf material was ground to fine powder for 1 min at  $30 \text{ s}^{-1}$  using a mixer mill (MM 2, Retsch) and a steel ball of 8 mm diameter. Homogenized powder ( $100 \pm 1$  mg) was weighed into a precooled 2 mL centrifuge tube and sequentially extracted with 1 mL of isoctane [containing 1:2000 (v/v) 6-methyl-5-penten-2-one as internal standard], vibrated (10 min, 30 rpm), sonicated (10 min,  $20$  °C) and centrifuged ( $13,000g$ , 10 min,  $20$  °C). The supernatant was transferred into GC vials and stored at  $-80$  °C until analysis.

The chemical analysis of the extracted volatile compounds was performed as described previously for the analysis of EOs (Karimi et al., 2020b). In brief, the volatile compounds of *Z. multiflora* were determined using an Agilent gas chromatograph 6890 N (GC–FID), fitted with a HP-5 column ( $30 \text{ m} \times 0.25 \text{ mm i.d.}$ , with a film thickness of  $0.5 \mu\text{m}$ ). The carrier gas was hydrogen. The injector and detector temperatures were set to  $250$  °C. The oven temperature was programmed at  $50$  °C for 2 min, heating from  $50$  to  $320$  °C at  $5$  °C  $\text{min}^{-1}$ , and held at  $320$  °C for

6 min. Mass spectrometry was carried out using an Agilent MSD 5975B, equipped with a  $30 \text{ m} \times 0.25 \text{ mm i.d.}$ ,  $0.5 \mu\text{m}$ , HP-5MS column, operating at  $70 \text{ eV}$  ionization energy and ion source temperature  $230$  °C, using the same temperature program as above. Helium was used as carrier gas. The compounds were identified by comparing of their mass spectra and retention indices with those of the internal reference libraries [Adams and NIST (SRD 69) databases], standard compounds and the previously published data. The retention indices of individual components were calculated using a series of n-alkanes (C8–C40). The relative percentage composition of individual compounds was computed from the GC peak areas obtained without using correction factors.

### 2.4. Statistical analysis

All statistical analyses were performed using the R software (version 4.0.2). One-way analysis of variance (ANOVA) was performed using least significant difference (LSD) method for the comparison of means and standard deviations ( $\pm$ ) of trace and mineral element concentration in the leaves and antifungal activity of EOs at the level of  $p < 0.05$ ,  $p < 0.01$  and  $p < 0.001$ . The Spearman's correlation analysis and Canonical correlation analysis (CCA) were used to analyze linear relationships between leaf elements concentration in *Z. multiflora* leaves, EO compounds and inhibitory index of fungal mycelium growth. Canonical correlation analysis is a multivariate ordination technique that analyses the relationships within and between groups of variables by comparing Spearman's rank correlation coefficients. The data were mean-centered and associations among variables were measured using CCA. The effects of heat, drought and UV-A radiation treatments were tested using two-way ANOVA followed by LSD tests to examine the differences between populations and treatments for all extracted volatile compounds. The Bonferroni test was performed to adjust for multiple testing. The normality was tested by the Shapiro-Wilk test.

## 3. Results

### 3.1. Inhibitory effect of *Z. multiflora* essential oils on pathogens

The EOs of *Z. multiflora* were effective against all studied fungi. Especially the carvacrol and thymol chemotypes exhibited growth inhibitory effects at low concentrations (Tables 1 and 2). When testing the EOs of all fourteen populations of *Z. multiflora* at  $30 \mu\text{L/mL}$ , antifungal activity against all fungi were found (Table 1). Mycelial growth of *C. lindemuthianum* was inhibited by 100 % by the carvacrol and thymol chemotype EOs (twelve populations), whereas the linalool chemotype EOs (populations from Siriz and Haneshk) inhibited growth by 40 % and 58 %. The mycelium growth of *F. sambucinum* was inhibited by more than 96 % by the carvacrol chemotype EOs and by 84%–90% by the thymol chemotype EOs; in contrast the EOs from the linalool chemotype showed 28 % and 61 % inhibition on *F. sambucinum*. The carvacrol and thymol chemotype EOs significantly affected *F. culmorum* colony diameter ( $> 65$  % inhibition), whilst the linalool chemotype EO had a 21 % inhibitory effect on *F. culmorum* colony diameter. Carvacrol and thymol chemotype EOs effectively inhibited the mycelium growth of *A. dauci* (by 38%–62%), while the linalool type EO was hardly inhibitory with 13 % maximum inhibition. Essential oils from carvacrol and thymol chemotypes also demonstrated a higher potential in terms of inhibition (55%–77%) on the mycelium growth of *B. cinerea*, whereas those of the two populations of the linalool type only showed 23 % and 46 % inhibition.

When determining minimum inhibitory concentration of the EOs of different chemotypes, the mycelial growth of *F. sambucinum*, *F. culmorum* and *B. cinerea* was completely stopped by  $1 \mu\text{L/plate}$  EOs from carvacrol and thymol chemotypes, while the EO of the linalool chemotype restrained the mycelial growth of these fungi at  $4 \mu\text{L/plate}$  (Table 2). While the linalool chemotype EO inhibited the mycelial

**Table 1**  
Antifungal activity of essential oils from plants of 14 *Z. multiflora* populations against five economically important plant pathogenic fungi.

EO samples	Latitude (N)	Longitude (E)	Chemotype	Inhibition of mycelial growth (%)				
				<i>C. lindemuthianum</i>	<i>F. sambucinum</i>	<i>F. culmorum</i>	<i>A. dauci</i>	<i>B. cinerea</i>
Arsenjan	29 ° 53' 49''	53 ° 16' 20''	carvacrol	100 ± 0 a <sup>a</sup>	96 ± 4 a	76 ± 9 ab	59 ± 8 ab	63 ± 1 cd
Ashkezar	31 ° 48' 49''	54 ° 00' 26''	carvacrol	100 ± 0 a	96 ± 3 a	71 ± 9 b	42 ± 3 cd	55 ± 14 d
Daarbast	26 ° 58' 02''	54 ° 01' 59''	carvacrol	100 ± 0 a	100 ± 0 a	83 ± 15 ab	47 ± 13 bcd	74 ± 1 ab
Darab	28 ° 44' 27''	54 ° 34' 41''	thymol	100 ± 0 a	84 ± 8 b	65 ± 16 b	45 ± 4 bcd	69 ± 4 abc
Fasa	28 ° 59' 27''	53 ° 42' 25''	thymol	100 ± 0 a	90 ± 9 ab	75 ± 5 b	46 ± 6 bcd	63 ± 4 cd
Gachooyeh	26 ° 58' 28''	53 ° 58' 06''	carvacrol	100 ± 0 a	100 ± 0 a	79 ± 6 ab	54 ± 8 abc	77 ± 2 a
Gezeh	27 ° 06' 35''	54 ° 04' 46''	carvacrol	100 ± 0 a	100 ± 0 a	70 ± 11 b	38 ± 3 d	72 ± 2 ab
Haneshk	30 ° 49' 16''	53 ° 18' 19''	linalool	58 ± 18 b	61 ± 19 c	21 ± 20 c	13 ± 5 e	46 ± 5 e
Hongooyeh	27 ° 06' 19''	54 ° 04' 07''	carvacrol	100 ± 0 a	100 ± 0 a	81 ± 9 ab	39 ± 14 d	70 ± 2 abc
Jandaq	33 ° 57' 44''	54 ° 31' 02''	carvacrol	100 ± 0 a	100 ± 0 a	97 ± 2 a	62 ± 6 a	62 ± 1 cd
Kemeshk	27 ° 03' 13''	53 ° 50' 41''	carvacrol	100 ± 0 a	100 ± 0 a	77 ± 8 ab	44 ± 11 dc	74 ± 2 ab
Konar Siah	27 ° 09' 05''	53 ° 57' 04''	carvacrol	100 ± 0 a	100 ± 0 a	77 ± 17 ab	55 ± 10 abc	76 ± 1 a
Siriz	30 ° 55' 43''	55 ° 57' 01''	linalool	40 ± 5 c	28 ± 2 d	21 ± 9 c	12 ± 1 e	23 ± 1 f
Taft	31 ° 42' 26''	54 ° 10'	carvacrol	100 ± 0 a	100 ± 0 a	85 ± 13 ab	56 ± 10 abc	66 ± 2 bc

<sup>a</sup> Different lowercase letters indicate significant differences in each column among the means [ $\pm$  SD] of antifungal activity by LSD tests ( $p < 0.05$ ) using 9 replicates.

**Table 2**  
Effect of essential oils from plants of three *Z. multiflora* chemotypes on five economically important plant pathogenic fungi.

Pathogens	Chemotype	EO ( $\mu$ L/plate)	Mycelial growth (mm)								
			0	0.1	0.2	0.4	0.6	0.8	1	2	4
<i>Colletotrichum lindemuthianum</i>	linalool		14.2 ± 0.4 <sup>a</sup>	13.1 ± 0.5	11.8 ± 0.2	11.4 ± 0.7	10.5 ± 0.7	6.2 ± 0.7	5.7 ± 0.8	0.0	0.0
	thymol		14.2 ± 0.4	10.5 ± 0.4	8.2 ± 0.6	6.7 ± 0.6	3.9 ± 0.8	0.0	0.0	0.0	0.0
	carvacrol		14.2 ± 0.4	8.7 ± 0.6	4.7 ± 0.4	2.7 ± 0.4	2.1 ± 0.2	0.0	0.0	0.0	0.0
<i>Fusarium sambucinum</i>	linalool		28.4 ± 2.4	27.2 ± 1	25.5 ± 1.6	24.5 ± 1	22.4 ± 1.9	22.1 ± 2.8	15.4 ± 2.6	5.8 ± 1.4	0.0
	thymol		28.4 ± 2.4	19.9 ± 3	19.7 ± 3.6	13.7 ± 0.8	6.8 ± 0.7	4.7 ± 0.6	0.0	0.0	0.0
	carvacrol		28.4 ± 2.4	20.9 ± 1.4	17.7 ± 1.3	11.1 ± 2.1	6.1 ± 0.6	3.7 ± 0.7	0.0	0.0	0.0
<i>Fusarium culmorum</i>	linalool		38.1 ± 1.2	36.9 ± 2	34.5 ± 0.5	30.6 ± 1.2	28 ± 2.3	23.1 ± 2.6	12.7 ± 1.2	4.1 ± 0.5	0.0
	thymol		38.1 ± 1.2	20.8 ± 1.2	14.9 ± 1.3	12.5 ± 0.9	5.8 ± 0.8	3.1 ± 0.7	0.0	0.0	0.0
	carvacrol		38.1 ± 1.2	17.6 ± 1.6	9.5 ± 1.7	6.2 ± 0.4	4.3 ± 0.8	2.2 ± 0.4	0.0	0.0	0.0
<i>Alternaria dauci</i>	linalool		16.1 ± 1.1	13.8 ± 0.6	12.8 ± 1	12.2 ± 0.6	11.1 ± 0.3	8.9 ± 0.3	5.2 ± 1.2	2.9 ± 0.5	0.0
	thymol		16.1 ± 1.1	12 ± 0.7	10.9 ± 0.5	9.2 ± 0.5	8.3 ± 0.6	5.4 ± 0.5	3.2 ± 0.5	0.0	0.0
	carvacrol		16.1 ± 1.1	10.5 ± 1.4	7.1 ± 0.6	5.6 ± 0.5	4.8 ± 0.2	2.3 ± 0.4	0.0	0.0	0.0
<i>Botrytis cinerea</i>	linalool		37.8 ± 1	37 ± 2.3	33.4 ± 1.4	29.3 ± 1.9	25.9 ± 1.3	20.1 ± 1.8	10.6 ± 1.8	4.9 ± 1.1	0.0
	thymol		37.8 ± 1	35.4 ± 1.2	29.9 ± 0.5	26 ± 1.5	7.6 ± 1.2	2.4 ± 0.7	0.0	0.0	0.0
	carvacrol		37.8 ± 1	27 ± 0.7	17.7 ± 0.4	14.5 ± 0.6	3.9 ± 0.8	2.3 ± 0.5	0.0	0.0	0.0

<sup>a</sup> Means [ $\pm$  SD] of 3 replicates of mycelial growth after 5–7 days.

**Table 3**  
Element concentrations in leaves from plants of *Z. multiflora* populations from 14 different regions in Iran.

Element <sup>a</sup>	Element concentration (ppm)						
	Arsenjan	Ashkezar	Daarbast	Darab	Fasa	Gachooye	Gezeh
B***	35 ± 2	44 ± 0.4	28 ± 1	60 ± 2	33 ± 0.9	30 ± 0.1	34 ± 2
Ca***	28,885 ± 813	13,894 ± 465	14,909 ± 39	27,116 ± 649	20,475 ± 146	15,852 ± 1473	16,602 ± 518
Cu***	10 ± 0.4	6 ± 0.4	8 ± 0.4	6 ± 0.2	6 ± 0.1	9 ± 3	6 ± 0.1
Fe***	1577 ± 59	855 ± 85	322 ± 102	802 ± 42	545 ± 13	392 ± 265	225 ± 34
K***	8373 ± 134	11,242 ± 42	12,176 ± 1033	5780 ± 159	7757 ± 144	14,578 ± 2534	11,262 ± 17
Mg***	3480 ± 90	3664 ± 125	3134 ± 41	2352 ± 86	2432 ± 52	2705 ± 399	2855 ± 351
Mn***	68 ± 2	57 ± 8	29 ± 1	38 ± 2	31 ± 0.1	34 ± 9	22 ± 0.6
Na***	293 ± 5	1014 ± 96	405 ± 118	354 ± 8	284 ± 4	353 ± 201	350 ± 26
P**	935 ± 8	1045 ± 7	1029 ± 103	496 ± 15	796 ± 19	1117 ± 260	863 ± 11
S	3039 ± 94	3633 ± 10	3102 ± 262	3222 ± 109	2862 ± 84	3461 ± 643	3181 ± 198
Zn***	37 ± 3	17 ± 1	34 ± 6	12 ± 0.6	19 ± 0.7	31 ± 7	40 ± 8
Element	Haneshk	Hongooye	Jandaq	Kemeshk	Konar Siah	Siriz	Taft
B***	49 ± 3	35 ± 2	58 ± 2	36 ± 4	33 ± 1	84 ± 17	45 ± 9
Ca***	17,635 ± 54	16,368 ± 2079	19,963 ± 269	17,019 ± 322	16,082 ± 1680	18,141 ± 1184	16,783 ± 1576
Cu***	5 ± 0.3	6 ± 0.3	11 ± 0.8	11 ± 2	8 ± 2	12 ± 0.9	8 ± 0.2
Fe***	894 ± 154	227 ± 10	1090 ± 6	535 ± 22	316 ± 136	1349 ± 135	1115 ± 135
K***	9846 ± 1239	11,752 ± 1486	10,880 ± 769	14,332 ± 1901	13,934 ± 3453	9714 ± 1177	11,147 ± 633
Mg***	3562 ± 801	2858 ± 526	3580 ± 532	3309 ± 241	3069 ± 211	4182 ± 9	3448 ± 158
Mn***	66 ± 15	24 ± 2	70 ± 0.3	44 ± 4	21 ± 6	72 ± 5	58 ± 9
Na***	406 ± 16	296 ± 99	2241 ± 257	571 ± 192	366 ± 55	511 ± 26	1443 ± 37
P**	944 ± 120	867 ± 79	917 ± 232	1147 ± 73	1144 ± 246	800 ± 148	1060 ± 37
S	3724 ± 26	3240 ± 373	3528 ± 355	3805 ± 373	3348 ± 758	3529 ± 122	3458 ± 372
Zn***	16 ± 3	43 ± 5	54 ± 4	29 ± 6	55 ± 11	76 ± 14	43 ± 7

<sup>a</sup> Significant differences among the means [ $\pm$  SD] of 3 replicates of single element concentrations from plants of 14 populations by ANOVA tests are given. Significant levels: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

growth of *C. lindemuthianum* at a final concentration of 2  $\mu\text{L}/\text{plate}$ , the carvacrol and thymol chemotype EOs stopped the mycelial growth at 0.8  $\mu\text{L}/\text{plate}$ . The mycelial growth of *A. dauci* was completely stopped by 4  $\mu\text{L}/\text{plate}$  EO from the linalool chemotype, whereas the carvacrol and thymol chemotype EOs restrained the mycelial growth of *A. dauci* at 1  $\mu\text{L}/\text{plate}$  and 2  $\mu\text{L}/\text{plate}$ , respectively.

### 3.2. Relationships between leaf element concentrations, EO compounds and antifungal activity

The most abundant concentrations of mineral and trace elements in *Z. multiflora* leaves were Ca, K, S, Mg, P, Fe, Na, Mn, B, Zn and Cu. Except for S, all element concentrations of *Z. multiflora* leaves from plants of the 14 populations showed significant differences (Table 3). To evaluate relationships between leaf element concentrations, EO relative amounts (see Karimi et al., 2020b) and antifungal activity, Canonical correlation analysis (CCA) and Spearman's correlation analysis were employed. The CCA indicates that the element concentrations in *Z. multiflora* leaves were positively or negatively correlated with the relative amounts of EO compounds. Furthermore, it showed that the EO compounds have a high potential in terms of inhibiting fungal mycelium growth (Fig. 2). The concentration of Mg, Mn, B, Fe, B, Cu and Zn were positively correlated with myrcene and linalool relative amounts, which were not strongly related to the inhibition of the fungal mycelium growth. The concentrations of K, Na and P were positively correlated with  $\gamma$ -terpinene and carvacrol relative amounts and the content of Ca showed a positive correlation with thymol and *p*-cymene relative amounts which in turn significantly correlated with the inhibition of the fungal mycelium growth.

The Spearman's correlation indicates that the concentration of B in *Z. multiflora* leaves was significantly and positively correlated with relative amounts of myrcene and linalool, while those showed a highly significant negative correlation with antifungal activity of all fungi (Table S1). Negative correlations were found between the concentration of Fe and relative amount of  $\alpha$ -pinene. The concentration of Mg showed a significant negative correlation with the relative amount of *p*-cymene, that of Mn a significant negative correlation with  $\gamma$ -terpinene, and the concentration of K was significantly and positively correlated with the relative amount of carvacrol. Furthermore, there were significant

positive correlations between the relative amounts of *p*-cymene,  $\gamma$ -terpinene, carvacrol and the inhibitory indices of fungal mycelium growth suggesting that there were significant increases of antifungal activity of EOs with increasing relative contents of *p*-cymene,  $\gamma$ -terpinene and carvacrol, whilst with increasing relative contents of myrcene and linalool, antifungal activity of EOs decreased. No significant linear correlation between thymol and antifungal activity was obtained, while the CCA indicated the antifungal activity of thymol.

### 3.3. Influence of drought and heat on volatile compounds

To determine the impact of various abiotic stresses on the production of volatile compounds, differently treated *Z. multiflora* samples were extracted with iso-octane and analyzed by GC-MS and GC-FID. In total, thirty-eight compounds were identified (Table 4). Heat and drought stress affected acclimatized *Z. multiflora* chemotypes differently in their main volatile composition (Fig. 3). The carvacrol chemotype (populations from Gezeh and Konar Siah) did not show significant changes in response to drought and heat stress with respect to its volatiles. In the linalool chemotype (Siriz population) the relative contents of linalool, *E-p*-mentha-2,8-dien-1-ol and 1,8-octanediol were significantly decreased in response to heat stress and combined drought and heat stress, but not to drought stress alone (Fig. 3C-E). At the same time, the heat stress and the combination of heat and drought stress significantly increased the relative content of carvacrol in the linalool chemotype (Fig. 3H). Drought and heat stress alone had no significant effect on the relative content of thymol,  $\gamma$ -terpinene and *p*-cymene in the linalool chemotype whilst the combination of heat and drought stress significantly increased the relative content of *p*-cymene,  $\gamma$ -terpinene and thymol (Fig. 3A & B & G).

### 3.4. Influence of UV-A radiation on volatile compounds

Increasing UV-A irradiation only slightly changed the volatile composition of *Z. multiflora* extracts (Table 5). The main volatile compounds in Gezeh and Konar Siah populations were carvacrol,  $\gamma$ -terpinene and *p*-cymene, which classified these populations as the carvacrol chemotype, while the main compounds in the Siriz population were linalool, *E-p*-mentha-2,8-dien-1-ol, 1,8-octanediol, thymol and

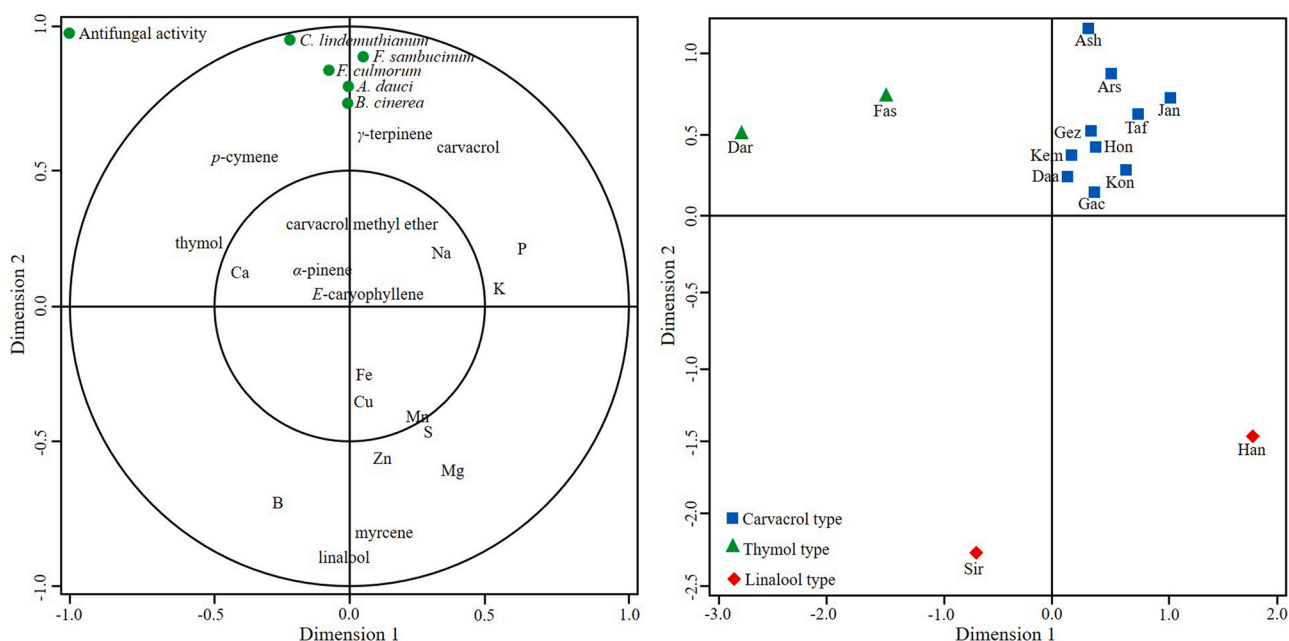


Fig. 2. Canonical correlation analysis (CCA) of leaf elements concentration, EOs compounds relative amounts and antifungal activity of EOs from *Z. multiflora* populations (left) and Score plot (PCA) obtained from the main variation of the EOs compositions and antifungal activities among populations (right).

Table 4

Relative content of solvent extracted volatile compounds from plants of *Z. multiflora* populations/chemotypes treated with different heat and drought stresses.

Compound	Retention indices			Relative content (%)											
				Gezeh (carvacrol) <sup>d</sup>				Konar Siah (carvacrol)				Siriz (linalool)			
	RI <sup>a</sup>	RI <sup>b</sup>	RI <sup>c</sup>	C <sup>e</sup>	D	H	H + D	C	D	H	H + D	C	D	H	H + D
$\alpha$ -thujene	926	924	929	1.1	1.2	1.8	1.9	1.1	1.1	1.8	2	tr	tr	0.7	1.4
$\alpha$ -pinene	937	932	939	1.1	0.9	1.1	1.3	0.7	0.7	1	1.2	0.7	0.8	0.9	1.3
sabinene	976	969	977	0.4	0.4	0.4	0.3	0.4	0.4	0.3	0.4	tr	tr	0.1	0.4
$\beta$ -pinene	979	974	979	0.3	0.3	0.2	0.3	0.3	0.3	0.3	0.2	0.4	0.3	0.4	0.5
myrcene	992	988	992	1.5	1.6	2.1	2.2	1.6	1.6	2.1	2.2	0.3	0.1	0.7	1.4
$\alpha$ -phellandrene	1007	1002	1005	0.1	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.1	tr	0.1	0.1
$\alpha$ -terpinene	1019	1014	1019	1.3	1.2	1.6	1.5	1.2	1.3	1.6	1.7	0.1	tr	0.6	1.6
<i>p</i> -cymene	1027	1020	1025	3.2	2.9	4.3	4.2	2.7	2.6	3.3	4.2	0.5	0.4	1.6	5.1
$\beta$ -phellandrene	1032	1025	1029	0.3	0.3	0.5	0.5	0.3	0.3	0.5	0.5	tr	tr	0.2	0.4
$\gamma$ -terpinene	1061	1054	1062	7.3	7.1	8.6	7.7	7.3	7.7	8.1	8.4	0.2	0.1	3.1	7
Z-sabinene hydrate	1070	1065	1070	0.9	0.9	1	1.1	0.8	0.8	1.1	1.1	tr	tr	0.4	0.9
Z-linalool oxide	1080	1070	1074	tr	tr	tr	tr	tr	tr	tr	tr	0.3	0.4	0.2	tr
terpinolene	1093	1086	1089	tr	tr	tr	tr	tr	tr	tr	tr	0.1	tr	0.1	tr
linalool	1100	1095	1100	2.2	0.5	0.5	0.4	0.4	0.4	0.6	0.4	58.3	61.5	43.1	14.1
<i>E</i> - $\gamma$ -caryophyllene	1104		1106	tr	tr	tr	tr	tr	tr	tr	tr	1.3	1.1	0.7	0.1
<i>p</i> -mentha-1(7),8-diene	1111		1107	tr	tr	tr	tr	tr	tr	tr	tr	0.3	0.2	0.1	tr
<i>p</i> -menth-2-en-1-ol	1123	1118		tr	tr	tr	tr	tr	tr	tr	tr	0.4	0.4	0.2	tr
1,3,8- <i>p</i> -menthatriene	1132		1119	tr	tr	tr	tr	tr	tr	tr	tr	0.8	0.7	0.5	0.1
<i>Z</i> - <i>p</i> -mentha-2,8-dien-1-ol	1135	1133		tr	tr	tr	tr	tr	tr	tr	tr	0.8	0.8	0.4	0.1
<i>E</i> - <i>p</i> -mentha-2,8-dien-1-ol	1154		1142	0.1	tr	tr	tr	tr	tr	tr	tr	8.2	7.5	3.6	0.8
<i>p</i> -mentha-1,5-dien-8-ol	1170		1171	tr	tr	tr	0.1	tr	tr	tr	tr	0.5	0.4	0.3	tr
borneol	1171	1165	1172	0.1	0.1	0.2	0.2	0.1	0.1	0.2	0.2	tr	tr	tr	0.1
4-terpineol	1181		1179	tr	tr	tr	tr	tr	tr	tr	tr	0.2	0.1	0.2	tr
<i>p</i> -cymenol	1189		1184	tr	tr	0.1	0.2	tr	tr	0.1	0.2	0.1	0.2	tr	tr
<i>E</i> - <i>p</i> -mentha-1(7),8-dien-2-ol	1200		1187	tr	tr	tr	tr	tr	tr	tr	tr	0.9	0.8	0.5	0.1
$\beta$ -cyclocitral	1209		1208	tr	tr	tr	tr	tr	tr	tr	tr	2.6	2.2	1.4	0.3
carvacrol methyl ether	1246	1241		0.2	0.3	0.1	0.2	0.2	0.2	0.1	0.2	tr	tr	0.4	0.2
linalool acetate	1256	1254		0.1	0.1	tr	tr	0.1	0.1	tr	0.1	1.4	1.6	0.5	0.2
geranial	1273		1273	tr	tr	tr	tr	tr	tr	tr	tr	0.3	0.1	0.3	tr
thymol	1291	1289	1295	2.5	0.2	0.3	0.3	0.2	0.2	0.3	0.3	3.2	2.5	4.2	25.2
carvacrol	1308	1298	1305	71.3	76.1	71.1	71.4	76.5	76.6	72.7	70.4	1.51	1.72	22.7	32.9
1,8-octanediol	1333	1339		0.2	tr	tr	tr	tr	tr	tr	tr	11.5	10.3	6.2	1.2
thymol acetate	1354	1349	1359	0.1	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	0.2
carvacrol acetate	1375	1370	1368	1.5	1.7	1.7	1.7	1.4	1.5	1.6	1.6	tr	tr	0.3	0.3
<i>E</i> -caryophyllene	1431		1427	1.3	1.8	1.4	1.5	1.6	1.2	1.5	1.7	2.3	2.7	2.6	2
$\alpha$ -humulene	1465		1452	tr	tr	0.1	0.2	tr	tr	tr	0.1	tr	tr	tr	tr
bicyclogermacrene	1508	1500	1507	1.2	0.9	1	0.9	1.3	1.2	0.9	0.8	0.6	0.8	0.3	0.4
spathulenol	1590	1577	1578	tr	tr	0.1	0.1	tr	tr	tr	0.1	tr	tr	tr	tr

tr: traces < 0.1 %. <sup>a</sup> RI, linear retention indices on the HP-5MS column, experimentally determined using a homologue series of n-alkanes. <sup>b,c</sup> Relative retention indices taken from Adams<sup>b</sup> and NIST<sup>c</sup>. <sup>d</sup> Populations (chemotypes). <sup>e</sup> Treatment: C, control; D, drought stress; H, heat stress; H + D, heat + drought stress. The values are presented as means of 10 replicates.

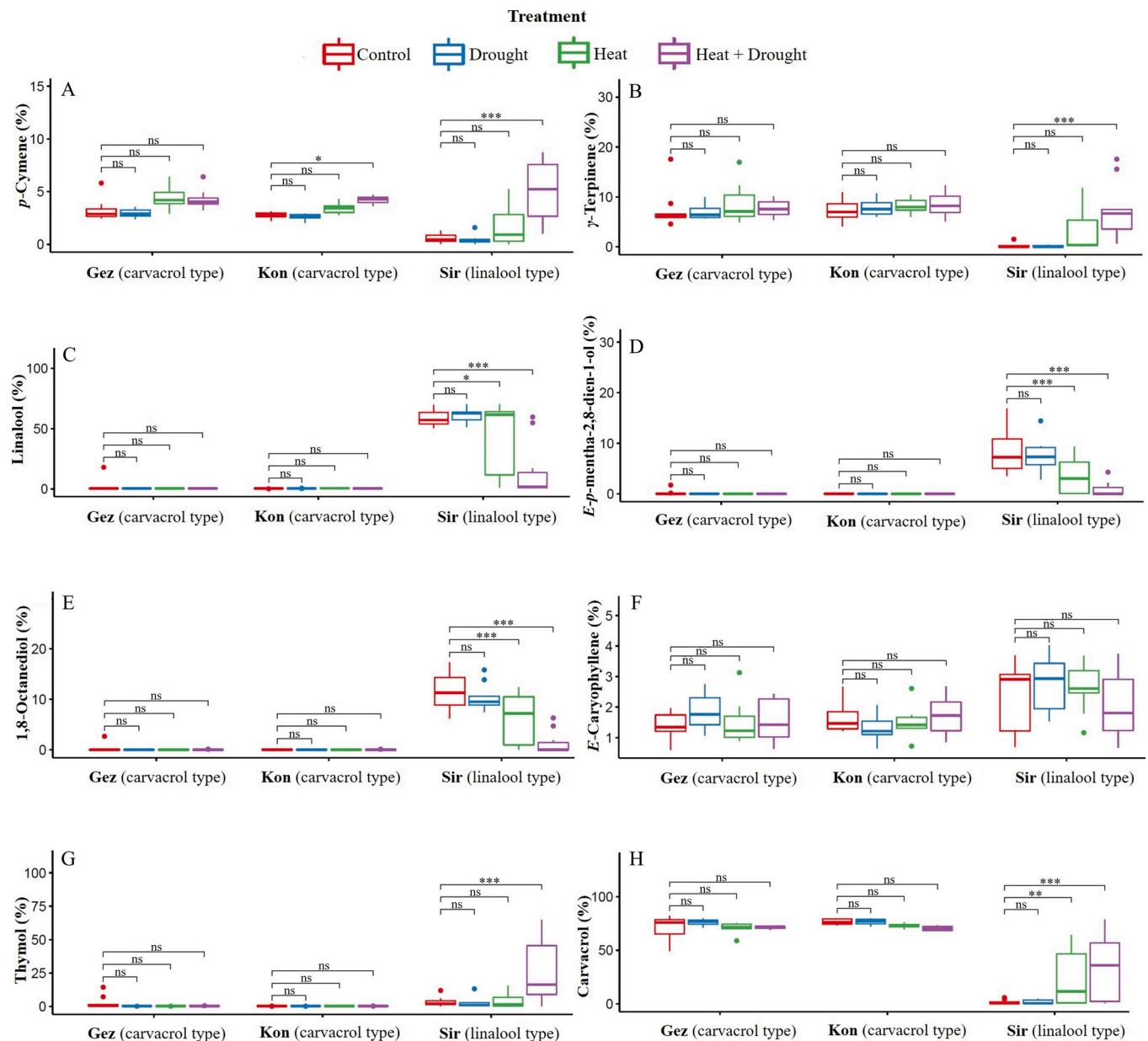
carvacrol. These differences in the volatile compounds remained visible in the plants grown from seeds from different Iranian populations in the growth chamber; however, no significant differences were observed in extracted volatile compounds in UV-A irradiated plants (Table S2). The relative content of the main component linalool was only reduced by trend in the linalool chemotype (population of Siriz), when plants were subjected to high and intermediate intensities of UV-A radiation, whilst the relative content of carvacrol was increased by trend. The different intensities of UV-A radiation did not change the relative content of other volatile compounds such as  $\gamma$ -terpinene, *p*-cymene, *E*-*p*-mentha-2,8-dien-1-ol, 1,8-octanediol and thymol.

#### 4. Discussion

The high rate of crop yield and food losses caused by microbial pathogens and the residual problem and toxicity of chemical pesticides to the living environment are demanding for alternative plant and food protection measures (Ogunnupebi et al., 2020). The use of plant EOs is an environmentally friendly alternative approach against pathogens and pests (Domingues and Santos, 2019). This study explored the potential of EOs of 14 *Z. multiflora* populations covering three chemotypes to inhibit a broad spectrum of agriculturally important fungal pathogens and elucidated the effects of leaf elements concentration, heat and drought stress and UV-light intensity on the production of antifungal

compounds.

Essential oils from plants of carvacrol and thymol chemotypes as well as carvacrol and thymol themselves inhibited the growth of five different fungal pathogens from a broader range of crop species (*C. lindemuthianum*, *F. sambucinum*, *F. culmorum*, *A. dauci* and *B. cinerea*) already at low concentrations, whilst EO of the linalool chemotype and linalool itself inhibited the fungal growth only weakly. Oregano and thyme oils strongly inhibited the mycelial growth of *A. dauci* and *C. lindemuthianum* (Pane et al., 2013), and the EO of *Origanum acutidens* containing carvacrol as main compound, as well as carvacrol and thymol alone, completely inhibited mycelial growth of 17 phytopathogenic fungi while *p*-cymene showed lower antifungal activity (Kordali et al., 2008). Several studies have revealed the strong antifungal activity of thymol and carvacrol, e.g. against *A. alternata*, and *B. cinerea* under in vitro and in vivo conditions, while linalool had a minor effect (Zhang et al., 2019; Shin et al., 2014; Feng et al., 2011). Contrariwise, the level of linalool production increased in strawberry fruits infected by *B. cinerea* and linalool fumigation inhibited the infection of fruits (Xu et al., 2019). Mehrparvar et al. (2016) reported that EO of *Z. multiflora* and *Satureja hortensis*, which contain carvacrol and thymol as main compounds, showed the highest antifungal activity against *Lecanicillium fungicola* and *Agaricus bisporus* whereas the EO of *Citrus limonum*, *Citrus aurantium* and *Artemisia dracunculoides* showed the lowest antifungal activity. This suggests that the antifungal activity of *Z. multiflora* EOs can



**Fig. 3.** Box plot graphics representing the median, 25-75 % quartiles, min and max (%) of 10 replicates of the main compounds (%) present in the essential oil from leaves of *Z. multiflora* chemotypes after drought and heat stress treatments. A) *p*-cymene; B)  $\gamma$ -terpinene; C) linalool; D) *E-p*-mentha-2,8-dien-1-ol; E) 1,8-octanediol; F) *E*-caryophyllene; G) thymol; H) carvacrol. Levels of statistical significance after LSD tests between means: \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ .

be attributed to the phenolic components and here particularly to thymol and carvacrol.

Secondary metabolites play an important role in the adaptation of plants to the environment including abiotic and biotic stresses. At the same time, they show promising activity against pathogens, pests and diseases. A previous study using field-collected *Z. multiflora* plants from different habitats in Iran, shown that the presence of carvacrol, thymol and linalool chemotypes could be related to different environmental factors (Karimi et al., 2020b). It suggests (following Thompson et al., 2007) that carvacrol and thymol chemotypes are present in adverse environments and that the EO phenols help the plants to cope with high temperatures and summer drought, while the linalool chemotype is better adapted to an early-winter freezing environment. To test the high temperature and summer drought hypothesis, plants of two *Z. multiflora* chemotypes were exposed to different abiotic stress conditions and analyzed with respect to changes in the volatile composition of extracts. Drought mimicking deficit irrigation treatments alone did not influence

the relative amounts of the main volatile compounds of the *Z. multiflora* chemotypes. The plants might have experienced only mild drought stress due to reduced irrigation with water when experiencing normal temperature (20 °C). Also in two oregano subspecies mild and moderate water stress conditions did not change the proportions of the main EO compounds carvacrol,  $\gamma$ -terpinene and *Z*- $\alpha$ -bisabolene (Morshedloo et al., 2017). In contrast, drought stress influenced the relative amounts of EO compounds and EO total yield of *Salvia officinalis*; moderate water deficit conditions increased and severe water deficit conditions decreased the linalool relative amount (Bettaieb et al., 2009). The percentages of EO compounds of rosemary, sage, lavender and basil including  $\alpha$ -pinene, D-limonene, eucalyptol and camphor were modified under drought stress (Kulak, 2020). In *Ocimum basilicum* L., drought conditions changed the relative amounts of EO components but not that of linalool (Mandoulakani et al., 2017). Over all, the responses to drought stress are specific for the specific EO compounds but also for the plant species under investigation and depend on the degree of drought



Table 5

Relative content of solvent extracted volatile compounds from plants of *Z. multiflora* populations/chemotypes irradiated with various levels of UV-A.

Compound	Retention indices			Relative content (%)											
				Gezeh (carvacrol) <sup>d</sup>				Konar Siah (carvacrol)				Siriz (linalool)			
	RI <sup>a</sup>	RI <sup>b</sup>	RI <sup>c</sup>	C <sup>e</sup>	LI	II	HI	C	LI	II	HI	C	LI	II	HI
<i>α</i> -thujene	926	924	929	1.2	1.3	1.3	1.3	1.2	1.3	1.2	1.3	0.4	0.2	0.5	0.5
<i>α</i> -pinene	937	932	939	0.8	0.7	0.9	0.8	0.6	0.9	0.7	0.8	0.6	0.4	0.6	0.7
sabinene	976	969	977	0.4	0.5	0.5	0.5	0.4	0.5	0.5	0.5	0.1	0.1	0.1	0.2
<i>β</i> -pinene	979	974	979	0.4	0.3	0.2	0.2	0.3	0.2	0.2	0.2	0.6	0.4	0.6	0.4
myrcene	992	988	992	1.6	1.7	1.6	1.7	1.6	1.7	1.6	1.7	0.7	0.4	0.7	0.8
<i>α</i> -phellandrene	1007	1002	1005	0.1	0.2	0.1	0.2	0.2	0.2	0.1	0.2	0.4	0.4	0.3	0.2
<i>α</i> -terpinene	1019	1014	1019	1.1	1.2	1.1	1.2	1.2	1.2	1.1	1.2	0.8	0.6	0.7	0.8
<i>p</i> -cymene	1027	1020	1025	2.6	2.7	2.6	2.6	2.4	2.5	2.7	2.6	1.8	1.1	1.9	2.2
<i>β</i> -phellandrene	1032	1025	1029	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.1	0.1	0.1	0.1
<i>γ</i> -terpinene	1061	1054	1062	6.2	6.7	6	7.3	6.6	6.8	6.4	7.2	2.6	1.3	2.6	3.3
<i>Z</i> -sabinene hydrate	1070	1065	1070	0.9	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.3	0.1	0.3	0.4
<i>Z</i> -linalool oxide	1080	1070	1074	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.3	0.1	0.2
terpinolene	1093	1086	1089	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	0.1	0.1
linalool	1100	1095	1100	0.5	0.3	0.4	0.4	0.4	0.4	0.5	0.4	44.6	49.9	38.4	33.9
<i>E</i> - <i>γ</i> -caryophyllene	1104		1106	tr	tr	tr	tr	tr	tr	tr	tr	0.6	0.8	0.4	0.5
<i>p</i> -mentha-1(7),8-diene	1111		1107	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	0.1	0.1
<i>p</i> -menth-2-en-1-ol	1123	1118		tr	tr	tr	tr	tr	tr	tr	tr	0.4	0.4	0.3	0.3
1,3,8- <i>p</i> -menthatriene	1132		1119	tr	tr	tr	tr	tr	tr	tr	tr	0.7	0.8	0.5	0.5
<i>Z</i> - <i>p</i> -mentha-2,8-dien-1-ol	1135	1133		tr	tr	tr	tr	tr	tr	tr	tr	0.5	0.8	0.5	0.5
<i>E</i> - <i>p</i> -mentha-2,8-dien-1-ol	1154		1142	tr	tr	tr	tr	tr	tr	tr	tr	5.6	10	5.1	5.7
<i>p</i> -mentha-1,5-dien-8-ol	1170		1171	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	0.2	0.2
borneol	1171	1165	1172	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr	tr	tr	tr
4-terpineol	1181		1179	0.1	tr	0.1	tr	tr	tr	tr	tr	0.1	tr	0.1	0.1
<i>p</i> -cymenol	1189		1184	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.1	0.1	tr
<i>E</i> - <i>p</i> -mentha-1(7),8-dien-2-ol	1200		1187	tr	tr	tr	tr	tr	tr	tr	tr	0.5	0.5	0.5	0.5
<i>β</i> -cyclocitral	1209		1208	tr	tr	tr	tr	tr	tr	tr	tr	1	1.2	1.2	1.5
carvacrol methyl ether	1246	1241		0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	tr	tr	0.2
linalool acetate	1256	1254		tr	tr	tr	tr	tr	tr	tr	tr	1.1	1.3	1.1	1
geranial	1273		1273	tr	tr	tr	tr	tr	tr	tr	tr	0.1	0.2	0.1	0.1
thymol	1291	1289	1295	0.2	0.1	0.2	0.1	0.2	0.2	0.2	0.2	14.3	10.6	13.4	12.4
carvacrol	1308	1298	1305	78.6	78.1	79.1	76.6	79	77.5	78.8	77.49	9	0.97	17.1	20.1
1,8-octanediol	1333	1339		tr	tr	tr	tr	tr	tr	tr	tr	7.5	11.3	6.7	7.3
thymol acetate	1354	1349	1359	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	tr	tr	0.1	tr
carvacrol acetate	1375	1370	1368	1.5	1.1	1.1	1.3	1	1.2	1.2	1.2	tr	tr	0.1	0.1
<i>E</i> -caryophyllene	1431		1427	1.6	1.5	1.3	1.7	1.3	1.8	1.3	1.5	2.6	2.3	2.6	2.2
<i>α</i> -humulene	1465		1452	tr	0.1	tr	tr	0.1	tr	tr	tr	0.1	tr	tr	tr
bicyclogermacrene	1508	1500	1507	0.9	1.2	1.3	1.2	1.1	1	0.7	1	0.7	0.6	0.8	0.8
spathulenol	1590	1577	1578	0.1	tr	tr	tr	0.1	tr	tr	tr	tr	tr	tr	tr

tr: traces < 0.1 %. <sup>a</sup> RI, linear retention indices on the HP-5MS column, experimentally determined using a homologue series of n-alkanes. <sup>b,c</sup> Relative retention indices taken from Adams<sup>b</sup> and NIST<sup>c</sup>. <sup>d</sup> Populations (chemotypes). <sup>e</sup> Treatment: C, Control (no UV-A radiation); LI, Low intensity UV-A radiation; II, Intermediate intensity UV-A radiation; HI, High intensity UV-A radiation. The values are presented as means of 10 replicates.

stress.

Heat and the combination of drought and heat reduced the relative content of linalool and increased that of carvacrol in *Z. multiflora* plants from the linalool chemotype; the combined heat and drought stress increased additionally the relative content of thymol, *γ*-terpinene and *p*-cymene. In the natural habitats of *Z. multiflora*, mean annual temperatures were negatively correlated with the relative linalool content whilst they were positively correlated with the relative carvacrol content (Karimi et al., 2020b). Similarly, in thyme, phenolic EO chemotypes dominate thyme populations in hot, dry locations (near the Mediterranean sea), while non-phenolic EO chemotypes were found farther inland, especially above 400 m elevation, i.e., in wetter, cooler climates (Granger and Passet, 1973).

The non-phenolic chemotypes are also more adapted to low temperatures in winter while phenolic chemotypes are sensitive to extremely cold temperatures. Phenolic EO chemotypes however, appear to be better adapted to summer drought than non-phenolic chemotypes (Thompson et al., 2007; Amiot et al., 2005).

High temperatures lead to a decrease of the linalool content in an *Ocimum basilicum* L. landrace, but did not affect the relative content of the main compound *Z*-methyl cinnamate (Tursun and Telci, 2020). The carvacrol chemotype of *Z. multiflora* grows in habitats with high temperature whilst that of the linalool chemotype grows in lower temperature environments (Karimi et al., 2020b). Moreover, the linalool

chemotype responded to heat and the combination of drought and heat by enhancing carvacrol (and thymol) and reducing linalool production. Hence, it was assumed that temperature directs the production of EO compounds of *Z. multiflora* (constitutively in the carvacrol and thymol chemotype and facultatively in the linalool chemotype) by modulating the monoterpene synthesis pathway in such a way that the geranyl cation, which serves as precursor for linalool, is converted to terpinene-4-yl cation, a precursor for *γ*-terpinene, *p*-cymene and carvacrol (Fig. 1). Further molecular research is needed to gain deeper insight into the role of terpene biosynthesis of *Z. multiflora* and the main components in EOs and into how different chemotypes were influenced under severe stress conditions.

Increased UV-A radiation did not significantly alter the relative contents of volatile compounds, although it reduced the relative content of linalool and increased the relative contents of *p*-cymene, *γ*-terpinene and carvacrol by trend in the linalool chemotype. In *Mentha piperita* UV-A radiation increased total EO, menthofuran and menthol relative contents significantly, when applied during the day (to act as UV-adsorbing pigments), whereas EO and menthol relative content decreased when applied during the night, where plants without white light (380–760 nm) provision responded as shade plants (Maffei et al., 1999). Verdaguer et al. (2017) conclude in their review on UV-A radiation effects on higher plants that plants exposed to UV-A do not employ alterations in total phenolic leaf compounds as a main protection

strategy, since hardly any changes are reported regardless of the plant life-form or geographic origin. However, changes in the production of individual phenolic compounds should be considered to understand the effects of UV-A radiation on plant metabolites. Other wavelengths might have stronger effects on EOs composition and need to be studied further. UV-B radiation affects the oil content and relative quantity of main compounds of lemon catmint and lemon balm essential oil, e.g., in lemon balm, it decreases the relative content of citronellal and geraniol and increases that of neral and geraniol (Manukyan, 2013). Tohidi et al. (2019) reported that different light treatments changed the percentages of EO components of thyme species; for instance, blue light treatment (460–475 nm) enhanced thymol relative content whilst white light (380–760 nm) enhanced relative linalool content in *T. migricus*. The combination of drought stress, high air temperatures and light intensities can increase the abundance of reactive oxygen species in plant leaves potentially damaging the photosynthetic apparatus (Miller et al., 2008), and specific monoterpene emissions might help to avoid permanent damage to the photosynthetic apparatus under stressful conditions (Haberstroh et al., 2018).

Mineral and trace elements are required for vital functions in plant metabolism. Their accumulation and leaf concentration varies due to their function in the plant and depends on plant species and genotype (Kara, 2009). Based on their mineral and trace element concentrations 18 herbs and herbal teas (such as peppermint, thyme, sage, lemon balm, etc.) could be classified into 5 groups (Kara, 2009). In many of these plants, the level of secondary metabolites depends on the level of specific mineral and trace elements. For example, in leaves and fruits of Ponkan mandarin, linalool synthase (CuSTS3-1 and CuSTS3-2) and linalool/nerolidol synthase (CuSTS4) only converted geranyl diphosphate (GPP) to the linalool in the presence of divalent cations  $Mn^{2+}$  and  $Mg^{2+}$ ; here linalool exhibited strong bacterial and antifungal activities against *Xanthomonas citri* subsp. *citri* and *Penicillium italicum* (Shimada et al., 2014). In this study, the most abundant elements in *Z. multiflora* leaves were Ca, K and Mg, while the abundant trace elements were Fe, Mn and Zn. In the CCA, the concentration of Mg, Mn, Fe, and Zn positively correlated with the relative amounts of myrcene and linalool, whereas the leaf elements concentration of K and P positively correlated with those of  $\gamma$ -terpinene and carvacrol; the content of Ca had a positive correlation with the thymol and *p*-cymene relative amount. Divalent cations such as  $Mn^{2+}$  and  $Mg^{2+}$ , which are present in higher amounts in the linalool chemotype of *Z. multiflora*, are required for converting GPP to the linalool (Shimada et al., 2014). Thiruvengadam et al. (2020) described a strong and positive correlation between Ca, Mg and the total phenolic content as well as a positive relation between Cu, Fe, Mn and total flavonoid content of *Lycium chinense* Miller. Moreover, trace element such as Cu, Zn, and Mn are essential co-factors for antioxidant potential of plants (Thiruvengadam et al., 2020). Biosynthesis and accumulation of bioactive molecules in medicinal and aromatic plants, which display various biological and physiological activities, are dependent on the availability and accessibility of mineral elements that cause varying effects in plant metabolism (Singh et al., 2017). Thus, considering mineral and trace element concentrations can help directing the mono- and sesquiterpene differentiation in medicinal and aromatic plants.

## 5. Conclusion

Essential oils of the carvacrol and thymol chemotypes of *Z. multiflora*, which contain the valuable phenolic compounds thymol and carvacrol, exhibited strong antifungal activities on a broad spectrum of important agricultural and food pathogens, whereas EO of the linalool chemotype inhibited fungal growth only weakly. Adjusting specific abiotic conditions such as low or high temperature or the combination of a specific temperature and water supply can be applied to modify the content of linalool or of the antifungal compound carvacrol in the linalool chemotype. Understanding the effect of environmental conditions on

populations and chemotypes development as well as that of abiotic stresses on the production of specific compounds by using environmental metabolomics approaches (Peters et al., 2018), can help fully exploiting the potential of aromatic plants as a potential source of natural sustainable fungicides in agriculture and food industry.

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## CRedit authorship contribution statement

Ali Karimi: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. Torsten Meiners: Conceptualization, Methodology, Validation, Writing - review & editing, Supervision, Project administration.

## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix B. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.indcrop.2021.113888>.

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