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Allelopathic activity from two Tunisian *Cupressaceae*

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Abstract : Medicinal plants possessed important biological activities that constitutes an important source for the development of bioactive compounds used as alternative to chemical pesticides**.** The present study investigated the allelopathic activities of *Tetraclinis articulata* and *Cupressus sempervirens* essential oils (EOs) extracted at vegetative, flowering and fructification stages. The EO chemical composition determined with by GC-MS revealed the predominance of oxygenated monoterpene classes of (50%) in *T. articulata* EO from fructification stage. However, *C. sempervirens* EOs were characterized by the abundance of sesquiterpene hydrocarbons class (64%) in EO from fructification stage. The EO from fructification stage inhibited at all concentrations germinated seeds and at highest concentrations radical elongation of *Raphanus sativus*. This EO inhibited also at highest concentrations germinated seeds and radical elongation of *Lattuca sativa. C. sempervirens* from flowering stage inhibited at highest concentrations seeds germination of *Lepidium sativum* and radical elongation of *Raphanus sativus.* Statistical analysis demonstrated that allelopathic activity these two Tunisian Cupressaceae EOs was attributed to the both monoterpene and sesquiterpene classes. Essential oils extracted from *T. articulata* and *Cupressus sempervirens* EOs could be used as a new alternative to chemical pesticides in agronomic fields.

Key words: *Tetraclinis articulata*; *Cupressus sempervirens*; allelopathic activity; essential oil.

1. Introduction

Weeds are defined as plants with any useless, troublesome and noxious plants growing out of place (Amri et al., 2012). Most commonly weeds compete with desired plants for space, nutriments, water, lights and pollinators (Vyvyan et al., 2002). This competition can be devastating. In fact, such weed infestations can change species composition so decreasing biodiversity vegetation, structure and ecosystem functions, biochemical cycles such as water and nutriment cycles (Nishida et al., 2005). Weeds disturbed sites may be toxic or irritant or pose some other danger to animals or humans. Weeds may harbor diseases, insect pests or vermin (Ouhaddou et al., 2022). They can impede regeneration of desired plants through allelopathic, suppressing their germination because of their physiological presence. Weeds can dilute genetic purity through hybridation, block and redirect water (Amri et al., 2012). According to (M'barek et al., 2016) weeds caused crop loss which reached 12%. Furthermore, the increase of weeds resulted in the increase of the herbicide uses. However, these chemical products caused dangerous several effects notably food residues, unpleased side effects and resistance weeds against synthetic agents (Ouhaddou et al., 2022). This has encouraged researchers to look for new alternative pesticides more efficient and respectful to human and environment. Recently, they have been considerable interest in biologically active compounds from plants as source of biopesticides. Among these biomolecules, EOs extracted from medicinal plants were considered as efficiency molecules to control weeds. In fact, Essential oil were more recommended because of their biodegradability, low coast and high availability. In nature, EO played an important role in the plant protection against microbial attack and sexual interaction. Accumulation of EOs in plant can play an important role in plant interaction by inhibition the germination and the development of other plants. Thus, the study of these secondary metabolites that inhibited or stimulate the germination of other plants was considered a part of defense mechanisms. For this reason, scientists explored EOs for weeds destruction and were considered EO as an important source of lead molecules in agriculture.

In this context, the use of secondary metabolites implicated in allelopathic interactions as sources for new agrochemical models could satisfy the requirements for crop protection and weeds managements (Abrahim et al., 2003). The present study aimed to study the allelopathic activities of *T. articulata and C. sempervirens* EOs extracted at three phenological stage and to determine the volatile compounds responsible for this activity.

2. Material and methods

2.1. Plant material

Tetraclinis articulata and *Cupressus sempervirens* used in this study are coniferous trees of Cupressaceae family native of North Africa and largely distributed in Tunisia. Areal parts of plants (terminal branches, leaves, flowers and fruits) were harvested from the forest of Saouaf in the North –East region of Tunisia (Latitude 36°13′41.80″N / Longitude 10°10′18.50″E) in 2020 at vegetative, flowering and fructification stages. Fresh samples were air dried at shade and ambient temperature (24 °C)

2.2. Extraction and analysis of essential oils

In order to extract EOs, dried samples of 50 g were hydrodistilled for 180 min in a Clevenger apparatus. The extraction time was optimized by kinetic survey during 30, 60, 90, 120, 180 and 210 min. Extractions were repeated three times and 6-methyl-5-hepten-2-one was used as an internal standard for the quantification of EO components An Agilent 7890A gas chromatograph (GC) was used for the analysis of EO. This system is coupled to an Agilent 5972C mass spectroscopy detector with electron impact ionization (70 eV). A HP-5 MS capillary column (30m×0.25 mm, coated with 5% phenyl methyl silicone, 95% dimethylpolysiloxane, 0.25mm film thickness; Hewlett-Packard, CA, USA) was used. The column temperature was programmed as follows: from 60 to 260 °C with a rate of 5 °C/min, from 260 to 340 °C with a rate of 40 °C/min. The carrier gas was helium N60 with a 0.9 mL/min flow rate; split ratio was 100:1. Scan time and mass range were 1 s and 50–550 m/z, respectively. The identification of compounds was based on mass spectra (compared with Wiley Registry 9th Edition/NIST 2011 edition mass spectral library)

2.3. Phytotoxic activity of the essential oils

The phytotoxic activity was evaluated on germination and root elongation of three different plant species: *Raphanus sativus* (radish), *Lepidum sativum* (garden cress) and *Latuca sativa* (lettuce) according to the method described by Mancini and Arnold (2009).These seeds were used in assays of phytotoxicity because of their easy germinability. Petri dishes were covered with five Whatman filter papers and impregnated with 7 mL of different concentrations of *T. articulata and C. sempervirens* EOs (2.5; 1.25; 0.625; 0.25; 0.125 and 0.062 µg ml^{-1}). Essential oils were dissolved in water-acetone mixture (99.5: 0.5, v/v). Seeds were surface sterilized for some seconds in ethanol (95%) than sown in petri dishes. Dishes were than incubated at 20°C with natural photoperiod. Control was performed with a mixture of water-ethanol. Seed germination was observed directly in petri dishes every 24 h. A seed was considered germinated when the protrusion of the root became evident (9). After 120 h, the effects on radicle elongation were measured in cm. Each determination was repeated three times, using Petri dishes containing 10 seeds each.

2.4. Statistical Analysis

The results of activities were expressed as mean (±Standard Deviation, SD) and statistically analyzed using the Student's t-test by MS-Excel software, Differences were considered significant at $p < 0.05$. IC_{50} was calculated by Graph Pad Prism non-linear regression equation. Data of antifungal were analyzed by one way ANOVA/Manova using SPSS software version 20. Mean values were compared using the Duncan multiple range test at $P \leq 0.05$. Result analyses of allelopathic activity were performed by the "Statistica v 5.1" software (Statsoft. Inc., EEUU).

3. Results

3.1. Essential oil chemical composition

The chemical composition of *T. articulata* EOs determined by GC-MS analysis was described by to Rguez et al. (2020). Results showed the richness of these EOs on α-pinene, bornyl acetate, caryophyllene and caryophyllene oxide. Oxygenated monoterpene class was the major class in all EOs (50.86% in EO from fructification stage) followed by monoterpene hydrocarbons (28% in EO from fructification stage). Sesquiterpene hydrocarbon class was more concentrated in EO from vegetative stage (38.33%). Bornyl acetate was the major compound identified at all phenological stage (Table 1).

Table 1. Chemical composition of *Tetraclinis articulata* essential oils at three phenological stages.

Compounds	% of total aroma		
	Vegetative stage	Flowering stage	Fructification stage
α-Pinene	$8.13^{c} \pm 0.20$	$18.50^{b} \pm 0.16$	$21.52^{a} \pm 1.77$
Camphene	$0.19^{a} \pm 0.06$	$0.23^{a} \pm 0.02$	nd
Verbenene	$0.24^{b} \pm 0.06$	$1.08^{a} \pm 0.1^{0}$	$1.08^{a} \pm 0.10$
β.-Myrcene	nd	$0.44^{a} \pm 0.03$	$0.34^{a} \pm 0.02$
P-Cimene	$0.14^{a} \pm 0.00$	$0.20^{a} \pm 0.01$	$5.2^{a} \pm 0.11$
Sabinene	nd	$3.65^{a} \pm 0.02$	$0.24^{b} \pm 0.02$
β-Terpinyl acetate	$3.25^{a} \pm 0.10$	$0.55^{b} \pm 0.04$	nd
Terpinolene	$0.24^{a} \pm 0.02$	nd	$0.57^{a} \pm 0.05$
Linalool	$0.36^{a} \pm 0.02$	nd	nd
α-Campholenal	$0.32^{c} \pm 0.01$	$1.22^{b} \pm 0.10$	$1.60^{a} \pm 0.01$
Pinocarveol	$0.32^{c} \pm 0.02$	$1.19^{b} \pm 0.07$	$1.44^{a} \pm 0.12$
Camphor	$1.43^{c} \pm 0.01$	$7.10^{a} \pm 0.38$	$5.36^{b} \pm 0.49$
Borneol	$3.82^{b} \pm 0.30$	$5.94^{a} \pm 0.09$	$6.04^{a} \pm 0.04$
Terpinen-4-ol	$0.40^{b} \pm 0.03$	$1.24^{a} \pm 0.04$	$1.28^{a} \pm 0.01$
P-Cymen-8-ol	nd	nd	$0.38^{a} \pm 0.05$
α-Terpineol	$0.36^{c} \pm 0.02$	$0.60^{b} \pm 0.05$	$0.87^{a} \pm 0.07$
Myrtenol	$0.44^{c} \pm 0.11$	$0.79^{b} \pm 0.06$	$1.27^{a} \pm 0.10$
Verbenone	$0.20^{c} \pm 0.01$	$0.47^{b} \pm 0.01$	$0.74^{a} \pm 0.06$
Cis-Carveol	$0.37^{a} \pm 0.03$	$0.63^{a} \pm 0.01$	$0.68^{a} \pm 0.06$
Cuminal	$0.32^{b} \pm 0.01$	nd	$0.46^{a} \pm 0.02$
Carvone	$1.96^{a} \pm 0.01$	$1.28^{b} \pm 0.10$	$1.78^{ab} \pm 0.01$
Bornyl Acetate	$17.22^{b} \pm 1.10$	$25.17^{a} \pm 0.30$	$26.3^{a} \pm 0.20$
α-Terpineol acetate	$1.45^{a} \pm 0.08$	$1.26^{a} \pm 0.01$	$1.15^{a} \pm 0.14$
α-Copaene	$1.57^{a} \pm 0.09$	$0.93^{ab} \pm 0.01$	$0.75^{b} \pm 0.04$
β-Elemene	$0.44^{a} \pm 0.01$	nd	$0.07^{b} \pm 0.01$
β-Maaliene	$2.36^{a} \pm 0.15$	nd	nd
Isoledene	nd^{c}	$1.33^{b} \pm 0.01$	$1.88^{a} \pm 0.10$
β-Caryophyllene	$16.10^{a} \pm 0.53$	$5.38^{b} \pm 0.09$	$5.17^{b} \pm 0.07$
β-ylangene	$0.24^{a} \pm 0.00$	nd	$0.13^{b} \pm 0.01$
α-Caryophyllene	$5.61^{a} \pm 0.12$	$1.75^{c} \pm 0.08$	$2.49^{b} \pm 0.10$
δ-Muurolene	$1.27^{a} \pm 0.03$	$0.48^{b} \pm 0.01$	nd
Germacrene D	$6.40^{a} \pm 0.05$	$1.83^{b} \pm 0.01$	$1.47^{b} \pm 0.01$
δ-Cadinene	$2.04^{a} \pm 0.01$	$1.47^{b} \pm 0.01$	nd
β-Cadinene	$3.86^{a} \pm 0.16$	$3.21^{b} \pm 0.1$	nd
Ledene Oxide-(II)	$0.54^{a} \pm 0.10$	nd	nd
Caryophyllene Oxide	$5.93^{a} \pm 0.01$	$5.57^{a} \pm 0.50$	$6.83^{a} \pm 0.50$
Aristolene Epoxide	$0.26^{a} \pm 0.02$	nd	$0.11^{a} \pm 0.01$
Spiro [4.4]nonan-2-one	$2.39^{a} \pm 0.18$	$1.34^{a} \pm 0.01$	$1.18^{a} \pm 0.01$
Cadina-1.4-Diene	$0.19^{b} \pm 0.01$	$1.49^{a} \pm 0.00$	nd
Cubenol	$3.11^{a} \pm 0.10$	nd	nd
Tau-Muurolol	$1.56^{a} \pm 0.08$	$1.17^{b} \pm 0.01$	nd
β-Cadin-4-en-10-ol	$0.15^{a} \pm 0.01$	$0.13^{a} \pm 0.00$	nd
α-Cadinol	$1.27^{a} \pm 0.0$	$1.22^{a} \pm 0.00$	nd
Unknown	1.25 ± 0.00	0.65 ± 0.01	0.43 ± 0.01
Total identified (%)	$96.47^{b} \pm 0.09$	$98.74^{a} \pm 0.06$	$98.40^{a} \pm 0.13$
Chemical classes			
Monoterpene hydrocarbons	$12.19^{c} \pm 0.14$	$24.66^{b} \pm 0.04$	$28.96^{a} \pm 0.25$
Oxygenated monoterpenes	$30.55^{c} \pm 0.60$	$47.82^{b} \pm 0.09$	$50.86^{a} \pm 0.19$
Sesquiterpene hydrocarbons	$38.33^{a} \pm 0.09$	$15.45^{b} \pm 0.07$	$11.21^{c} \pm 0.07$
Oxygenated sesquiterpenes	$15.4^{a} \pm 0.05$	$10.92^{b} \pm 0.12$	$8.12^{c} \pm 0.16$
Essential oil yield	$0.21^{b} \pm 0.00$	$0.25^{a} \pm 0.01$	$0.17^{c} \pm 0.01$

Components are listed in order of elution in polar column (HP-Innowax); nd: not detected. Means in the same column and followed by the same letter are not significantly different at $p < 0.05$ using Duncan's multiple range test.

Cupressus sempervirens EOs chemical composition was described by (Rguez et al., 2018). EOs were dominated by sesquiterpene hydrocarbons class (64.51% in EO flowering stage) followed by monoterpene hydrocarbons class (26% in EO from flowering stage). Germacrene D, β-caryophyllene and α-pinene were major compounds of EOs extracted from all phenological stage (Table 2).

Table 2. Chemical composition of *Cupressus sempervirens* essential oils at three phenological stages.

Compounds	% of total aroma		
	Vegetative stage	Flowering stage	Fructification stage
α-Thujene	$0.15^{b} \pm 0.01$	$0.36^{a} \pm 0.03$	nd^{b}
α-Pinene	**$14.75^{b} \pm 0.75$**	**$22.92^{a} \pm 2.94$**	**$16.19^{b} \pm 0.48$**
Camphene	nd^{b}	$0.33^{a} \pm 00.00$	nd^{b}
β-Pinene	$0.17^{a} \pm 0.18$	nd^{a}	nd^{a}
Myrcene	$0.15^{a} \pm 0.37$	nd^{a}	nd^{a}
δ-3-Carene	nd^{c}	$1.30^{b} \pm 0.08$	$5.75^{a} \pm 0.04$
Limonene	$0.97^{b} \pm 0.07$	$1.30^{a} \pm 0.04$	$1.35^{a} \pm 0.00$
p-Cimene	$0.14^{a} \pm 0.13$	nd^{a}	nd^{a}
δ-Terpinene	$0.30^{b} \pm 0.04$	$0.32^{b} \pm 0.02$	$0.40^{b} \pm 0.00$
α-Terpinolene	$1.02^{b} \pm 0.07$	$0.91^{c} \pm 0.03$	$1.69^{a} \pm 0.02$
***trans*-Pinocarveol**	$0.37^{a} \pm 0.14$	$0.39^{a} \pm 0.02$	nd^{b}
α-Phellandrene-8-ol	$0.67^{a} \pm 0.18$	$0.38^{b} \pm 0.01$	nd^{c}
Terpinene-4-ol	$0.95^{a} \pm 0.07$	$0.61^{b} \pm 0.15$	$0.32^{c} \pm 0.00$
α-Terpineol	$0.32^{b} \pm 0.04$	$0.16^{c} \pm 0.01$	$0.93^{a} \pm 0.00$
Carvacrolmethylether	$0.84^{a} \pm 0.05$	$0.87^{a} \pm 0.00$	$0.67^{b} \pm 0.00$
Borneol.acetate	$0.54^{a} \pm 0.02$	$0.43^{b} \pm 0.01$	$0.38^{c} \pm 0.00$
m-Mentha-1.8-diene	nd^{b}	$0.32^{a} \pm 0.01$	nd^{b}
α-Terpinylacetate	$0.42^{a} \pm 0.42$	nd^{a}	$0.35^{a} \pm 0.00$
Alloaromadendrene	nd^{c}	$0.48^{b} \pm 0.01$	$1.00^{a} \pm 0.05$
(+)-4-Carene	$4.96^{a} \pm 1.32$	$3.60^{a} \pm 0.09$	$6.63^{a} \pm 0.03$
Ylangene	nd^{b}	$0.25^{a} \pm 0.01$	nd^{b}
α-Copaene	$0.72^{b} \pm 0.03$	$0.82^{a} \pm 0.02$	$0.67^{c} \pm 0.0$
β-Bourbonene	nd^{b}	$0.16^{a} \pm 0.01$	nd^{b}
***Cis*-muurola-3.5-diene**	$0.45^{b} \pm 0.00$	$0.51^{a} \pm 0.01$	$0.43^{c} \pm 0.00$
α-Farnesene	nd^{b}	$0.21^{a} \pm 0.06$	nd^{b}
(+)-.β.-Funebrene	$2.47^{b} \pm 0.11$	$3.06^{a} \pm 0.08$	$2.63^{b} \pm 0.02$
β-Caryophyllene	$5.29^{a} \pm 0.21$	$5.29^{a} \pm 0.19$	$3.39^{b} \pm 0.04$
β-Ylangene	$0.54^{a} \pm 0.15$	nd^{b}	nd^{b}
β-Cubebene	nd^{b}	$0.58^{a} \pm 0.15$	$0.43^{a} \pm 0.21$
δ-Cadinene	$1.73^{b} \pm 0.04$	$0.77^{c} \pm 0.02$	$2.75^{a} \pm 0.03$
α-Caryophyllene	$4.46^{a} \pm 0.24$	$4.21^{a} \pm 0.08$	$2.36^{b} \pm 0.03$
epi-Bicyclosesquiphellandrene	$4.03^{b} \pm 0.12$	$2.44^{c} \pm 0.09$	$6.83^{a} \pm 0.10$
α-Amorphene	nd^{c}	$2.86^{a} \pm 0.07$	$1.47^{b} \pm 0.09$
δ-Muurolene	$2.95^{a} \pm 1.07$	nd^{b}	nd^{b}
Germacrene D	**$18.38^{c} \pm 0.18$**	**$20.66^{b} \pm 0.38$**	**$24.82^{a} \pm 0.14$**
Longipinene	$1.31^{a} \pm 0.33$	$1.18^{a} \pm 0.04$	nd^{b}
α-Ylangene	$0.41^{ab} \pm 0.82$	nd^{b}	$0.87^{a} \pm 0.05$
α-Muurolene	$2.15^{ab} \pm 0.67$	$1.72^{b} \pm 0.02$	$2.57^{a} \pm 0.04$
δ-Cadinene	$2.86^{a} \pm 0.11$	nd^{b}	nd^{b}
(+)-β-Gurjunene	nd^{c}	$3.29^{a} \pm 0.06$	$2.15^{b} \pm 0.03$
β-Cadinene	$7.39^{a} \pm 0.33$	$7.06^{a} \pm 0.16$	$4.84^{b} \pm 0.07$
β-Muurolene	$0.32^{b} \pm 0.00$	$0.29^{c} \pm 0.00$	$0.43^{a} \pm 0.01$
α-Cadinene	nd^{b}	nd^{b}	$0.24^{a} \pm 0.00$
Ledeneoxide-(II)	$0.35^{a} \pm 0.02$	$0.16^{b} \pm 0.08$	nd^{c}
Cadinol	nd^{b}	nd^{b}	$0.25^{a} \pm 0.01$
Caryophylleneoxide	$0.46^{a} \pm 0.02$	$0.32^{b} \pm 0.00$	nd^{c}
Aromadendreneoxide	$0.31^{a} \pm 0.03$	$0.25^{a} \pm 0.11$	nd^{b}
α-Cedrol	$0.81^{a} \pm 0.33$	$5.99^{b} \pm 0.07$	$5.61^{c} \pm 0.09$
Drimenol	$0.37^{a} \pm 0.06$	$0.40^{a} \pm 0.00$	nd^{b}
Cubenol	$0.18^{ab} \pm 0.22$	$0.27^{a} \pm 0.00$	nd^{b}
Muurolol	$0.39^{a} \pm 0.01$	$0.22^{b} \pm 0.00$	nd^{c}
α-Cadinol	$1.23^{a} \pm 0.31$	$0.40^{c} \pm 0.00$	$0.65^{b} \pm 0.01$
Sclareol	$0.30^{a} \pm 0.64$	nd^{a}	nd^{a}
Unknown	1.02 ± 0.01	0.77 ± 0.00	0.46 ± 0.00
Chemical Classes			
Monoterpenehydrocarbons	$16.63^{c} \pm 0.17$	$26.53^{a} \pm 0.34$	$23.69^{b} \pm 0.05$
Oxygenated monoterpenes	$5.13^{a} \pm 0.06$	$4.79^{b} \pm 0.03$	$4.34^{b} \pm 0.01$
Sesquiterpenehydrocarbons	$60.42^{b} \pm 0.21$	$59.59^{b} \pm 0.10$	$64.51^{a} \pm 0.16$
Oxygenatedsesquiterpenes	$4.87^{c} \pm 0.02$	$8.06^{a} \pm 0.01$	$6.51^{b} \pm 0.01$
Others	$1.43^{a} \pm 0.01$	$1.02^{a} \pm 0.01$	$1.1^{a} \pm 0.00$

Components are listed in order of elution in polar column (HP-Innowax); nd: not detected. In the same column, means followed by the same letter are not significantly different at ($P \leq 0.05$) using Duncan's multiple range test.

3.2. Allelopathic activity

In this study, *T. articulata* EOs from vegetative, flowering and fructification stages were evaluated for their activity against germination and radical elongation of radish (*R. sativus* L.), lettuce (*L. sativa* L.) and garden cress (*L. sativum* L.). Results in Table 3 showed that *T. articulata* EOs affected the germination and the radical elongation of *R. sativus* and *L. sativa*. However, *L. sativum* seeds were not affected by thuya EOs. The germination and radical elongation of *R. sativus* and *L. sativa* appeared sensitive to *T. articulata* EO from fructification stage. In fact, at all doses used (0.062 µg mL^{-1} to 2.5 µg mL^{-1}), radish seeds germination were inhibited significantly (Table 3).

Table 3. Phytotoxic activity of the essential oils of Tetraclinis articulata against germination and radicle elongation of Raphanus sativus, Lepidium sativum and Lactuca sativa. Data expressed in centimeter were obtained after 120 h of sowing.

Raphanus sativus **Germinated seeds**			**SD (cm)**	***Raphanus sativus*** **Radical elongation**			**SD (cm)**
Doses	***vg***	***fl***	***fr***	**Doses**	***vg***	***fl***	***fr***
Control	10.0±0.00	10.00±0.00	10.00±0.00	**Control**	9.30±0.10	9.30±0.10	9.30±0.10
0.062 µg/ml	9.30±0.20	10.00±0.00	9.30±0.60*	**0.062 µg/ml**	9.30±0.70	9.30±0.70	9.30±0.02
0.125 µg/ml	10.0±0.00	10.00±0.00	9.30±0.60*	**0.125 µg/ml**	9.30±0.50	9.00±0.80	9.00±0.20
0.25 µg/ml	9.60±0.50	9.70±0.80	9.00±0.00*	**0.25 µg/ml**	9.20±0.50	9.30±0.60	9.00±0.70
0.625 µg/ml	10.00±0.00	9.70 ±0.50	8.30±0.20**	**0.625 µg/ml**	9.30±0.30	9.10±0.80	4.40±0.70**
1.25 µg/ml	10.0±0.00	10.00±0.00	8.30±0.50**	**1.25 µg/ml**	9.40±0.30	9.50±0.30	3.00±0.30**
2.5 µg/ml	9.30± 0.50	9.60± 0.50	8.00±0.00**	**2.5 µg/ml**	9.80±0.50	9.20±0.20	3.60±0.40**
Lattuca sativa **Germinated seeds**			**SD (cm)**	***Lattuca sativa*** **Radical elongation**			**SD (cm)**
Doses	***vg***	***fl***	***fr***	**Doses**	***vg***	***fl***	***fr***
Control	10.00±0.00	10.00±0.00	10.00±0.00	**Control**	1.40±0.10	1.20±0.20	1.50±0.30
0.062 µg/ml	10.00±0.06	10.30±0.20	0.90±0.30	**0.062 µg/ml**	1.00±0.30	1.30±0.30	1.40±0.30
0.125 µg/ml	10.30±0.60	9.30±0.50	9.90±0.30	**0.125 µg/ml**	1.10±0.20	1.10±0.20	1.50±0.30
0.25 µg/ml	9.70±0.30	9.30±0.20	9.97±0.30	**0.25 µg/ml**	1.20±0.30	1.40±0.10	1.45±0.30
0.625 µg/ml	9.74±0.50	10.00±0.00	9.63±0.40	**0.625 µg/ml**	1.22±0.30	1.20±0.40	1.30±0.40
1.25 µg/ml	9.70±0.30	10.00±0.60	6.73±0.60**	**1.25 µg/ml**	1.35±0.20	1.30±0.10	1.10±0.50
2.5 µg/ml	10.00±0.70	9.30±0.70	5.91±0.40***	**2.5 µg/ml**	1.20±0.20	1.10±0.20	0.80±0.20**
Lepidium sativum **Germinated seeds**			**SD (cm)**	***Lepidium sativum*** **Radical elongation**			**SD (cm)**
Doses	***vg***	***fl***	***fr***	**Doses**	***vg***	***fl***	***fr***
Control	8.30±0.60	8.30±0.60	8.30±0.60	**Control**	7.00±0.60	7.00±0.60	7.00±0.60
0.062 µg/ml	8.00±0.60	8.22±0.30	7.70±0.66	**0.062 µg/ml**	6.50±0.05	7.22±033	7.60±0.90
0.125 µg/ml	7.70±0.60	8.66±0.52	8.00±0.80	**0.125 µg/ml**	6.40±0.80	6.65±0.55	7.40±0.80
0.25 µg/ml	8.30±0.60	8.30±0.60	8.60±0.86	**0.25 µg/ml**	6.00±0.80	7.30±0.66	7.70±0.90
0.625 µg/ml	8.30±0.60	7.70±0.60	8.70±0.60	**0.625 µg/ml**	6.40±0.90	6.85±0.62	7.20±0.70
1.25 µg/ml	8.33±0.22	8.30±0.60	8.00±0.40	**1.25 µg/ml**	6.90±0.75	7.50±0.50	7.00±0.40
2.5 µg/ml	8.70±0.60	7.30±1.20	7.50±0.65	**2.5 µg/ml**	6.50±0.90	7.75±0.70	6.8±0.60

At highest concentrations (0.625 µg mL^{-1}, 1.250 µg mL^{-1} and 2.5 µg mL^{-1}), thuya EO inhibited significantly radish radical elongation. Statistical analysis showed that phytotoxic activity against *Raphanus sativus* seeds germination was positively correlated with monoterpene hydrocarbons class (p=0.27) and some monoterpene compounds notably α-pinene (p=0.3), bornyl acetate and camphor (p=0.7). Phytotoxic activity of thuya EO against seeds elongation was statistically attributed to sesquiterpenes hydrocarbons (p=0.64), oxygenated sesquiterpenes (p=0.81) and β-caryophyllene sesquiterpene hydrocarbon compound (p=0.54) (Figure 1). Phytotoxic activity of thuya EO from fructification stage against *L. sativa* was positively correlated with sesquiterpene hydrocarbons class (p=0.55), sesquiterpene oxygenated (p=0.73) and β-caryophyllene (p=0.44). Anti-radical elongation of *T. articulata* EO against *L. sativa* was positively correlated to sesquiterpene hydrocarbons class (p=0.79), sesquiterpene oxygenated (p=0.9) and β-caryophyllene (p=0.7).

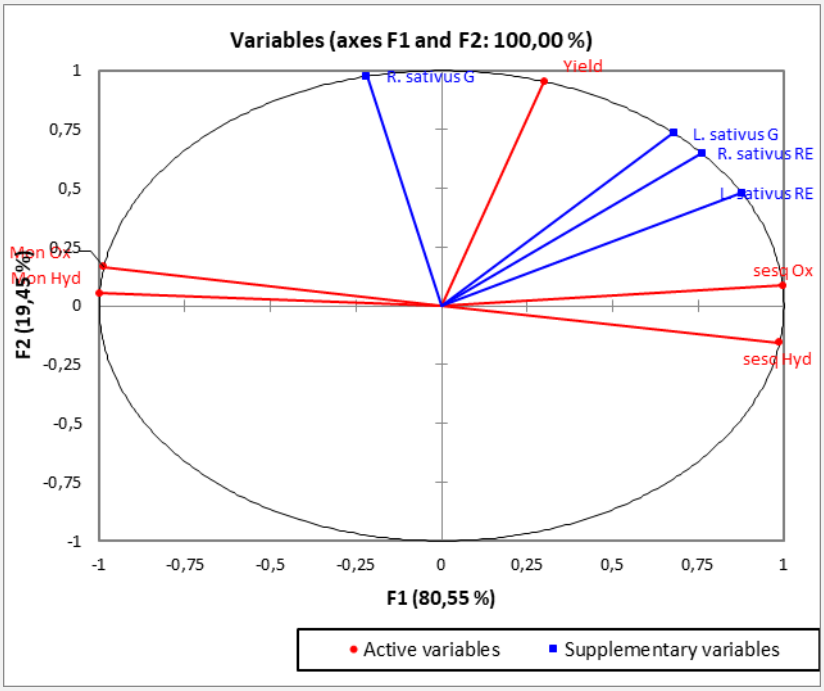

Figure 1: Principal component analysis biplot of phenological stages of *Tetraclinis articulata* according to essential oil composition and allelopathic activity.

Phytotoxic activity against radish, lettuce and garden cress of *C. sempervirens* EOs were presented in table 4. Results showed that EOs from flowering and fructification stage inhibited radical elongation of radish at 1.25 µg mL^{-1} and 2.5 µg mL^{-1}. *C. sempervirens* EO from flowering stage at highest tested concentrations (0.625 µg mL^{-1}, 1.250 µg mL^{-1} and 2.5 µg mL^{-1}) inhibited *Lepidium sativum* radical elongation However, *C. sempervirens* EOs extracted from all phenological stages didn't affect the germination and the radical elongation of *L. sativa* (Table 4).

Table 4. Phytotoxic activity of the essential oils of Cupressus sempervirens harvested at three phenological stages against germination and radicle elongation of Raphanus sativus, Latuca sativa and Lepidium sativum. Data expressed in centimeter were obtained after 120 h of sowing.

***Raphanus sativus* Germinated seeds ± SD**				***Raphanus sativus* Radical elongation ± SD (cm)**			
Doses (µg ml^{-1})	***vg***	***fl***	***fr***	**Doses (µg ml^{-1})**	***vg***	***fl***	***fr***
Control	9.30 ± 0.60	10.00 ± 0.00	9.70 ± 0.60	**Control**	3.50 ± 0.20	3.5 ± 0.20	3.50 ± 0.20
0.062	9.20 ± 0.30	9.90 ± 0.20	9.30 ± 0.60	**0.062**	3.30 ± 0.10	2.10 ± 0.20	2.30 ± 0.30
0.125	9.30 ± 0.00	9.70 ± 0.50	9.70 ± 0.60	**0.125**	3.40 ±0.02	2.10 ± 0.20	2.30 ± 0.01
0.25	9.30 ± 0.10	9.60 ± 0.58	8.50 ± 0.50	**0.25**	3.30 ± 0.60	1.90 ± 0.08	2.30 ± 0.20
0.625	9.30 ± 0.50	9.00 ± 0.00	9.00 ± 0.50	**0.625**	2.40 ± 0.22	2.80 ± 0.10	2.50 ± 0.35
1.25	9.30 ± 0.00	9.70 ± 0.00	9.30 ± 0.50	**1.25**	2.50 ± 0.80	1.80 ± 0.04**	2.20 ± 0.20*
2.5	9.60 ± 0.30	9.30 ± 0.70	9.00 ± 0.00	**2.5**	2.40 ± 0.20	1.80 ± 0.02**	2.40 ± 0.20*
***Latuca sativa* Germinated seeds ± SD**				***Latuca sativa* Radicle elongation ± SD (cm)**			
Doses (µg ml^{-1})	***vg* *Ethiopian***	***fl* *Black***	***fr* *Green***	**Doses (µg ml^{-1})**	***vg* *Ethiopian***	***fl* *Black***	***fr* *Green***
Control	10.00 ± 0.00	10.0 ± 0.00	10.00 ± 0.20	**Control**	0.50 ± 0.03	0.60 ±0.03	0.70 ±0.04
0.062	9.70 ± 0.40	9.70 ± 0.60	10.00 ± 0.00	**0.062**	0.40 ± 0.02	0.60 ±0.02	0.70 ±0.30
0.125	9.30 ± 0.80	9.30 ±0.80	9.00 ± 0.90	**0.125**	0.40 ± 0.20	0.60 ±0.11	0.70 ±0.03
0.25	9.00 ± 0.82	9.30 ± 0.82	9.30 ± 0.60	**0.25**	0.40 ± 0.20	0.60 ±0.02	0.70 ±0.07
0.625	9.70 ± 0.60	10.00 ± 0.00	9.40 ± 0.80	**0.625**	0.40 ± 0.20	0.60 ±0.02	0.70 ± 0.04
1.25	9.40 ± 0.40	10.00 ± 0.00	10.00 ± 0.00	**1.25**	0.30 ± 0.20	0.60 ±0.02	0.70 ±0.44
2.5	9.50 ± 0.90	9.70 ± 0.60	9.70 ± 0.60	**2.5**	0.30 ± 0.20	0.60 ±0.12	0.70 ±0.55
***Lepidium sativum* Germinated seeds ± SD**				***Lepidium sativum* Radicale elongation ± SD (cm)**			
Doses (µg ml^{-1})	***vg* *Ethiopian***	***fl* *Black***	***fr* *Green***	**Doses (µg ml^{-1})**	***vg* *Ethiopian***	***fl* *Black***	***fr* *Green***
Control	8.50 ± 0.00	8.30 ± 0.60	8.70 ± 0.20	**Control**	3.40 ± 0.02	3.60 ± 0.02	3.60 ±0.03
0.062	9.00 ±0.80	7.70 ± 0.60	6.30 ± 0.20	**0.062**	3.50 ± 0.02	3.75 ± 0.20	3.20±0.02
0.125	8.00 ± 0.70	8.70 ± 0.60	8.50 ± 1.00	**0.125**	3.20 ± 0.22	3.88 ± 0.40	3.20 ±0.12
0.25	9.00 ± 1.00	8.67 ± 0.60	8.00 ± 0.80	**0.25**	3.10 ± 0.02	3.80 ± 0.40	3.40 ±0.30
0.625	9.00 ± 1.00	7.70 ± 0.20*	8.70 ± 0.20	**0.625**	3.40 ± 0.01	3.87 ± 0.00	3.50 ±0.30
1.25	7.70 ± 0.60	4.70 ± 0.50**	8.70 ± 0.60	**1.25**	3.34 ± 0.05	3.60 ± 0.01	3.50 ± 0.22
2.5	8.70 ± 0.60	3.50 ± 0.50**	8.00 ± 0.72	**2.5**	3.90 ± 0.50	3.66 ± 0.02	3.30 ±0.33

Statistical analysis showed that phytotoxic activity of *C. sempervirens* EOs from flowering and fructification stages against *R. sativum* elongation showed that this activity was positively correlated with monoterpene oxygenated (p=0.35) and sesquiterpene hydrocarbon (p=0.23) class and (+)-4-carene oxygenated monoterpene compound (p=0.52). Phytotoxic activity of *C. sempervirens* EO from flowering against *L. sativum* was statically contributed to sesquiterpene hydrocarbon class (p=0.44) and sesquiterpene hydrocarbon compounds epi-bicyclosesquiphellandrene (p=0.61) and α-muurolene (p=0.73) (Figure 2).

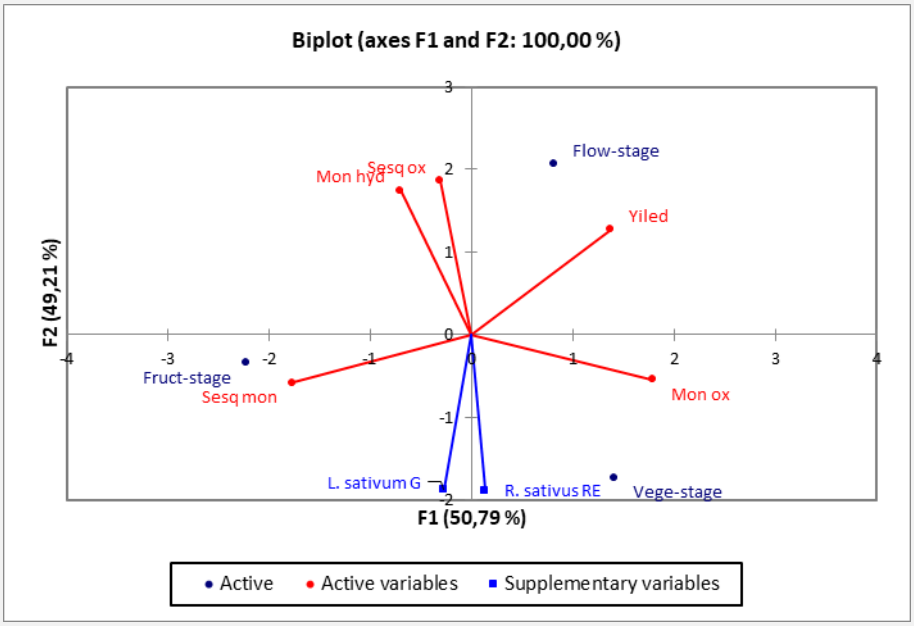

Figure 2: Principal component analysis biplot of phenological stages of *Cupressus sempervirens* according to essential oil composition and allelopathic activity.

4. Discussion

In this study, we prospected the effect of phenological stage on the phytotoxic activity of T. articulata and *C. sempervirens* along with its valorization for weed control. *T. articulata* EO from fructification stage inhibited seeds germination of *R. sativus* at all tested concentrations and at highest concentrations radical elongation of *R. sativus* and seeds germination and radical elongations of *L. sativa*. *C. sempervirens* EOs from flowering and fructification stages inhibited radical elongation of *R. sativus* and only EO from flowering stage inhibited seeds germination of *L. sativum.* According to our knowledge, only study of (M'barek et al., 2018) interested to the phytotoxic analysis of *T. articulata* EO. In fact, authors demonstrated that this EO inhibited seeds germination of *Sinapis arvensis* L and *Phalaris canariensis* L. Many research works described *C. sempervirens* EO phytotoxicity. Ismail et al. (2013) showed a significant phytotoxic effect of this EO against germination and seedling growth of four weeds: *Sinapis arvensis*, *Trifolium campestre*, *Lolium rigidum* and *Phalaris canariensis*. Statistical analysis showed that *T. articulata* EO phytotoxic activity against *Raphanus sativus* sand *Latuca sativa* was attributed to monoterpene class presented by camphor, bornyl acetate and α-pinene and sesquiterpene class presented by β-caryophyllene. However, phytotoxic activities of *C. sempervirens* EO was statistically attributed to oxygenated monoterpenes presented by (+)-4-carene and sesquiterpene hydrocarbons presented by epi-bicyclosesquiphellandrene and α-muurolene. Herbicidal effects of EOs against weeds have been previously reported and their phytotoxicity was generally attributed to the allelopathic properties of some terpenes notably mono and sesquiterpenes (Bourkhiss et al., 2010). According to (Muscolo et al., 2001), terpenes inhibited cell division and induced structural breaks and decomposition in roots (De Matino et al., 2012). Terpenes have been also reported for their effects on mitochondrial respiration (Rolin de Almeida et al., 2010) and for disruption of cellular membranes (De Matino et al., 2010). Terpenes compounds caused destruction in some organelles such as mitochondria, accumulation of lipid globules in the cytoplasm (De Almeida et al., 2010). Many authors suggested that terpenes inhibited germination and plant root growth through generation of ROS induced oxidative stress. Rguez et al (2020) described that EOs caused anatomical and physiological changes in plant seedling leading to accumulation of lipid globules in the cytoplasm, reduction in some organelles such mitochondria, inhibition of DNA synthesis and cell well alteration (Twvrkoski et al., 2002). Nishida et al. (2005) explained that the effect of an EO on seed germination and seedling growth is often explained in terms of the individual effects of some

constituents or the synergic effects of different EO compounds seeing that EO is a mixture of many different compounds.

5. Conclusion

T. articulata EO extracted at fructification stage and *C. sempervirens* EOs extracted at flowering and fructification stage showed a good antifungal activity against seeds germination and radical elongation of tested seeds. These EOs can be used as an alternative of chemical pesticides against weeds especially in organic farming production system.

6. References

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