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PHYTOTOXIC EFFECTS OF THYMUS VULGARIS AND CITRUS SINENSIS ESSENTIAL OILS ON SEED GERMINATION OF SEVEN CEREAL VARIETIES

Fatma Zohra HAMDANI[1,2]*, Namira MHAMEDI BOUZINA[2], Imane KRAOUCHE[2]

[1]Laboratory of Production and Protection of Crops of Chlef region, Hassiba Benbouali University of Chlef, Algeria
[2]Faculty of Nature and Life Sciences, Hassiba Benbouali University of Chlef, Algeria
*Corresponding Author: f.hamdani@univ-chlef.dz

ABSTRACT

In this study, we evaluated the allelopathic effects of the essential oils of *Thymus vulgaris* and *Citrus sinensis* on the seeds (seed germination and root length) of seven varieties of cereals (durum wheat: VITRON, SIMETO, CHEN'S, OUED BARED; soft wheat: MAWNA, ARZ; and barley: SAIDA). The chemical composition of the essential oils was determined using gas chromatography/mass spectrometry (GC/MS). The main constituents of the essential oils were carvacrol (48.56%) for *Thymus vulgaris* and β-pinene (30%) for *Citrus sinensis*. The results of the seed bioassay showed that the essential oils of *Thymus vulgaris* and *Citrus sinensis* exhibited a phytotoxic effect at high concentrations (1 and 0.1 mg/ml). The essential oils suppressed seed germination and root length. Germination of seeds was inhibited by 100% at concentrations of 1 and 0.1 mg/ml for both essential oils. A similar inhibitory effect was observed regarding root length; *Thymus vulgaris* essential oil reduced root length at 1, 0.1, and 0.01 mg/ml. The data suggest a greater effect of *Thymus vulgaris* essential oils than *Citrus sinensis*. Our study confirmed the phytotoxic effects of *Thymus vulgaris* and *Citrus sinensis* essential oils. Further studies are necessary for these essential oils to be used as bioherbicides. The research needs to focus on determining the herbicidal effects on cereal weeds and the minimum inhibitory dose that does not cause damage to host plants under field conditions.
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Key words: *Citrus sinensis, Thymus vulgaris, Essential oil, Germination, cereals.*

INTRODUCTION

The losses caused by weeds are one of the main limiting factors for staple crops worldwide. Interest in pesticides of natural origin (particularly plant-based) as an alternative to chemical pesticides has grown, as they are environmentally friendly, biodegradable, non-toxic and have a specific action, thus attracting considerable attention (Tripathi and Dubey, 2004 ; Isman ,2000; Ishii,2006; Ito and al.,2023). The yield of major crops, as well as the quality of harvested products, are severely compromised by weed infestation (Đorđević and al.,2022). In recent years, research has focused on exploring new ecological compounds with herbicidal activity for crop protection and weed management (Araniti and al., 2014). Particularly on cereals, Đurđić et al.(2016) considers, the wheat as the most important grainy plant used in human nutrition and it is the second on the ladder of overall grain production, after the corn Essential oils represent alternatives to chemical herbicides and have been widely studied as valuable ecological compounds with herbicidal activity for weed management (Araniti and al, 2018, Sarić-Krsmanović and al, 2023). The phytotoxicity of certain essential oils against weed seed germination has been proven (Tworkoski, 2002; Ibanez and Blazquez , 2020). However, the phytotoxic activity of essential oils must be selective, so that they do not harm crops. Therefore, besides the study of the herbicidal activity of essential oils, it is also necessary to know their potential effect on crops. Among essential oils, many have been identified as allelochemicals and tested for phytotoxicity(Kostina-Bednarz and al., 2023). The herbicidal effect of *Citrus sinensis* and *Thymus vulgaris* essential oils has been reported by Nepomuceno and Salgueiro, (2012) and Alexa and al, (2018). Zheljazko and al, (2021), *Thymus vulgaris* essential oils showed promise as a potential suppressor of seed germination and radicle growth of the weed species *Raphanus sativus, Lactuca sativa,* and *Lepidium sativum*. Our objective was to evaluate the phytotoxic effect of *Citrus sinensis* and *Thymus vulgaris* essential oils on cereal seeds considered as strategic crops in Algeria.

MATERIALS AND METHODS

Cereal seeds

Mature, healthy seeds of 7 cereal varieties donated by the Chlef cereals and pulses cooperative (CCLS) were used: 4 durum wheat varieties (VITRON, SIMETO, CHEN'S, OUED BARED), 2 soft wheat varieties (MAWNA, ARZ) and one barley variety (SAIDA).

Plant essential oils

Fresh *Citrus sinensis* and *Thymus vulgaris* fresh leaves were used for isolation of the essential oil, air-dried plant material was submitted to water distillation for 3 h using a Clevenger-type apparatus according to the European Pharmacopoeia method. The essential oils are stored in opaque bottles in the refrigerator at a temperature of 4°C and protected from light.

Gas chromatography (GC) analysis

The chemical composition of the essential oils was determined using gas chromatography/mass spectrometry (GC/MS). The operating conditions were as follows: the carrier gas was helium at a flow rate of 1 ml/min, the injector temperature was 250°C and 0.1 µl was injected in splitless mode for 0.1 min onto an HP5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm); the column temperature was programmed from 60°C to 260°C at a rate of 5°C/min with a final hold time of 10 min. For the Agilent 5973 model mass spectrometer, the temperature is: interface (280°C), source (230°C), quadrupole (150°C); ionization energy is 70 eV. Kovats indices of the compounds were calculated using the retention time of a series of n-alkanes.

Biological test

Using bioassays (% seed germination and root growth) we investigated the phytotoxic effect of the essential oils of *Thymus vulgaris* and *Citrus sinensis* on the seeds of 7 varieties of cereals. A total of 20 seeds were placed in 90mm diameter Petri dishes, containing a layer of filter paper (Whatman N01). The essential oil was emulsified with Tween20 (v/v 0.05%) and dissolved in distilled water. to obtained the final concentration: 0.0001, 0.001, 0.01, and 1 mg/ml of *Citrus sinensis* and *Thymus vulgaris* essential oils. 4 ml of the solution obtained was used to impregnate the germinating seeds. Distilled water mixed with Tween 20 (v/v 0.05 %) served as a control. The petri dishes were placed in a growth chamber at 25°C ± 2°C in dark for 10 days. The seed germination and elongation of radicel was observed in Petri dishes every 24 hours.

Statistical analyses

The results were subjected to an analysis of variance (ANOVA). Mean comparison tests were performed using the Fisher method at the 5% level and the software used to process the data was XLSTAT software

RESULTS AND DISCUSSION

Chemical composition of *Citrus sinensis* and *Thymus vulgaris* essential oils.

Essential oils were isolated from the leaves of *Citrus sinensis* and *Thymus vulgaris* by hydro distillation. Qualitative and quantitative analysis of essential oil chemical profiles is reported in Table 1. GC/MS analysis identified and characterized 26 compounds representing 95.72% of the *Citrus sinensis* essential oil and 22 compounds representing 91.75 % of the total *Thymus vulgaris* essential oil. The essential oil of *Citrus sinensis* was characterized by high levels of β - pinene (30%) while content of other components ranged from 9.37% to 0.07%. 15 components were present at less than 1%. Chi et al, (2020) and Mohammed et al, (2024) reported a similar number of constituents in *Citrus sinensis* essential oil, but with a different percentage of main compounds; in particular, a higher content of limonene. The essential oil of *Thymus vulgaris* was characterized by high levels of carvacrol (48.56%) and thymol (17.54%) and other components ranging from 8.70 to 0.09%. Five components were present at less than 1%. These results are in accordance with those previously reported. by Alexa and al., (2018) who reported that the major component of thyme essential oil was thymol.Vinciguerra et al. (2019) reported that p-cymene (36.36%) and thymol (24.35%) were the main components of *Thymus vulgaris* essential oil.

Phytotoxicity of *Citrus sinensis* and *Thymus vulgaris* essential oils

The results of seed bioassay showed that seed germination and length of roots of durum wheat, common wheat and barley varieties was inhibited at high concentrations (1 and 0.1 mg/ml) of *Thymus vulgaris* and *Citrus sinensis* essential oils. *Thymus vulgaris* essential oil show a strong inhibitory power (100%) at concentrations of 1, 0.1 and 0.01 mg/ml with VITRON, SIMETO and MAWNA varieties and 1 and 0.1 mg/ml for CHEN'S, OUED BARED, ARZ and SAIDA varieties (Figure 1). Sarić-Krsmanović and al, (2023) report the inhibiting effect that essential oils can have on seed germination. A comparable effect was observed on length of roots at levels of 1; 0.1 and 0.01 mg/ml, resulting in a 50% reduction in length of roots compared to the control (Table 2). The inhibitory effect was confirmed by statistically significant differences between all test concentrations (for seed germination and length of roots) and the control across the seven cereal varieties. However, no statistically significant difference was found for the low concentrations (0.001, 0.0001, and 0.00001 mg/ml). The effect of the high concentration used on the inhibition of seed germination was reported by Dorđević and al., (2022) for *S. glauca*, the allelopathic effect expressed through the inhibition of seed germination was more pronounced only after treatments with the highest used concentrations of extracts. Similar effect was observed with *Citrus sinensis* essential oils at the concentration 1 mg/ml (seed germination and length of roots) (Figure 2) (Table 3). The data suggest a greater effect of *Thymus vulgaris* essential oils compared to *Citrus sinensis* essential oils. The literature highlights a good phytotoxic capacity for *Thymus vulgaris* essential oil (Rolli et al. ,2014). The high phytotoxicity recorded for the essential oil of *Thymus vulgaris* compared with that of *Citrus sinensis* may be related to its high thymol and carvacrol content. Effectively Synowiec et al., (2017) report that the most phytotoxic group consisted of four essential oils, namely *Carum carvi*, *Thymus vulgaris*, *Mentha piperita* and *Salvia.officinalis* principally composed of oxygenated monoterpenes, while the second least phytotoxic group consisted of *S. canadensis* essential oil, rich in mono- and sesquiterpene hydrocarbons. Abd-ElGawad and al ,(2020) have mentioned that α- and β-pinene, 1,8 cineole, linalool, and carvacrol were the most effective allelopathic monoterpene compound.It has been suggested that the effect of essential oils can disrupt the permeability of the cell membrane structure. Such a phenomenon is due to the penetration of monoterpenes through the cell wall and cell membrane, or causes a leakage of cellular potassium that inhibits glucose-dependent respiration (Mutlu et al., 2011). Varietal sensitivity to essential oils was different, with the VITRON variety being the most sensitive to *Thymus vulgaris* and *Citrus sinensis* and essential oils, followed by CHEN'S, OUED BARED, MAWNA, ARZ and SAIDA, in contrast to SIMETO, which recorded the highest percentage of seed germination. Abrahim et al. (2000) and Batish et al. (2004), reported that reduction of a plant's respiratory activities by monoterpenes reduces the amount of photosynthesis and disrupts germination as well as radicle growth.

Table 1. Chemical composition of *Citrus sinensis* and *Thymus vulgaris* essential oils

Components	*RI*	*Citrus sinensis* Contents (%)	*Thymus vulgaris* Contents (%)
α-thujene	930	1.49	1.0
α -pinene	939	2.04	5.69
camphene	953	-	0.31
sabinene	976	-	tr
β - pinene	979	30	0.4
myrcene	991	4.52	1.40
α phellandrene	1008	0.68	0.21
3 carene	1012	6.41	0.15
(+)-4-carene	1035	2.77	-
limonene	1035	9.37	0.30
β -ocimene	1070	0.74	0.1
1,3,6-octatriene,3,7-dimethyl-	1070	8.59	-
γ- terpinene	1080	4.42	8.70
cis sabinene hydrate	1094	0.21	-
terpinolene	1096	2.53	0.10
α -campholenal	1124	0.42	
linalool	1128	1.24	6.20
camphor	1143	-	-
borneol	1165	-	0.1
terpineol-4	1177	2.14	-
α terpineol	1189	0.07	0.1
citronellol	1226	1.05	-
carvacrol	1300	-	48.56
thymol	1309	-	17.54
(Z) β-elemene	1393	0.44	**-**
(E) β - elemene	1398	8.97	**-**
Caryophyllene	1417	3.48	**-**
trans- caryophyllene	1417	-	**0.50**
***α* -humulene**	1474	1.33	**0.10**
γ-cadinène	1513	0.56	**0.09**
caryophyllene oxide	1606	0.23	**-**
β sinensal	1706	0.72	**-**
α sinensal	1750	0.3	-
Total identified (%)		95.72	91.7

RI : retention index ; Tr: Trace (value less than 0.01%)

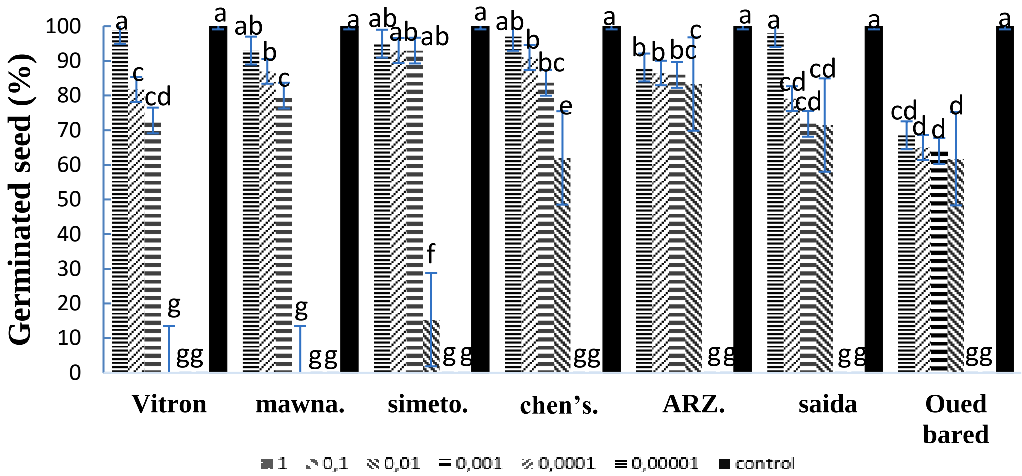

Figure 1. Effects of different concentrations of Thymus vulgaris essential oil on seed germination of 7 varieties of cereals. Means sharing the same letter are not significantly different at the 5% level of significance

Table 2. Effects of different concentrations of *Thymus vulgaris* essential oil on radicle length (mm) of 7 varieties of cereals

Concentration of essential oil (1mg/ml)	Vitron	Mawna	Simeto	Chen's	ARZ	Saida	Oued bared
1	0±0.00[d*]	0±0.00[d]	0±0.00[d]	0±0.00[d]	0±0.00[d]	0±0.00[d]	0±0.00[d]
0,1	0±0.00[d]	0±0.00[d]	0±0.00[d]	0±0.00[d]	0±0.00[d]	0±0.00[d]	0±0.00[d]
0,01	0±0.00[d]	0±0.00[d]	4,05±0.09[c]	4,15±1.06[c]	6±0.10[c]	5,88±0.89[c]	5,7±0.06[c]
0,001	0±0.00[d]	5,17±0.24[c]	4,57±0.16[c]	6,55±2.03[bc]	6,85±1.06[bc]	6±0.56[b]	6,15±0.36[b]
0,0001	4,3 ± 0.25[c]	5,62±0.06[b]	6,6±1.26[b]	7,37±1.07[b]	7,37±0.8[b]	6,23±0.17[b]	6,38±2.12[b]
0,00001	5,25 ± 0.30[b]	6±0.21[b]	8,1±1.13[ab]	7,4±0.06[b]	8,43±0.14[ab]	6,35±1.08[b]	6,48±0.04[b]
Control	6,02 ± 0.31[a]	8,05±0.06[a]	9,7±0.05[a]	9,55±0.15[a]	10,05±0.24[a]	9,7±0.44[a]	9,37±1.05[a]

* Within each column, means sharing the same letter were not significantly different at the 5% level of significance

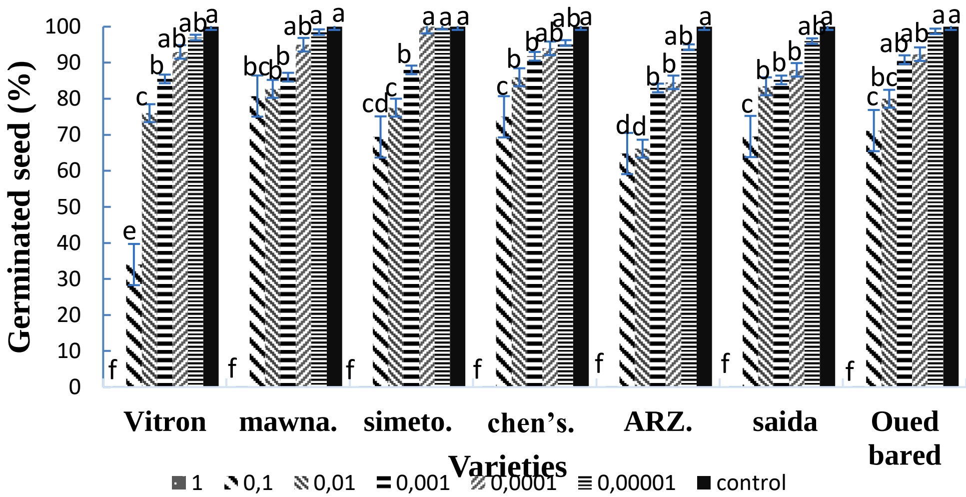

Figure 2. Effects of different concentrations of *Citrus sinensis* essential oil on seed germination of 7 varieties of cereals. Means sharing the same letter are not significantly different at the 5% level of significance

Table 3. Effects of different concentrations of *Citrus sinensis* essential oil on radicle length (mm) of 7 varieties of cereals.

Concentration of essential oil (1mg/ml)	**Vitron**	**Mawna**	**Simeto**	**Chen's**	**ARZ**	**Saida**	**Oued bared**
1	0,05±0.24^{d}*	0,05±0.03^{d}	0,12±0.05^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{c}
0,1	1,5±0.08^{c}	6,62±1.65^{c}	5,23±0.99^{c}	6,52±0.07^{c}	4,6±1.92^{c}	5,72±0.89^{c}	6,6±0.14^{bc}
0,01	4,05±1.35^{b}	6,87±0.55^{c}	6,5±0.47^{c}	6,57±0.15^{c}	5,72±0.35^{c}	6,45±1.24^{bc}	6,75±0.88^{bc}
0,001	4,3±0.07^{b}	7,52±0.78^{b}	7,62±0.21^{b}	7,35±0.06^{bc}	6,7±0.70^{bc}	6,87±0.58^{b}	8,12±0.45^{b}
0,0001	4,57±0.89^{b}	7,42±1.13^{b}	7,82±0.34^{b}	8,52±0.75^{b}	7,95±1.87^{b}	7,25±0.27^{b}	8,57±2.13^{ab}
0,00001	5,77±1.54^{a}	8±0.84^{a}	8,57±3.12^{ab}	8,95±2.47^{a}	9,52±0.03^{ab}	7,9±0.46^{ab}	9,04±2.76^{a}
Control	6,02 ± 0.31^{a}	8,05±0.06^{a}	9,7±0.05^{a}	9,55±0.15^{a}	10,05±0.24^{a}	9,7±0.44^{a}	9,37±1.05^{a}

* Within each column, means sharing the same letter were not significantly different at the 5% level of significance

CONCLUSION

Our study confirmed the phytotoxic effect of *Thymus vulgaris* and *Citrus sinensis* essential oils used in higher doses on seed germination and length of roots *in vitro*. further studies are necessary for essential oils of *Thymus vulgaris* and *Citrus sinensis* to be used as bioherbicides. The research needs to focus on determining the herbicidal effect on cereal weeds and the minimum inhibitory dose that cannot cause damage to host plants under field conditions.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Abd-ElGawad, A. M., El Gendy, A. E. N. G., Assaeed, A. M., Al-Rowaily, S. L., Alharthi, A. S., Mohamed, T. A., ... & Elshamy, A. I. (2020). Phytotoxic effects of plant essential oils: A systematic review and structure-activity relationship based on chemometric analyses. *Plants*, *10*(1), 36.

Abrahim, D., Braguini, W. L., Kelmer-Bracht, A. M.& Ishi-Iwamoto E. L. (2000) Effects of four monoterpenes on germination, primary root growth and mitochondrial respiration of maize, Journal of chemical ecology, 26, 611-623.

Alexa, E., Sumalan, R. M., Danciu, C., Obistioiu, D., Negrea, M., Poiana, M. A., ... & Dehelean, C. (2018). Synergistic antifungal, allelopatic and anti-proliferative potential of *Salvia officinalis* L., and *Thymus vulgaris* L. essential oils. *Molecules*, *23*(1), 185.

Araniti F, Marrelli M, Lupini A, Mercati F, Statti GA & Abenavoli MR (2014) Phytotoxic activity of *Cachrys pungens* Jan, a Mediterranean species: separation, identification and quantification of potential allelochemicals. Acta Physiol Plant 36:1071–1083

Araniti F., Landi M., Lupini A., Sunseri F., Guidi L.& Abenavoli M.R.,(2018). *Origanum vulgare* essential oils inhibit glutamate and aspartate metabolism altering the photorespiratory pathway in *Arabidopsis thaliana* seedlings, Journal of Plant Physiology, Volume 231,

Batish, D. R., Setia, N., Singh, H. P. &Kohli, R. K. (2004) Phytotoxicity of lemon-scented eucalypt oil and its potential use as a bioherbicide, Crop Protection, 23, 1209-1214

Chi, P. T. L., Van Hung, P., Le Thanh, H., & Phi, N. T. L. (2020). Valorization of Citrus Leaves: Chemical Composition, Antioxidant and Antibacterial Activities of Essential Oils. *Waste and Biomass Valorization*, *11*(9). https://doi.org/10.1007/s12649-019-00815-6

Đorđević, T., Đurović-Pejčev, R., Stevanović, M., Sarić-Krsmanović, M., Radivojević, L., Šantrić, L., & Gajić-Umiljendić, J. (2022). Phytotoxicity and allelopathic potential of *Juglans regia* L. leaf extract. Frontiers in Plant Science, 13, 986740.

Đorđević, T., Gajić, U. J., Sarić-Krsmanović, M., Radivojević, L., Đurović-Pejčev, R., Stevanović, M., & Vuković, M. (2022). Antioxidant activity of *Juglans regia L*. and *Rumex obtusifoliu*s L. leaf extracts and screening for their allelopathic potential. Pestic. Phytomed. (Belgrade), 37(1), 9–19. DOI: https://doi.org/10.2298/PIF2201009D

Đurðic, I., Govedarica, B., & Milic, V. (2016). Testing living capabilities of seeds of wheat varietys from banja luka (cold test). *Poljoprivreda i Sumarstvo*, *62*(2), 29.

Ibanez, M.D.& Blazquez, M.A., (2020). Phytotoxic effects of commercial essential oils on selected vegetable crops: Cucumber and tomato. Sustainable Chemistry and Pharmacy 15 100209

Isman MB (2000) Plant essential oils for pest and disease management. Crop Prot 19:603–608

Ishii H. (2006). Impact of fungicide resistance in plant pathogens on crop disease control and agricultural environment. *Japan Agricultural Research Quarterly*, 40 : 205–211.

Ito, M., Sperandio, N. C., da Silva, Y. H., Boeloni, J. N., Ignacchiti, M. D., de Avelar, B. R., ... & Martins, I. V. (2023). Chemical composition and effect of essential oils of *Thymus vulgaris* and *Origanum vulgare* on adults and ovigerous masses of *Pseudosuccinea columella*. *Natural Product Research*, 1-5.

Kostina-Bednarz, M., Płonka, J., & Barchanska, H. (2023). Allelopathy as a source of bioherbicides: challenges and prospects for sustainable agriculture. In *Reviews in Environmental Science and Biotechnology* (Vol. 22, Issue 2). https://doi.org/10.1007/s11157-023-09656-1

Mohammed, I. O., Alrasheid, A. A., & Hussein Ayoub, S. M. (2024). GC-MS Analysis and Study of the Antimicrobial Activity of *Citrus paradisi*, *Citrus aurantifolia*, and *Citrus sinensis* Peel Essential Oils as Hand Sanitizer. *International Journal of Microbiology*, *2024*. https://doi.org/10.1155/2024/4957712

Mutlu, S., Atici, O., Esim, N., Mete, E., 2011. Essential oils of catmint (*Nepeta meyeri* Benth.) induce oxidative stress in early seedlings of various weed species. Acta Physiologiae Plantarum 33, 943–951.

Nepomuceno Ribeiro, J. P., & Salgueiro Lima, M. I. (2012). Allelopathic effects of orange (*Citrus sinensis L.*) peel essential oil. *Acta Botanica Brasilica*, 256–259. http://www.scielo.br/pdf/abb/v26n1/25.pdf

Rolli, E., Marieschi, M., Maietti, S., Sacchetti, G., & Bruni, R. (2014). Comparative phytotoxicity of 25 essential oils on pre- and post-emergence development of *Solanum lycopersicum* L.: A multivariate approach. Industrial Crops and Products, *60*, 280–290. https://doi.org/10.1016/j.indcrop.2014.06.021

Sarić-Krsmanović, M., Gajić-Umiljendić, J., Radivojević, L., Šantrić, L., Đorđević, T., & Đurović-Pejčev, R. (2023). Sensitivity of Cuscuta species and their hosts to Anethum graveolens essential oil. *Pesticidi i fitomedicina= Pesticides and Phytomedicine*, *38*(1), 33-39.

Synowiec, A., Kalemba, D., Drozdek, E., & Bocianowski, J. (2017). Phytotoxic potential of essential oils from temperate climate plants against the germination of selected weeds and crops. Journal of Pest Science, 90, 407-419.

Tripathi P.&Dubey N. (2004). Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables. *Postharvest Biology and Technology*. 32: 235–245

Tworkoski, T., (2002). Herbicide effects of essential oils. Weed Sci. 50, 425–431

Vinciguerra, V., Rojas, F., Tedesco, V., Giusiano, G., & Angiolella, L. (2019). Chemical characterization and antifungal activity of *Origanum vulgare*, *Thymus vulgaris* essential oils and carvacrol against Malassezia furfur. *Natural Product Research*, *33*(22), 3273–3277. https://doi.org/10.1080/14786419.2018.1468325

Zheljazkov, V. D., Jeliazkova, E. A., & Astatkie, T. (2021). Allelopathic effects of essential oils on seed germination of barley and wheat. *Plants*, *10*(12), 2728.