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ALLELOPATHIC EFFECT OF THE ESSENTIAL OIL OBTAINED FROM HYSSOP (*HYSSOPUS OFFICINALIS* L., FAM. *LAMIACEAE*)

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Abstract

The paper aimed to present the evaluation of the allelopathic effect of the essential oil (EO) of hyssop (Hyssopus officinalis L.) obtained from a new Romanian variety ('Catalina' variety), on seed germination and seedling growth. As biological material, seeds from two weed species were used: green foxtail (Setaria viridis), Johnson grass (Sorghum halepense) and two vegetable species: lettuce ('May King' variety) and spinach ('Matador' variety). The main compounds identified in EO were: cis-pinocamphone (34.57%), trans-pinocamphone (13.73%), along with ß-pinene (13.32%), β-phellandrene (9.51%) and germacrene-D (5.38%). The obtained results demonstrated that EO had an allelopathic effect, inhibiting/stimulating seed germination and subsequent seedling development. However, the concentrations that showed inhibition/stimulation effect were different depending on the seed species tested. The study demonstrated possible allelopathic effects of EO from hyssop 'Cătălina', on weed and vegetable seed germination. The obtained results can be used in the future, for the development of commercial products with bioherbicidal action, but additionally, thorough research is needed regarding the mechanism of action, EO encapsulation, testing in field conditions, etc.

Key words*: EO, Hyssopus officinalis, 'Catalina' variety, chemical composition, germination and seedling growth.*

INTRODUCTION

Allelopathy refers to the inhibitory or stimulatory effect of one plant species on the germination, growth, and metabolism of another plant species, due to the release of allelochemicals into the surrounding environment (Maurya et al., 2022). Allelochemicals are substances extracted from plants, which have the ability to synthesize secondary metabolites such as phenolic acids, phenols, flavones, flavonoids, saponins, coumarins, which accumulate in small quantities in the cells of various organs (roots, leaves, flowers, and seeds). These can release allelochemical compounds into the surrounding environment through leaching, root exudation, volatilization, decomposition of residues, or organic matter, in both natural and agricultural systems (Sakihama et al., 2002). Allelopathy is a complex process, and to date, a number of allelochemicals have been identified, which have been the subject of many studies, including species from the fam. Poaceae (Hierro et al., 2021; Fatholahi et al. 2020; Favaretto et al., 2018). However, very little data is known about the transport and biodegradation of allelochemicals in the soil or about the genetic populations of species with allelopathic properties (Acheuk et al., 2022).

The ability of allelochemical compounds to affect germination, inhibit, or delay the growth of other plants can be a problem for agricultural production due to weed interference with crop plants. However, these plant metabolites can also act as natural bioherbicides. Establishing practical ways to use allelochemicals in the field, the rapid adaptation of weeds to avoid them, the diversity of soil microbial communities, all these aspects should be researched in the future. The research conducted so far has shown that allelopathy has good potential for application in agricultural practice. Many crops with allelopathic potential have been used in agricultural production, but their application is limited to small-scale, regional areas (Kesraoui et al., 2022). Weed management involves using multiple techniques to limit the infestation of agricultural crops and minimize competition. These techniques have evolved to mitigate production losses and intervene only when a problem is identified (Abd-ElGawad et al., 2021). Currently, researchers are addressing weed control through physical, chemical, and biological methods. Weed control carried out in an environmentally friendly manner is considered a challenge. Natural resources provide new approaches for producing environmentally friendly and safe bioherbicides that are effective against weeds. One of these resources is represented by medicinal plants that produce essential oils (EOs), complex mixtures of secondary metabolites containing mono-, di-, and sesqui-terpenes, in addition to hydrocarbons (Assaeed et al., 2020; Abd-ElGawad et al., 2019). EOs and their volatile compounds are at the forefront due to their phytotoxicity and allelopathy, combined with rapid degradation in the environment, which can have practical applications in agriculture, horticulture, and ecology (Stan (Tudora) et al., 2022). They can be used as natural alternatives to synthetic herbicides and pesticides or as additives to improve crop quality and yield (Soares et al., 2023). The allelopathic effect of hyssop EO refers to its ability to influence the growth and development of other plants by releasing chemical substances into the surrounding environment. Thus, its antiviral, antiseptic, anti-inflammatory, and antispasmodic properties can be beneficial for human health as well as for weed control or crop stimulation. According to a study, hyssop EO exhibited inhibitory effects on the germination and growth of wheat, barley, oats seedlings, as well as on their photosynthesis and respiration. This effect was attributed to the high content of thymol and carvacrol, which acted as allelopathic agents. The study suggests that hyssop EO can be used as a natural herbicide in combating monocotyledonous weeds (Zheljazkov et al., 2021). Another study showed that the same type of EO had a stimulatory effect on the germination and growth of seedlings of dill, fennel and basil. The study suggests that hyssop EO can be used as a natural additive to improve the quality and yield of dicotyledonous crops (Raut & Karuppayil, 2014). In the study conducted by Almeida et al., in 2010, the main monoterpenic compounds identified in hyssop EO were β-pinene, *iso*- and *trans*-pinocamphone, compounds with phytotoxic activity on *Raphanus sativus, Lactuca sativa, Lepidium sativum*.

The use of EOs obtained from MAPs (Medicinal and Aromatic Plants) can be an important component in integrated weed management systems, and the identified bioactive compounds could be used as potential bioherbicides (Maurya et al., 2022). Currently, more than 1400 biopesticides have been registered worldwide (Balog et al., 2017), with a smaller number registered in Europe due to the complex regulations within the EU.

Thus, in the EU, there are approximately 60 biopesticides available, compared to over 200 biopesticides on the North American market (Kumar et al., 2021). At the beginning of this decade, the global biopesticide market exceeded 4 billion USD, with expected doubling by the year 2025 (Rakshit et al., 2021).

In this context, the aim of this study was to evaluate the EO obtained from the first Romanian variety of hyssop ('Cătălina' variety), aiming to assess: the extraction yield, compound analysis using GC/MS (chemical composition, identification of major compounds and compound classes), and its allelopathic capacity (% germination, radicle and plumule length).

MATERIALS AND METHODS

The hyssop EO, 'Cătălina' variety, was obtained through hydrodistillation. The plant material required for hydrodistillation was cultivated on the lands within INMA Bucharest (Băneasa area), on a reddish-brown forest soil, under the climatic conditions of the year 2021. The crop was established using seedlings produced by SCDL Buzău, following all technological steps. The aerial parts of the plants (inflorescences and shoot tips) were processed when harvested in the flowering stage. The extraction yield was calculated using the formula:

$$\text{Extraction yield} = V / M \text{ (ml/kg)}$$

where:

V = the volume of essential oil obtained from the sample of green plant (ml);

M = the mass of the sample of medicinal plants (kg).

The chemical composition of hyssop EO. The concentration of compounds was determined by GC/MS analysis. The analysis conditions were as follows: Gas chromatograph Agilent Technologies type 7890 A GC system, MS Agilent Technologies type 5975 C Mass Selective Detector; HP 5MS column 30 m x 0.25 mm x 0.25 µm (5% Phenylmethylsiloxane); injector temperature 250°C, detector temperature 280°C; Temperature regime: 25°C (10 degrees/min) to 280°C (const. 5.5 min); mobile phase - helium 1 ml/min; injected volume - 0.1 µl EO; split ratio - 1:100.

The biological material used in the experiments consisted of seeds from two weed species:

- Green foxtail (*Setaria viridis* L., fam. *Poaceae)*, an annual grass weed with drought resistance, heat-loving characteristics, forming sparse tufts. It prefers calcium-poor, light soils, rich in nutrients, in warm regions;
- Johnson grass (*Sorghum halepense* L., fam. *Poaceae)*, an invasive perennial grass weed that forms rhizomes and prefers warm areas. It reproduces through seeds and rhizomes, making it difficult to control,

and two vegetable species:

- Lettuce (*Lactuca sativa* L., fam. *Asteraceae*, 'May King' variety), an early variety with medium-sized heads, light green in color, intended for outdoor cultivation. The crop can be established both in spring and autumn. Sowing period: March, August, September. Planting distances: 40 cm/25-30 cm.
- Spinach (*Spinacia oleracea* L., fam. *Amaranthaceae,* 'Matador' variety), a semi-early variety with a vegetation period of 45 days. The rosette is medium-sized, with slightly wrinkled, oval, fleshy leaves, short-petioled, dark green in color. It is resistant to low temperatures, can overwinter in the field, and produces floral stems late. It is sown in the field in February-March or August-October. Planting distances: 15-20 cm/3-5 cm. It is used for fresh consumption as well as for processing.

The experimental protocol aimed to: sterilize the seeds by immersing them for 3 minutes in a solution of NaOCl, followed by rinsing with sterile distilled water and drying on sterile filter paper. Hyssop EO was homogenized with Tween 80 (1:1 V/V) and tested at four concentrations: 100, 150, 250, and 300 µl, using sterile distilled water, while Tween 80 in water was used as a control. In Petri dishes, 20 seeds and 5 ml of each concentration of solution were placed on sterilized filter paper, followed by sealing with Parafilm and incubation in a plant growth chamber (climatic chamber with Fitotron system, model KK 115), at 27ºC, with a humidity range of 70-80%. After 5 days, the germinated seeds (radicle > 2mm) were counted, and after another 12 days (at the end of the experiment), the lengths of the radicle and plumule were measured. Each concentration was tested in 3 replicates. The parameters monitored were germination (%), radicle length and plumule length (cm).

The formula was applied to calculate germination, expressed in percentage (Siddiqi et al., 2007):

Germination = (number of germinated seeds/total number of seeds)× 100, (%)

The statistical processing for the interpolating the experimental data regarding the influence of hyssop EO action on the seeds, was performed using the MathCAD Soft 2000 and Excel programs from the MS Office 2007 package (MathCad, 2000).

RESULTS AND DISCUSSIONS

Extraction yield of hyssop EO, 'Cătălina' variety

Following the hydrodistillation process of the fresh plant material, represented by inflorescences and shoot tips, the hydrolate (a mixture of essential oil and floral water) was obtained. The hyssop EO, thus obtained, was decanted, filtered, and then stored in dark-colored bottles and kept in a cool, dry place. The extraction yield obtained was 10 ml of EO per 1 kg of fresh plant material and 2.0 liters of floral water per 1 kg of plant material, for 'Cătălina' variety. The quantity and especially the quality of the essential oil and floral water obtained from the new variety of hyssop were influenced by the type of soil, agricultural techniques applied, as well as by the agro-climatic conditions present in the production year (2021).

Chemical profile of hyssop EO, ′Cătălina′ variety

Specialized literature (Stan (Tudora) et.al, 2022) mentions compounds characteristic for hyssop EO to include *iso-* and *trans*-pinocamphone, pinocamphone, and their precursor, β-pinene (Figure 1). Among other constituents are pinocarvone, sabinene, germacrene-D, germacrene D-4-ol, α- and β-phellandrene, thymol, carvacrol, elemol, limonene, linalool, and 1,8-cineole (Fathiazad & Hamedeyazdan, 2011; Ogunwande et al., 2011*)*.

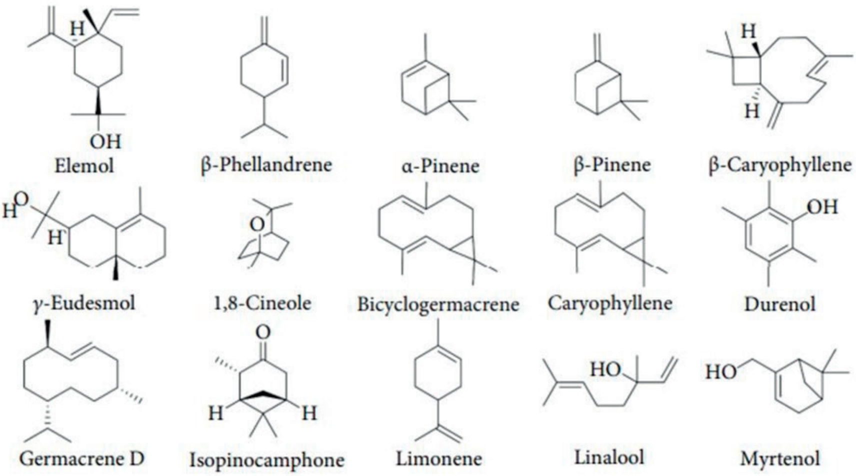

Figure 1. Chemical structures of the main bioactive compounds in hyssop EO (Source: Sharifi-Rad et al., 2022)

The chemical composition of hyssop EO is globally monitored by ISO 9841:2013.

In this document, 13 compounds are recognized as standards, and pinocamphone, *iso*-pinocamphone, and β-pinene are considered to be the most abundant compounds (40-90%).

For the EO obtained from the plants of the new variety of hyssop (produced in 2021), 16 compounds were identified, representing 99.99% of the total separated compounds.

The major compounds were: *cis*-pinocamphone (34.67%), *trans*-pinocamphone (13.73%), β-pinene (13.32%), germacrene-D (5.38%).

In addition to these, other compounds were also identified, but in variable amounts: β-phellandrene (9.51%), β-elemene (5.24%), α-phellandrene (2.64%), myrcene (2.72%), pinenol (1.37%), β-caryophyllene (1.92%), aromadendrene (1.70%), thujenol (1.28%), elemol (1.17%) (Table 1).

The main classes of compounds were: monoterpenes (84.58%), especially oxygenated ones (53.92%), and sesquiterpenes (15.41%), especially sesquiterpenes hydrocarbonate (16.24%) (Table 1).

Table 1. Chemical composition of the EO isolated from aerial part of the hyssop (*Hyssopus officinalis* L., fam. *Lamiaceae*), 'Cătălina' variety

Compound name[a]	**EO Hyssop ′Cătălina′ variety**	
	RT	**Area %**
β-Pinene	*9.59*	*13.32*
α-Phellandrene	10.66	2.64
Myrcene	13.45	2.72
D-Limonene	14.75	1.43
β-Phellandrene	*15.10*	*9.51*
β-Ocimene	17.23	1.04
Carenol	21.36	2.87
trans-Pinocamphone	25.16	13.73
cis-Pinocamphone	25.94	34.67
Pinenol	26.49	1.37
β-Caryophyllene	27.44	1.92
Aromadendrene	28.59	1.70
Germacrene-D	*30.09*	*5.38*
β-Elemene	30.64	5.24
Thujeol	31.75	1.28
Elemol	37.73	1.17
Total major compounds	**16 compounds identified 99.99%**	
Monoterpenes hydrocarbons	30.66	
Oxygenated monoterpenes	53.92	
Monoterpene	**84.58**	
Sesquiterpenes hydrocarbons	14.24	
Oxygenated sesquiterpenes	1.17	
Sesquiterpenes	**15.41**	

[a]Compounds identified based on retention index.

In the study conducted by Khan et al., in 2012, for the EO obtained from hyssop from Kashmir, the main compounds were: myrcene (2.24%), terpinene (1.03%), (Z)-sabinene hydrate (2.02%), linalool (1.04%), terpinen-4-ol (2.55%), germacrene-D (1.61%), and elemol (3.43%). The study also showed that the extraction yield was affected by the harvest stage, as young plants in the pre-flowering stage had a lower yield in obtaining EO compared to older plants, in full bloom and post-bloom. It was also observed that pinocamphone (the main compound in hyssop oil) gradually increases until after the post-flowering stage, while β-pinene, quantitatively in the plant, first increases and then decreases after the stage of full flowering. Hyssop EO from Bulgaria has as main constituents: 1,8-cineole (39.6%-48.2%), *iso*-pinocamphone (16.3%-28.0%), and pinene (9.4%-11.4%). A similar composition was found in a population of hyssop growing in eastern Serbia, with 1,8-cineole (36.4%), pinene (19.6%), and *iso*-pinocamphone (15.3%) being the most important compounds (Dzamic et al., 2013). Another study conducted on EO from Bulgaria, carried out by Hristova et al. in 2015, found 29.1% *iso*-pinocamphone, 11.2% *trans*-pinocamphone, and 18.2% β-pinene, while the concentration of these compounds in the EO from our study was 34.67%, 13.73%, and 13.32%, respectively (Table 1). The results of the study conducted by Venditti et al., in 2015 on two subspecies of hyssop from Italy (subsp. *officinalis* and subsp. *aristatus*) showed the presence of monoterpenic ketones such as pinocamphone, *iso*-pinocamphone, and methyl eugenol in the EOs. In another study conducted in Italy on EOs of hyssop cultivated at two different altitudes (100 m and 1000 m), the percentage variation of the main compounds was observed: 34-18.5% pinocamphone, 3.2-29.0% *iso*-pinocamphone, 10.5-10.8% β-pinene, 7.4-9.6% α-phellandrene, thus demonstrating the significant effect that the environment has on the composition of EOs (Sas-Piotrowska et al., 2010). In the study conducted on EO obtained from the aerial parts of hyssop plants cultivated in Egypt (Hussein et al., 2015), 33 compounds were identified, representing 99.99% of the total EO. The major compounds were *cis*-pinocamphone (26.85%), β-pinene (20.43%), and *trans*-pinocamphone (15.97%). The study conducted in Poland by Zawiślak in 2013 showed that the main compounds were: *cis*-pinocamphone (33.52%-37.13%), *trans*-pinocamphone (23.43%-28.67%), β-pinene (7.89%-8.12%), elemol (5.86%-8.95%), germacrene-D (3.23%-4.65%), and caryophyllene (2.67%). The Romanian EO of *H. officinalis* mainly contains aliphatic fatty acids: eicosadienoic acid (0.68%), linolenic acid (63.98%), arachidic acid (2.64%), stearic acid (10.73%), and palmitic acid (15.60%) (Benedec et al., 2002). In the study conducted by Jop et al. in 2021, the compounds *cis*-pinocamphone, *trans*-pinocamphone, and β-pinene were confirmed as the main three active, dominant compounds in the EO of *H. officinalis*, which is in accordance with the results of other researches (Hajian-Maleki et al., 2019; Kizil et al., 2010; Mahboubi et al., 2011;), but in different proportions. Monoterpenes (pinocamphone, *iso*-pinocamphone, etc.), the main representatives of this type of EO, are relatively rarely detected in larger quantities in EOs of other species. In terms of numbers (the most), 44 compounds have been identified in hyssop EO. In addition to the compounds already mentioned, other monoterpenes (β-pinene, pinocarvone, myrcene, etc.) have also been encountered, but in small concentrations. Thus, the biological variability of hyssop EO is relatively low, as indicated by available data (Hüsnü & Buchbauer, 2020). Although there are numerous studies regarding the successful use of EOs in weed control, there are constraints that limit their practical application. The role of EO composition is not clearly defined, and the mechanisms of action and selectivity are poorly understood, thus limiting their implementation. Additionally, studies regarding the potential side effects of these EOs on beneficial soil microorganisms are still lacking (De Mastro et al., 2021). In conclusion, all the data presented above support and confirm the results obtained in the present study regarding the chemical composition of the EO obtained from the first Romanian variety of hyssop, 'Cătălina' variety.

The allelopathic activity of EO of hyssop (Hyssopus officinalis L.), 'Cătălina' variety

According to Abd-ElGawad et al. (2021), hyssop essential oil containing monoterpenes such as β-pinene, *iso*-pinocamphone, and *trans*-

pinocamphone as main compounds, exhibits significantly higher phytotoxic activity on certain target plant species (*Raphanus sativus, Lactuca sativa, Lepidium sativum*). The monoterpenes (carvacrol, terpinene, thymol, etc.) are known to disrupt the cell membrane and affect photosynthesis, cellular respiration, and mitosis (De Mastro et al., 2021; Werrie et al., 2020). However, not much is known about the mechanism of action on plant metabolism (Lins et al., 2019), but it seems that they have cytotoxic effects that harm several plants (Acheuk et al., 2022; Sampaio et al., 2021). Similar to other monoterpenes, carvacrol has strong inhibitory effects on germination and seedling growth (Atak et al., 2016). Linalool is a monoterpene that affects multiple metabolic pathways, reducing photosynthesis, respiratory activity, and altering water status (Singh et al., 2002).

The effect of hyssop EO on seed germination

The results regarding the effect of treatment with EO obtained from hyssop on the germination of different seed species are presented in Figure 2.

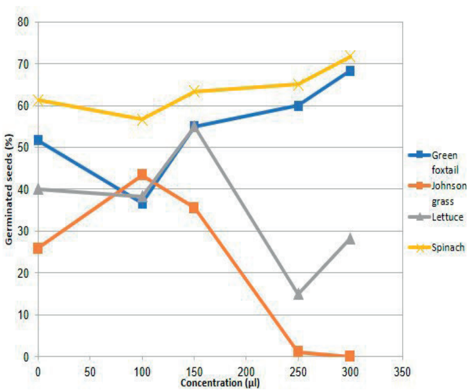

Figure 2. The effect of EO of hyssop (*Hyssopus officinalis* L.), 'Cătălina' variety, on seed germination

For Green foxtail, it is observed that up to the concentration c=100µl, the effect is inhibitory, leading to a reduction in germinated seeds (38%). Then, as the concentrations increase, the effect switches to stimulation of germination, reaching 70% germinated seeds at c=300 µl.
For Johnson grass, the situation is the opposite. At c=100 µl, germination is stimulated (42%), then it slightly decreases to 35% at c=150 µl. At c>150µl, there is a significant reduction in germination, with almost complete inhibition observed. Thus, at c=300 µl, only 2-3% of seeds are germinated.
For lettuce, there is an interval of concentration between 100-150 µl where the percentage of germinated seeds increases to 55%, indicating stimulation of germination. This is followed by a sudden decrease in germination (below 15%) at c=150-250 µl, indicating inhibition.
For spinach, at c>100 µL, there is an increase in the percentage of germinated seeds with the concentration used in the treatment (stimulation effect), reaching 72% for c=300 µl.
In the study conducted by Zheljazkov et al., 2021, it was demonstrated that *H. officinalis* EO applied at a dose of 90 µl completely suppressed the radicle growth of barley and wheat and germination (%), compared to the control. It is interesting to note that the same EO applied at a dose of 10 µl did not reduce germination in wheat, but it did reduce the number and length of radicles, seedlings, and vigor index. In another study conducted by Argyropoulos et al., 2008, the effect of mint EO (*M. spicata*) on the weed species *Setaria verticillata* was evaluated, and the results showed that the EO (with 82% *trans*-piperitone oxide) severely inhibited all tested species.

The effect of hyssop EO on radicle length

The experimental results aimed at assessing the effect of hyssop EO on radicle length (cm) are presented in Figure 3.

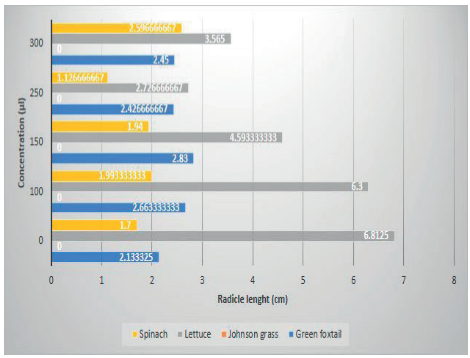

Figure 3. The effect of EO of hyssop (*Hyssopus officinalis* L.), 'Cătălina' variety, on radicle length

For Green foxtail, the treatment at c=100-150 µl had a stimulating effect, with an increase in radicle length of 2.83 cm. As the concentration of EO in the tested solutions increased, the effect was reversed, leading to a reduction in radicle growth. Thus, at c=350 µl, the radicle growth was only 2.45 cm. Similar results were obtained by Koiou et al., 2020, with tests conducted on *Setaria verticillata*, showing that germination and root length were mostly reduced as the concentrations of compounds in EOs increased, but not proportionally.

For Johnson grass, the result is consistent with the previous one, meaning that at c>100 µl, complete inhibition of radicle growth was observed. For lettuce, the effect is inhibitory, and it is noticeable that as the concentration of EO increases, the radicle length decreases, so that at c=250 µl, the radicle is only 2.73 cm compared to the control (6.81 cm). For spinach, at c=100-150 µl (radicle length is 1.99 cm), the effect is stimulatory, then decreases to 1.12 cm (c=250 µl, and finally increases to 2.60 cm (c=300 µl).

Specialized literature mentions few studies related to the action of *H. officinalis* EO on the germination and radicle growth of some garden vegetable species (*Raphanus sativus, Lactuca sativa,* and *Lepidium sativum*) (Zheljazkov et al., 2021; Hristova et al., 2015).

In the tests conducted with hyssop EO, it did not inhibit the germination of *Lactuca sativa* L. and *Lolium perenne* L. seeds, although it slightly decreased (≈20%) root and leaf growth in *L. perenne* (De Elguea-Culebras et al., 2017). Amri et al., in 2013, demonstrated the phytotoxic capacity of various EOs and attributed to the compound 1,8-cineole (also present in hyssop EO) a strong allelopathic effect. Additionally, the increased content of pinocarvone in hyssop EO could also be linked to certain allelopathic effects observed previously for various carvone isomers (Vokou et al., 2003).

The effect of hyssop EO on plumule length

The results obtained from the treatment with hyssop EO on plumule length in two weed species and two vegetable species are presented in Figure 4.

De Elguea-Culebras et al. in 2017 reported that 1,8-cineole (53%) and β-pinene (16%) are the major bioactive compounds present in hyssop EO, which have insecticidal effects on *Spodoptera littoralis* (cotton leafworm). Additionally, they reported that this type of EO does not have an inhibitory effect on the germination of *Lactuca sativa* L. var. *carrascoy* and *Lolium perenne* L. seeds, but it does have a slight inhibitory effect on root and leaf growth in this weed.

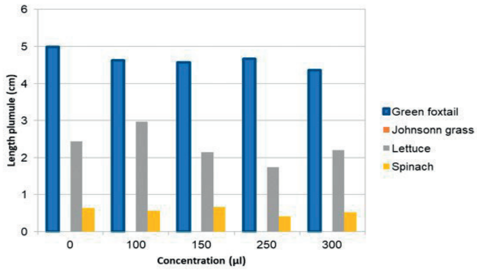

Figure 4. The effect of EO of hyssop (*Hyssopus officinalis* L.), 'Cătălina' variety, on plumule length

As shown in Figure 4, for green foxtail, the treatment effect was insignificant, with results similar to the control. However, a slight inhibitory effect on plumule length (up to 0.5-0.6 cm maximum) is noticeable at all tested concentrations.

For Johnson grass, the results correlate with those obtained previously. Even though the germination process was initiated and even stimulated at c=100 µl, subsequent development of radicles and plumules was not allowed, resulting in a total inhibition of further seedling growth.

For lettuce, at c=100 µl, the response was the most favorable, as it shows a stimulation of 0.5 cm in plumule length. However, as the concentration increases, the length of the plumule decreases.

The experimental results obtained for spinach show no variation, meaning that the effect is not significant compared to the control.

The studies conducted have shown that monoterpenes, which are the major compounds in EOs, inhibit seed germination and subsequent seedling growth. These are secondary metabolites commonly described for their bioherbicidal activity (De Mastro et al., 2021; Kumar et al., 2020). The experimental results obtained from tests conducted with the aqueous extract obtained from the aerial parts of hyssop, on the species *Cucumis sativus* L. and *Triticum aestivum*, showed that it had an inhibitory effect on germination and root elongation (*T. aestivum* > *C. sativus*) (Islam et al., 2022; Dragoeva et al., 2010). In the study conducted by Tkachova et al. (2022), the influence of the allelopathic activity of aqueous extracts (leaves, stems, flowers) of hyssop on the soil, in the rhizosphere zone, was demonstrated, affecting the root growth of *Lepidium sativum* L. Typically, the accumulation of hyssop EO occurs in the flowers and leaves, and to a lesser extent in the stems. The accumulation of substances with inhibitory effects happens gradually, with the plant being capable of accumulating essential oil starting from the second year of cultivation. On average, the allelopathic activity index of water-soluble biologically active substances from the above-ground organs of hyssop plants in the first and second years of growth had a stimulatory effect at a concentration of 1:50 and an inhibitory effect when using plants from the third year of growth at a concentration of 1:10. This result confirms that the highest amount of allelochemical substances, which can negatively affect the growth and development of other plants, accumulates starting from the third year of cultivation. It should be noted that despite the difference in allelopathic activity of extracts obtained from different organs of the hyssop plants, the trend of its increase occurs with each year of cultivation in monoculture.

In conclusion, the most likely cause of the allelopathic activity of the new variety of hyssop ('Cătălina' variety), whether it is inhibition or stimulation of seed germination and subsequent seedling growth, is attributed to the action of the main compounds (*cis*- and *trans*-pinocamphone, β-pinene, germacrene-D), as well as to the synergistic action among them and the secondary compounds identified in smaller quantities in this EO (α and β-phellandrene, β-elemene, myrcene, β-caryophyllene, aromadendrene, pinenol, thujenol, elemol).

CONCLUSIONS

The results of this study have shown that the EO obtained from the new variety of hyssop ('Cătălina' variety) contains the main compounds: *cis*- and *trans*-pinocamphone, β-pinene, germacrene-D, along with other secondary compounds found in smaller quantities (α and β-phellandrene, β-elemene, myrcene, β-caryophyllene, aromadendrene, pinenol, thujenol, elemol).

Furthermore, the allelopathic effect of this EO is either inhibitory or stimulatory on seed germination and subsequent seedling growth, with the effect being influenced by the concentration used and the species to which the treatment is applied.

Further research should focus on harnessing the cultivation of the new variety of hyssop, developing stable, environmentally friendly, and effective bioherbicides that can be used in field conditions to control a wide range of target weeds. The use of allelopathy in agriculture can be achieved by incorporating allelopathic plant residues into the soil or through mulching, applying EOs obtained from plants with allelopathic effects, and using artificially produced allelochemicals (through organic synthesis, fermentation, tissue cultures, etc.). Importance should also be given to promoting the production of bioherbicides and raising awareness among farmers about their ecological benefits.

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