Research Article Open Access

Composition of the essential oil of *Thuja occidentalis* under different growth conditions.

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Submission Date: September 23rd, 2025; **Acceptance Date:** November 24th, 2025; **Publication Date:** November 27th, 2025

Please cite this article as: Hakobjanyan A., Karapetyan A., Ghahramanyan A., Yeghiazaryan A., Gasparyan A., Mayrapetyan K. Composition of the essential oil of *Thuja occidentalis* under different growth conditions. *Bioactive Compounds in Health and Disease* 2025; 8(11): 517-526. DOI: https://doi.org/10.31989/bchd.v8i11.1787

ABSTRACT

Background: *Thuja occidentalis* essential oil is valuable in an antimicrobial treatment. Its main component, thujone, has biological activity against bronchial catarrh, cystitis, psoriasis, and rheumatism. At the same time, thujone is distinguished by its neurotoxic effect. *Thuja occidentalis* varieties, such as *Thuja occidentalis* 'Pyramidalis', are widely used in urban green spaces.

Objective: This study aims to estimate the influence of growth conditions and plant age on *Thuja occidentalis* 'Pyramidalis' leaves' essential oil composition.

Methods: The study was conducted on *Thuja occidentalis'* Pyramidalis' plants grown in hydroponic and soil conditions. Red and black slags were used as hydroponic growth substrates. Soil plants and some of the black slag plants were 5-year-old, and red slag plants, and the other half of the black slag plants were 4-year-old. Essential oil was obtained from fresh needle leaves of the plants via hydrodistillation. Essential oil component composition was estimated by gas chromatography-mass spectroscopy.

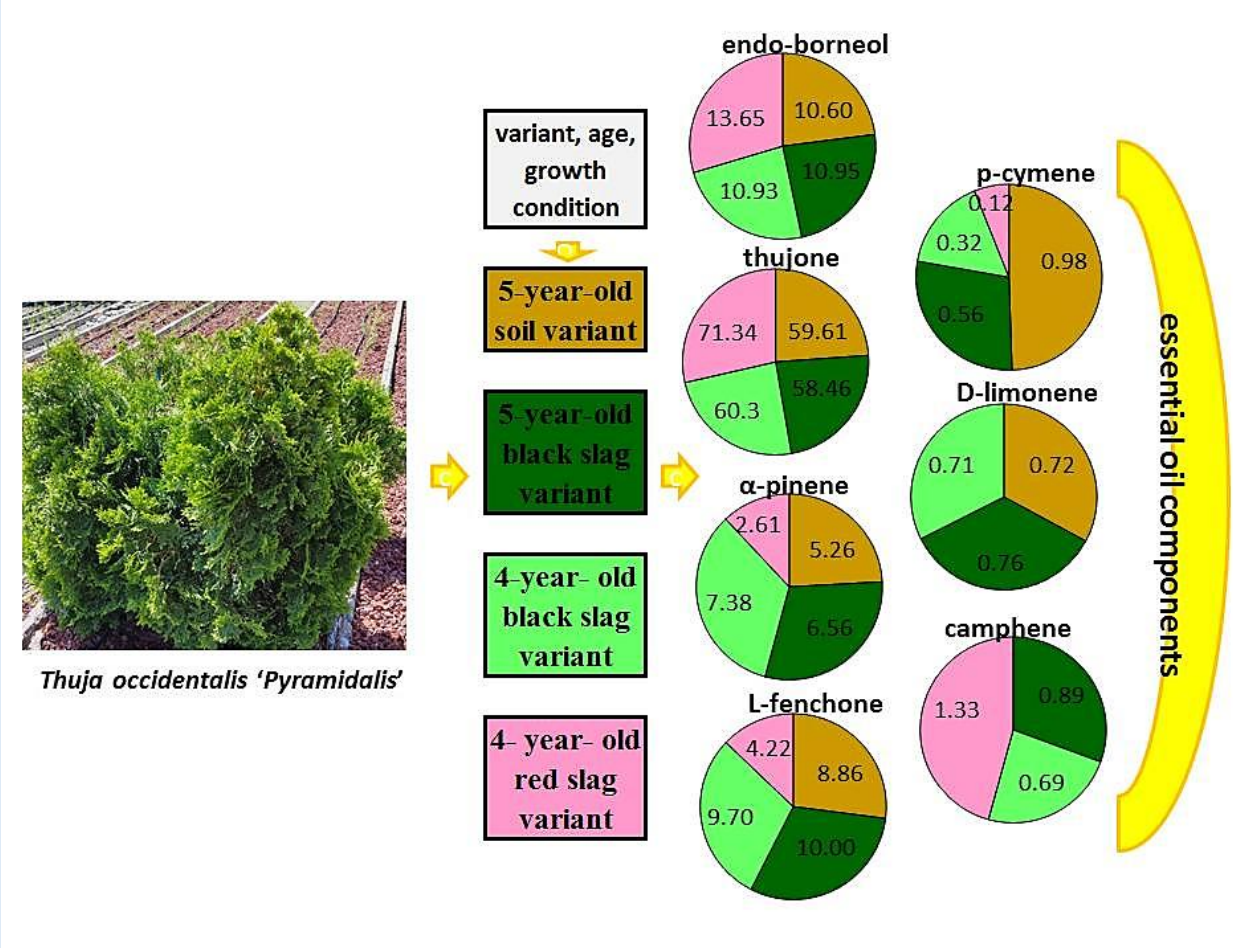
Results: Received data showed that the highest thujone content was observed in plants grown on red slag (71.34%), while the other variants had similar low thujone content (soil plants – 58.46%, 5-year-old black slag plants – 59.61%, and 4-

year-old black slag plants – 60.3%). The essential oil from 4-year-old plants does not contain β-pinene or terpinen-4-ol. At the same time, the highest content of α-pinene was recorded in 4-year-old black slag plants' essential oil (7.38%) and the lowest in 4-year-old red slag plants' essential oil (2.61%); the other two variants' results were close to those of 4-year-old black slag plants.

Novelty: For the first time, the essential oil composition of *Thuja occidentalis* cultivated on various hydroponic substrates was investigated and compared to that of plants grown in soil.

Conclusion: According to the results, *Thuja occidentalis* 'Pyramidalis' plants grown on black slag have similarities with soil plants in essential oil composition, while red slag plants show strong differences. Growth conditions affect the composition of essential oils. Plant age also influences it.

Keywords: thujone, pinene, red and black slag, Ararat Valley, hydroponics



Graphical Abstract: Composition of the essential oil of *Thuja occidentalis* under different growth conditions

MATERIALS AND METHODS

Thuja occidentalis 'Pyramidalis' plants were grown in hydroponic and soil conditions in the Ararat Valley of Armenia. Red and black slags were used as hydroponic growth substrates. The soil-grown plants were 5-year-old (n = 5) and were irrigated once a week with artesian water. Three variants of hydroponically grown plants were studied: (1) 5-year-old plants cultivated in black volcanic slag (n = 7), (2) 4-year-old plants grown in black volcanic slag (n = 10), and (3) 4-year-old plants grown in red volcanic slag (n = 10). The hydroponic plants were nourished with a nutrient solution authored by G. Davtyan [10].

Essential oil was obtained from fresh needle leaves of the plants in July 2024 by hydrodistillation [11]. 400g of leaves were placed in a flask, covered with water, and heated for 4 hours, using a refrigerator to condense and collect the resulting essential oil. The essential oil components of the studied variants were analyzed by gas chromatography-mass spectrometry.

RESULTS AND DISCUSSION

Monoterpenes, which are part of essential oils, are used to develop new active compounds in the drug industry. They have a broad spectrum of biological activity, good bioavailability, and the capacity to cross the blood–brain barrier (BBB). Due to their versatile reactivity and chirality, leveraging monoterpenes in synthesis enables the creation of new bioactive compounds with improved pharmacological properties, contributing to drug discovery, the optimization of new therapeutic agents, and agrochemical development [12].

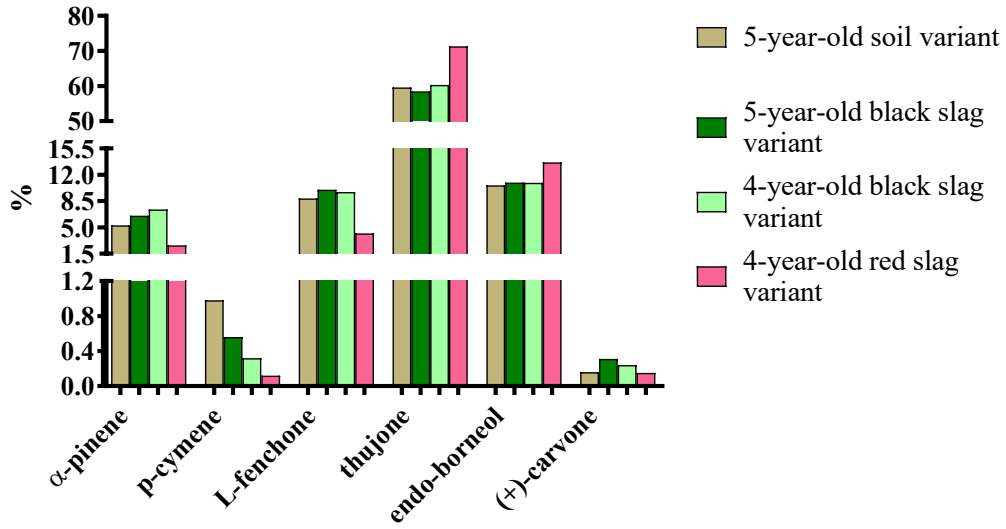
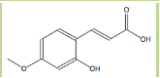
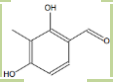
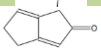
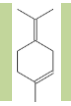
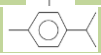
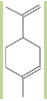
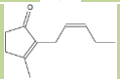
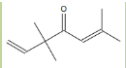
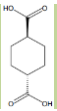
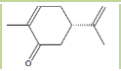
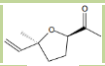
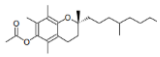
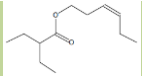
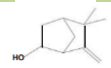
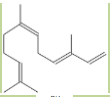
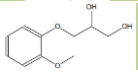
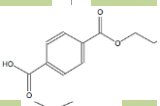
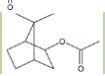
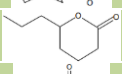
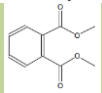


Figure 1. The content of some components that are found in essential oils of all variants.

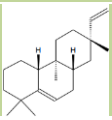
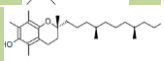
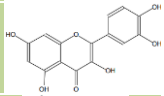
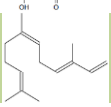
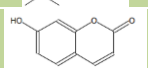
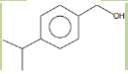
According to a study, the essential oil composition of thuja grown in red slag differed significantly from that of the other variants, which may be attributed to differences in the composition of red and black slag and soil, as well as to their varying capacities to absorb environmental heat [9, 13]. The highest content of thujone (71.3%) and endo-borneol (13.7%), and the lowest content of α-pinene (2.6%), p-cymene (0.1%), and L-fenchone (4.2%), were recorded in the red slag variant. Age has a low influence on their content, according to the results of the black slag variants (Fig. 1, Table 1).

Table 1: The content of all components of essential oils with release times and chemical formulas.

Compounds	RT	5-year-old soil variant, %	5-year-old black slag variant, %	4-year- old black slag variant, %	4- year- old red slag variant, %	Formula
2-hydroxy-4-methoxycinnamic acid	12.94	0.27	-	-	-	
(1S)-2,6,6-trimethylbicyclo[3,1,1]hept-2-ene	12.94	-	0.26	-	-	
2,4-dihydroxy-3-methylbenzaldehyde	12.94	-	-	0.23	-	
bicyclo[3,2,0]hepta-2,6-diene	12.94	-	-	-	0.10	
bicyclo[3,1,0]hex-2-ene, 4-methyl-1-(1-methylethyl)-	13.28	0.55	-	-		
bicyclo[3,1,0]hex-2-ene, 2-methyl-5-(1-methylethyl)-	13.28	-	0.69	0.63		
4,5-dihydro-2(1H)-pentalenone	13.28	-	-	-	0.25	
α-pinene	**15.31**	**5.26**	**6.56**	**7.38**	**2.61**	
(1R)-2,6,6-trimethylbicyclo[3,1,1]hept-2-ene	16.18-16.19	-	-	1.20	0.37	
β-pinene	16.19	0.81	1.06	-	-	
cyclohexene, 1-methyl-4-(1-methylethylidene)-	***17.53-17.54***	***0.31***	***0.21***	***0.25***	**-**	
p-cymene	**17.93-17.94**	**0.98**	**0.56**	**0.32**	**0.12**	
D-limonene	***18.16***	***0.72***	***0.76***	***0.71***	-	
***cis*-jasmone**	18.16	-	-	-	0.21	
1,5-heptadien-4-one, 3,3,6-trimethyl-	***19.67-19.69***	***1.30***	***1.23***	***0.94***	**-**	
trans-1,4-cyclohexanedicarboxylic acid	19.67	-	-	-	0.23	
α-terpinyl acetate	20.42	-	-	-	0.13	
citraconic acid	20.43	-	-	0.43	-	
bicyclo[2,2,2]oct-7-en-2-one, 5-methylene-	20.45	-	0.57	-	-	
fenchol, exo-	20.46	0.39	-	-	-	
L-fenchone	**21.28-21.31**	**8.86**	**10.00**	**9.7**	**4.22**	
thujone	**22.48-22.55**	**59.61**	**58.46**	**60.30**	**71.34**	
endo-borneol	**22.90-22.94**	**10.60**	**10.95**	**10.93**	**13.65**	

Compounds	RT	5-year-old soil variant, %	5-year-old black slag variant, %	4-year- old black slag variant, %	4- year- old red slag variant, %	Formula
(+)-carvone	**24.16-24.18**	**0.16**	**0.31**	**0.24**	**0.15**	
3-butyn-1-ol	24.59	-	-	-	0.10	
unknown	**25.71-25.75**	**0.39**	**0.58**	**0.17**	**0.12**	
***trans*-arbusculone**	25.83	-	-	0.63	0.88	
terpinen-4-ol	25.85	0.85	0.62	-	-	
α-tocopheryl acetate	***28.54-28.55***	***0.27***	***0.31***	**-**	***0.20***	
cis-3-hexenyl 2-ethylbutyrate	28.64	-	0.28	-	-	
bicyclo[3,1,1]hept-2-ene, 3,6,6-trimethyl-	29.64	-	-	-	0.56	
methanol, (1,4-dihydrophenyl)-	29.65	0.41	-	-	-	
camphene	***30.71-30.72***	**-**	***0.89***	***0.69***	***1.33***	
camphenol, 6-	30.73	1.21	-	-	-	
(E,Z)-α-farnesene	30.99	-	-	-	0.23	
L-α-terpineol	33.50	-	-	0.53	-	
cinnamyl alcohol,Z-	33.50	-	-	-	0.28	
bicyclo[2,2,2]oct-7-en-2-one, 5-methylene-	33.51	0.22	-	0.22[1]	-	
bicyclo[2,2,1]heptane, 2,2-dimethyl-3-methylene-, (1S)-	33.51	-	0.21	-	-	
guaiphenesin	34.91	0.28	-	-	-	
(1R)-2,6,6-trimethylbicyclo[3,1,1]hept-2-ene	34.91	-	0.51	-	-	
cyclohexene, 1-methyl-4-(1-methylethenyl)-, (S)-	34.91	-	-	0.35	-	
4-(butoxycarbonyl)benzoic acid	34.91	-	-	-	0.13	
bicyclo[2,2,1]heptan-2-ol, 7,7-dimethyl-, acetate	43.29	-	0.23	-	-	
δ-octalactone	43.29	-	-	-	0.09	
dimethyl phthalate	43.30	0.46	-	-	-	
(Z,Z)-α-farnesene	43.30	-	-	0.15	-	

[1] RT=29.64

Compounds	RT	5-year-old soil variant, %	5-year-old black slag variant, %	4-year- old black slag variant, %	4- year- old red slag variant, %	Formula
rosa-5,15-diene,ent-	54.72-54.73	0.78	-	0.93	-	
(+)-α-tocopherol	54.73	-	1.23	-	-	
quercetin	54.73	-	-	-	0.39	
(E,Z)-α-farnesene	55.88	-	-	3.07	-	
7-hydroxycoumarin	55.89	-	3.51	-	-	
tricyclo[3,2,1,0(2,4)]octane, 8-methylene-, (1α,2α,4α,5α)-	55.89	-	-	-	2.32	
p-cymen-7-ol	55.91	5.31	-	-	-	

Thujone has a toxic effect on brain, liver, and kidney cells. Its excessive ingestion can lead to convulsions, restlessness, vomiting, rapid heart rate, renal impairment, and epileptic seizures. However, its neurotoxic effects are considered fully reversible [14]. From this point of view, red slag plants' essential oil is more toxic than that of the other variants.

Endo-borneol ((+)-borneol) has been shown to inhibit microglial-mediated neuroinflammation by modulating the TLR4-NFκB signalling pathway, thereby attenuating neuronal damage and apoptosis in murine models of status epilepticus. These findings suggest its potential for use in epilepsy-modification therapy. In traditional Chinese medicine, (+)-borneol is extensively utilized in formulations targeting mental disorders. Notably, it can cross the blood–brain barrier and exerts neuroprotective effects by inhibiting oxidative stress and apoptosis, indicating its therapeutic relevance in various neurological disorders [15]. Borneol in combination with ferulic acid, an agent often used to treat vascular diseases and to prevent thrombosis, suppressed the abnormally increased BBB permeability caused by cerebral ischemia in mice [16]. The highest content was found in the red slag variant (13.7%) (Fig. 1, Table 1). It can be hypothesized that the high concentration of endo-borneol may reduce the neurotoxic effects of thujone in the essential oil of plants grown in red slag. Age didn't influence the borneol content.

The antibacterial properties of α-pinene against certain microorganisms depend on its concentration. It also has a synergistic effect with antibiotics. It was found that the pure α-pinene compound, with the highest concentration of the positive enantiomer, was more effective against strains of *E. coli* and *S. aureus* than the oil with the predominance of the α-pinene positive form, indicating that the other components of the essential oil interfere with α-pinene activity [17]. The bicyclic monoterpene L-fenchone exhibits significant antimicrobial activity and may serve as a promising therapeutic candidate for the treatment of oral candidiasis [18]. In rat models of constipation-predominant irritable bowel syndrome, fenchone increased body weight, the number of fecal pellets, fecal moisture content, and intestinal transit rate. It also promoted the relative abundance of *Lactobacillus* while decreasing the relative abundance of *Bacteroides*, *Enterococcus*, and *Escherichia-Shigella* at the genus level [19]. At the same time, the acute toxicity study of fenchone in rats showed significant increases in

hemoglobin, red blood cells, alkaline phosphatase, and alkaline transaminase, and significant decreases in serum triglycerides, cholesterol, and uric acid levels [20]. Carvone, as an anticonvulsant, reduces the frequency and severity of epileptic seizures. As an analgesic, it modulates mechanisms responsible for pain perception. Carvone exhibits antimicrobial and antifungal activities, as well as strong antispasmodic effects by inhibiting smooth muscle contractions, which makes it useful in the treatment of gastrointestinal and respiratory disorders. Carvone may regulate blood glucose levels. (+)-carvone exhibits moderate activity against *Escherichia coli*, *Salmonella typhimurium*, and *Staphylococcus aureus* [12]. The lowest contents of L-fenchone (4.2%) and α-pinene (2.6%) were in red slag plants' oil, making it less active against bacteria, and the highest contents of them and carvone were recorded in both black slag variants. With age, the content of α-pinene decreases (7.4% in the 4-year-old black slag variant and 6.6% in the 5-year-old black slag variant) (Fig. 1, Table 1). Soil variant's fenchone content was similar to Kovalenko's results [21], while α-pinene content prevailed over them. The lowest levels of carvone were recorded in the soil (0.16%) and red slag (0.15%) variants. In black slag variants, it increased with age by 1.3 times (from 0.24% to 0.31%).

p-Cymene, found in various plant species, possesses notable antioxidant, anti-inflammatory, antimicrobial, and anticancer properties. It has been shown to inhibit cytokine signalling 3 (SOCS3) expression during intestinal inflammation and to modulate both antioxidant and immunomodulatory pathways, thereby preserving epithelial barrier integrity and maintaining the mucus layer. Furthermore, p-cymene shows activity against food poisoning. Its antioxidant capacity helps prevent cellular damage and aging, with potential implications for neurodegenerative diseases such as Alzheimer's disease. Additionally, p-cymene exhibits antinociceptive effects, enhancing analgesia and potentiating the pharmacological efficacy of co-administered therapeutic agents [22]. In this study, the highest p-cymene content was recorded in soil plants (1.8 times higher than in 5-year-old black slag plants, 3.1 times higher than in 4-year-old black slag plants, and 8.2 times higher than in 4-year-old red slag plants). As can be seen, with age, its content increases in black slag plants (from 0.32% to 0.56%) (Fig. 1, Table 1).

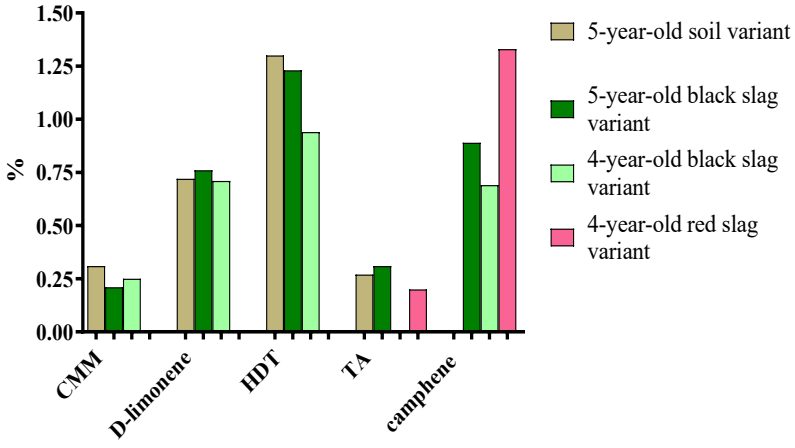


Figure 2. The content of some chemicals that are found in the essential oils of three different variants. (CMM - cyclohexene, 1-methyl-4-(1-methylethylidene), HDT - 1,5-heptadien-4-one,3,3,6-trimethyl, αTA - α-tocopheryl acetate)

Not all compounds of essential oils are common for all studied variants (Fig. 2, 3, Table 1). For example, cyclohexene, 1-methyl-4-(1-methylethylidene), D-limonene, 1,5-heptadien-4-one, 3,3,6-trimethyl- are common in soil plants and black slag plants, and absent in red slag plants. Camphene is found only in hydroponically grown plants. Camphene shows anti-leishmanial and hepatoprotective activities [23]. The highest content of camphene was registered in red slag plants (1.3%) (1.9 times higher compared with the same age black slag plants). At the same time, its amount increased by 1.3 times in black slag plants.

One of the most valuable properties of D-limonene is its ability to inhibit microbial biofilm formation. Its antibiofilm activity against *Streptococcus pyogenes* is 95%, against *Streptococcus mutans* is 94.88%, and against *Escherichia coli* is 92%, which is an expressive result, given that more than 80% of recurrent microbial diseases and chronic infections are associated with biofilm formation. As most plant essential oils contain D-limonene [24], essential oils are valuable in antimicrobial treatments. In this study, the content is approximately similar for soil and black slag plants (Fig. 2, Table 1).

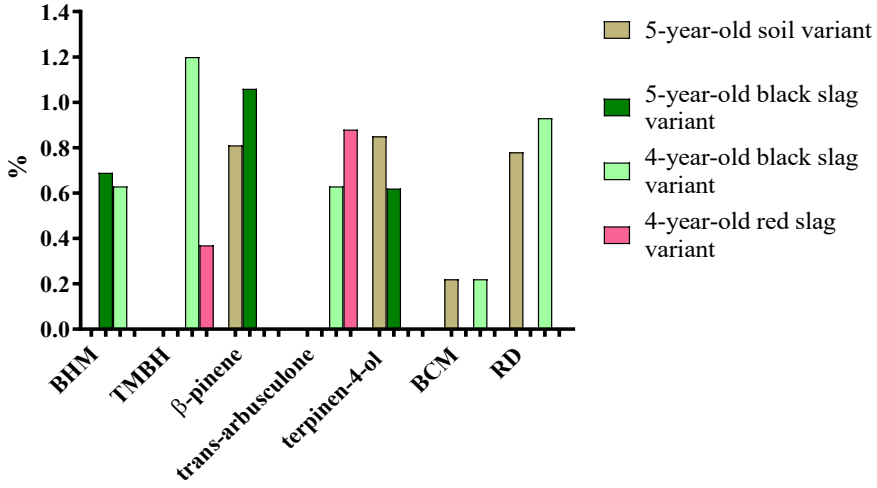


Figure 3. The content of some components that are found in the essential oils of two different variants. (BHM - bicyclo[3,1,0]hex-2-ene,2-methyl-5-(1-methylethyl), (1R)-2,6,6-trimethylbicyclo[3,1,1]hept-2-ene, TMBH - (1R)-2,6,6-trimethylbicyclo[3,1,1]hept-2-ene, BCM - bicyclo[2,2,2]oct-7-en-2-one,5-methylene, RD - rosa-5,15-diene,e

Seven oil compounds are found only in two variants of the study (Fig. 3, Table 1), and there is no algorithm to explain why only two variants contain them. β-pinene and terpinen-4ol exist only in 5-year-old soil and hydroponic plants, where β-pinene prevails in the hydroponic variant, while terpinen-4ol prevails in the soil variant.

Generally, in 5-year-old soil and hydroponic plants' essential oils, there are 23 components, in 5-year-old black slag plants' essential oil, 22, and in 5-year-old red slag plants' oil, 24 components (Table 1).

CONCLUSION

According to results, *Thuja occidentalis* '*Pyramidalis*' plants grown on black slag have similarities with soil plants in essential oil composition, while red slag plants show substantial differences. In red slag plants, oil neurotoxic thujone levels are higher than in other variants, making it more toxic to human organisms; at the same time, camphene levels are also higher, providing benefits against leishmaniosis and hepatoprotection.

Growth conditions affect the composition of essential oils. Plant age also influences it.

List of Abbreviations: CMM: cyclohexene, 1-methyl-4-(1-methylethylidene), HDT: 1,5-heptadien-4-one,3,3,6-trimethyl, αTA: α-tocopheryl acetate, BHM: bicyclo[3,1,0]hex-2-ene,2-methyl-5-(1-methylethyl), (1R)-2,6,6-trimethylbicyclo[3,1,1]hept-2-ene, TMBH: (1R)-2,6,6-trimethylbicyclo[3,1,1]hept-2-ene, BCM: bicyclo[2,2,2]oct-7-en-2-one,5-methylene, RD: Rosa-5,15-diene,ent.

Acknowledgments: The work was supported by the Science Committee of MESCS RA, in the frames of the research project № 21T-4D262 and by basic funding from the G.S. Davtyan Institute of Hydroponics Problems.

Authors' Contribution: All authors contributed to this study.

Competing interests: The authors declare no conflict of interest.

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