# THESIS

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# ALLELOPATHIC EFFECT OF LEAF EXTRACTS AND ESSENTIAL OILS OF *ARTEMISIA* SPECIES ON SEED GERMINATION AND SEEDLING GROWTH OF WHITE MUSTARD *(SINAPIS ALBA)*

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## Table of contents

## 1. Introduction and objectives

This study aims to investigate and analyse the allelopathic effect of plant extracts and essential oils of six species of *Artemisia* genus (*A. absinthium* L., *A. alba* Turra, *A. annua* L., *A. pontica* L., *A. scoparia* Waldst. & Kit., and *A. vulgaris* L.) on seed germination and seedling growth of white mustard *(Sinapis alba).*

The excessive use of pesticides and herbicides, and their persistence in the environment have caused serious problems, posing a huge threat to the ecosystem and human health (Wang et al., 2008; Li et al., 2021; Raffa, Chiampo, 2021). Plant species with allelopathic effects have emerged as a potential strategy for the development of ecologically friendly bioherbicides against weeds (Lopes et al., 2022). Allelochemicals related to allelopathy belong to the classes of phenolics, terpenoids and alkaloids, and can inhibit or stimulate the germination and/or growth of plants (Latif et al., 2017; Macías et al., 2019; Lopes et al., 2022). Plant-derived allelochemicals do not exert residual of toxic effects, therefore, they are considered as the perfect alternative to synthetic herbicides (Cheng, Cheng, 2015; Li et al., 2021). They have broad application prospects and high research value (Li et al., 2021).

The *Artemisia* genus includes more than 350 species and produces terpenoids, coumarins, flavonoids, sterols, glycosides and polyacetylenes, which are biologically active against malaria, cancer, viruses, hemorrhagy, sudoresis, free radicals, angina, ulcer, hepatitis and other diseases that might be treated by such compounds (Tan et al., 1998; Ferreira, Janick, 2004). The genus has been widely studied, including the investigation of the allelopathic effect of the species (Chirca, Fabian, 1973; Duke et al., 1987; Marchant, Cooper, 1987; Chen, Leather, 1990; Lydon et al., 1997; Inderjit, Foy, 1999; Schulz et al., 2007; Derwich et al., 2009; Kaur et al., 2010; Kaur, Batish, 2010; Mei et al., 2010; Kiplimo et al., 2016; Kapoor et al., 2019; Raveau et al., 2020; Souto et al., 2021; Maurya et al., 2022).

In the current study, the inhibitory effect of ethanol plant extracts and essential oils of *Artemisia* species on seed germination and seedling growth was examined and analysed, revealing the differences and interactions between the potency of the taxa and volume.

# 2. Literature review

## *2.1. Allelopathy*

The term allelopathy was created by Molisch (1937) to refer to biochemical interactions between all types of plants including microorganisms. According to his discussion, he coined the term to cover both inhibitory and stimulatory reciprocal biochemical interactions (Rice, 2012). The first edition of Rice's book on allelopathy (Rice, 1974) defined the term as any direct or indirect harmful effect by one plant (including microorganisms) on another through production of chemical compounds released into the environment (Rice, 2012). The interaction can occur between seeds, between a plant and a seed, or between plants. Normally, chemicals are emitted through roots, washed down from leaves by dew or rain, or released during the natural decomposition and assimilation of leaves. Allelopathic species might produce volatile compounds that elicit a physiological response in the reached target species. Complete inhibition is not required to classify a species as allelopathic; even the postponement of seed germination or a reduction in the growth rate of a competing species could provide the necessary advantage for the allelopathic species over the other (Ferreira, Janick, 2004).

### *2.1.1. Bioherbicidal potential of plant species with allelopathic effects*

Pesticides are chemical compounds used for eliminating pests; among them, herbicides are compounds particularly toxic to weeds. Their excessive use and persistence in the environment have caused serious problems, such as pollution of water, soil, air, and agricultural products, posing a huge threat to the ecosystem and human health (Wang et al., 2008; Li et al., 2021; Raffa, Chiampo, 2021). Therefore, to reduce the use of chemical pesticides and fertilizers and to develop new, safe, effective and pollution-free substitutes, the concepts of "organic products" and "ecological farming" were proposed (Li et al., 2021). Plant species with allelopathic effects have emerged as a potential strategy for the development of ecologically friendly bioherbicides against weeds (Lopes et al., 2022).

Allelochemicals related to allelopathy belong to the classes of phenolics, terpenoids and alkaloids (Latif et al., 2017; Macías et al., 2019; Lopes et al., 2022). Allelochemicals can inhibit or stimulate the germination and/or growth of plants, and increase the resistance of crops to biotic and abiotic stress. Plant-derived allelochemicals do not exert residual of toxic effects. Therefore, they are considered as the perfect alternative to synthetic herbicides (Cheng, Cheng, 2015; Li et al., 2021). In the late nineteenth century, Stickney and Hoy observed that under the

black walnut tree *(Juglans nigra)* the vegetation was very sparse compared with that under other commonly used shade trees, and that crops did not grow under or very near it. According to the claim of Hoy, this condition is mainly caused by the poisonous character of the water dripping from the tree, the juice of the leaf being poisonous (Stickney and Hoy, 1881; Rice, 2012). The mode of action of allelochemicals may include germination inhibition; interference with seedling or root growth; reduction in chlorophyll content, photosynthetic rate, mineral absorption and carbon flux; interference with enzymatic activity; inhibition of cell division; respiration and protein synthesis (Jabran, 2017; Lopes et al., 2022).

#### *2.1.2. Allelopathic interference in seed germination and seedling growth*

Thus far, several commercial herbicides have been discovered from plants or plant extracts all over the world. Cook et al. (1966) reported the isolation of strigol from cotton root (*Gossypium hirsutum)* secretions, a crystalline germination stimulant which effectively promoted the seeds germination of *Striga asiatica*. The "suicidal germination" of *Striga asiatica* and other parasitic weeds was induced by strigol treatment in the absence of hosts (Cook et al., 1966; Hsiao et al., 1988). The study of Evidente et al. (2007) revealed that the trigoxazonane secreted by *Trigonella foenum-graecum* root can effectively inhibit the germination of *Orobanche crenata* (a parasitic weed in the field of leguminous crops) (Evidente et al., 2007; Li et al., 2021). Funke (1941) stated that beet seeds produce substances that show inhibitory effect on the growth of *Agrostemma githago* but not that of white mustard. He examined several other cases of selective toxicity as well and came to the conclusion that such activity is the reason for the exclusive presence of certain weed species in cultivated fields. Funke suggested that much more careful research is needed due to the potential economic importance of this phenomenon (Rice, 2012). All things considered, plant-derived allelochemicals have broad application prospects and high research value (Li et al., 2021).

### *2.2. Plant extracts*

Extraction is the separation of biologically active components of a plant by utilizing selective solvents through standard methods. The objective of every extraction process is to isolate the soluble plant metabolites, while retaining the insoluble cellular marc (residue) (Handa et al., 2008; Azwanida, 2015). The products thus acquired from plants are complex mixtures of metabolites and aimed for oral or external use in liquid or semi-solid states, or, once the solvent is removed, in dry powder form. These products pertain to classes of preparations known as fluid extracts, decoctions, tinctures, infusions, pilular (semi-solid) extracts or powdered

extracts. The preparation, obtained through standardized extraction procedures for crude drugs derived from medicinal plants, may be suitable for use as medicinal agents in the form of fluid extracts and tinctures, or in any dosage form such as capsules and tablets after further processing. These extracts comprise a complex mixture of numerous medicinal plant metabolites, including glycosides, alkaloids, flavonoids, terpenoids and lignans. A plant extract may be further processed in order to be utilized as a modern drug through several techniques of fractionation to separate individual chemical components (e.g. vincristine, vinblastine) (Handa et al., 2008).

The quality of an extract is dependent on the used plant material, the solvent used for extraction, the method of extraction (technology), the equipment type utilized in the process, and the ratio of crude drug to extract. Therefore, the use of appropriate plant material, extraction solvent, extraction method, technology and manufacturing equipment is essential to produce a good quality extract, along with adherence to good manufacturing practices (Handa et al., 2008).

In the extraction of biologically active ingredients from plant material, the following sequential steps are involved: size reduction, extraction, filtration, concentration, and drying. The extraction of plant material can be carried out through cold aqueous percolation, hot aqueous extraction (decoction) or solvent extraction (cold or hot) (Handa et al., 2008).

### *2.2.1. General methods of plant extraction*

Maceration entails soaking crude drug (whole or coarsely powdered) in a sealed container with a solvent and allowed to stand at room temperature for at least 3 days with frequent agitation. Once the soluble matter has dissolved, the mixture is then strained, the damp solid material is pressed, and the combined liquids are clarified by decantation or filtration subsequent to a standing period (Handa et al., 2008; Azwanida, 2015).

Infusion uses the same principle as maceration; the crude drug is soaked in cold or boiling water, but for a shorter period of time. Fresh infusions are dilute solutions containing the readily soluble constituents of plant samples (Handa et al., 2008; Azwanida, 2015).

Digestion is a form of maceration, which is employed when moderately increased temperature is not considered objectionable, therefore, gentle heat is used during extraction. As a result, the solvent's effectiveness is enhanced (Handa et al., 2008).

Decoction is only suitable for extracting heat-stable, water-soluble components, hard plant materials (e.g. barks and roots) and in comparison to maceration and infusion, decoction typically results in more oil-soluble compounds. During this process, the crude plant material is boiled in a specified volume of water for a defined time, followed by cooling, then straining or filtration. The initial ratio of crude drug to water is fixed, such as 1:4 or 1:16; subsequently, the volume is reduced to one-fourth of the original through boiling. The concentrated extract is then filtered and utilized as such or processed further (Handa et al., 2008; Azwanida, 2015).

Percolation shares a similar fundamental principle (Azwanida, 2015). In this method a narrow, cone-shaped vessel (open at both ends) called percolator is used (Handa et al., 2008) into which dried powdered samples are packed, boiling water is added and then macerated for a period of 2 hours. The process is generally done at a moderate rate (e.g. 6 drops/min) until complete extraction before evaporation to get concentrated extracts (Rathi et al., 2006; Azwanida, 2015). The process of percolation is most frequently used in the preparation of fluid extracts and tinctures (Handa et al., 2008).

Soxhlet extraction or hot continuous extraction involves a finely ground sample placed in a "thimble" or porous bag made from cellulose or strong filter paper, which is placed in the thimble chamber of the Soxhlet apparatus. In the bottom flask the extracting solvent is heated, which vaporizes into the sample thimble, condenses in the condenser, then drips back into the thimble, and extracts the crude drug by contact. When the level of liquid reaches the siphon arm, the liquid emptied into the bottom flask again and the process is continued until a drop of solvent from the siphon tube leaves no residue upon evaporation (Handa et al., 2008; Azwanida, 2015).

Counter-current extraction (CCE) is a procedure in which wet raw plant sample is pulverized using toothed disc disintegrators to prepare a fine slurry. Generally in the form of a fine slurry, the plant sample is moved in one direction within a cylindrical extractor where it comes into contact with the solvent for extraction. The greater the distance covered by the initial sample, the higher the concentration of the extract. Optimizing the quantities of solvent and sample, as well as their flow rates, allows complete extraction to be achieved. This is a highly efficient method, posing no risk from high temperature and demanding minimal time. As a result, a sufficiently concentrated extract emerges from one end of the extractor, while the marc (practically free of visible solvent) is discharged from the other end (Handa et al., 2008).

Microwave assisted extraction (MAE) uses microwave energy to aid in the partitioning of analytes from the sample matrix into the solvent (Trusheva et al., 2007). Microwave radiation interacts with the dipoles of polarizable and polar materials (e.g. samples and solvents), inducing heat near the surface of these materials, with heat subsequently transferred by conduction. Dipole rotation of the molecules induced by microwave electromagnetic fields disrupts hydrogen bonding, thus facilitating the migration of dissolved ions and promoting solvent penetration into the matrix (Kaufmann, Christen, 2002). In the case of non-polar solvents, inadequate heating takes place as energy is solely transferred through dielectric absorption (Handa et al., 2008; Azwanida, 2015).

Ultrasound-assisted extraction (UAE) or sonication extraction entails the utilization of ultrasound frequencies ranging from 20 kHz to 2000 kHz (Handa et al., 2008). The mechanical impact of acoustic cavitation induced by ultrasound enhances both the permeability of cell walls and the surface contact between samples and solvents. The application of ultrasound alters the chemical and physical properties of the materials, leading to the disruption of the plant cell wall. This promotes the release of compounds and improves the mass transport of solvents into the plant cells (Dhanani et al., 2017). This procedure is simple and employs relatively low-cost technology suitable for both small and larger-scale phytochemical extraction (Azwanida, 2015).

Supercritical fluid extraction (SFE) uses supercritical fluid (also known as dense-gas) which is a substance that displays physical characteristics of both liquid and gas at its critical point. Factors such as pressure and temperature play essential roles in driving a substance into its critical region. Supercritical fluids behave predominantly like gases but possess the solvent-like attributes of liquids. One example of a supercritical fluid is carbon dioxide ($CO_2$) which transforms into a supercritical (fluid) state at temperatures exceeding 31.1 °C and pressures surpassing 7380 kPa. There is a growing interest in supercritical $CO_2$ (SC-$CO_2$) extraction due to its effectiveness as a solvent for nonpolar analytes and $CO_2$, as well as its ready availability at a low cost and low toxicity. Despite its limited solubility for polar compounds, SC-$CO_2$ can be modified by adding small amounts of methanol and ethanol to enable the extraction of polar compounds. As $CO_2$ vaporizes at ambient temperature, SC-$CO_2$ also produces concentrated analytes. The strength of supercritical-solvents can be readily adjusted by changing the pressure, temperature or by adding modifiers, thereby reducing the time of extraction (Patil et al., 2014; Azwanida, 2015).

### *2.3. Essential oils*

Essential oils are natural, volatile, complex compounds formed by aromatic plants as secondary metabolites, characterized by a strong odour. They usually have a wide spectrum of bioactivity, due to the presence of numerous active ingredients or secondary metabolites working through various modes of action. Secondary metabolism in plants plays a role in their survival and acts as a chemical protection. Essential oils take an important part in the defence of plants as insecticides, antifungals, antivirals and antibacterials, and they induce the reduction of herbivores' appetite for such plant. They also may favour the dispersion of pollens and seeds by attracting beneficial insects, and repel harmful ones (Abad et al., 2012).

Chemically, essential oils are very complex mixtures, containing about 20-60 components at different concentrations. They consist 2-3 major elements at quite high concentrations (20-70%), compared to other elements present in very small amounts. As a general rule, these major elements are the essential oil's biological features. These components include two groups with different biosynthetic origin, all of which are characterized by their low molecular weight: the main group is terpenes, the other is phenol-derived aromatic and aliphatic components (Abad et al., 2012).

The constituents of the essential oil may be affected by many other factors besides the genetic potential and inherited properties of the accession, such as growing conditions, organic differentiation, stage of development, harvesting time and method of extraction (Müller-Riebau et al., 1997).

Various extraction methods can be used to extract essential oils from different parts of several plants. The choice of extraction method, and the manufacturing of essential oils generally depend on the botanical material used, including the state and form of the material. One of the primary factors determining the quality of essential oil is the extraction procedure used. An inappropriate method can alter action or cause damage of chemical signature of oils, which leads to loss in natural characteristics and bioactivity. Essential oil extraction can be carried out by using several methods (Tongnuanchan, Benjakul, 2014).

#### *2.3.1. General methods of essential oil extraction*

Steam distillation is the most widely used method to extract essential oil from plants (Reverchon, Senatore, 1992). 93% of the essential oil proportion can be extracted by steam distillation, while the remaining 7% by other methods through further extraction (Masango,

2005). During steam distillation the plant sample is heated by steam, and as a consequence of heat application, the cell structure of plant material bursts and breaks down, thus the essential oils or aromatic compounds are released (Perineau et al., 1992; Babu, Kaul, 2005; Tongnuanchan, Benjakul, 2014).

Hydrodistillation has been used as a standard method of extraction of essential oils from plant material, such as flower or wood, to isolate nonwater-soluble substances with high boiling point. During hydrodistillation, the plant sample is completely immersed in water, and then boiled. The surrounding water prevents the oils extracted from overheating, thus protecting it to a certain degree. As a result of the process, the essential oil and steam vapour are condensed to an aqueous fraction. With this method, the plant material can be distilled at a temperature below 100 °C, which is an advantage (Tongnuanchan, Benjakul, 2014).

Hydrodiffusion is used for extraction from already dried plant sample, which is not damaged at boiling temperature (Vian et al., 2008). This extraction method is a type of steam distillation, being only different in the inlet way of steam into the container of still. This process is superior to steam distillation due to higher oil yield and shorter processing time with less steam used. In the case of hydrodiffusion, steam is applied from the top of plant sample, while in the process of steam distillation, it is entered from the bottom. Hydrodiffusion reduces the steam temperature to below 100 °C, and can be carried out under vacuum or low pressure (Tongnuanchan, Benjakul, 2014).

For delicate or fragile flower materials, which do not tolerate the heat of steam distillation, the use of conventional solvent extraction has been introduced. The different solvents that can be used for extraction include ethanol, methanol, hexane, acetone or petroleum ether (Areias et al., 2000; Pizzale et al., 2002; Koşar et al., 2005). In this extraction method, the solvent is mixed with the plant material and then heated, which is followed by filtration. As a consequence, the filtrate is concentrated by solvent evaporation. From the concentrate, which is concrete (a combination of essential oil, fragrance and wax) or resin (resinoid), it is mixed with pure alcohol and distilled at low temperatures. The fragrance is absorbed by the alcohol, and when the alcohol has evaporated, the aromatic absolute oil remains. This process is relatively time-consuming, making the oils more expensive than that extracted by other methods (Li et al., 2009; Tongnuanchan, Benjakul, 2014).

Conventional methods including steam distillation and solvent extraction have some deficiencies, such as large amount or organic solvents, long preparation time (Deng et al.,

2005), low extraction efficiency, degradation of unsaturated compounds, loss of some volatile compounds, and toxic solvent residue found in the extract (Jiménez-Carmona et al., 1999; Glišić et al., 2007; Gironi, Maschietti, 2008). Thereby, for essential oil extraction, supercritical fluids have been regarded as an alternative medium, carbon dioxide ($CO_2$) being the most commonly used, due to its modest critical conditions (Jiménez-Carmona et al., 1999; Señoráns et al., 2000). Carbon dioxide turns into liquid under high-pressure conditions, which can be utilized as a very safe and inert medium to extract aromatic molecules from raw plant material, since no solvent residue remains in the final product (Hawthorne et al., 1993; Tongnuanchan, Benjakul, 2014).

Pressurized hot water or subcritical water has been implemented for extraction under dynamic conditions (temperature range from 100 to 374 °C, pressure high enough to maintain water under liquid state). According to Jiménez-Carmona et al. (1999), in terms of volume of essential oil/1 g of plant material, the efficiency of continuous subcritical water extraction was 5.1 times higher than that of hydrodistillation. This method also provides more valuable essential oil, it is quicker, and enables substantial savings of costs, regarding plant material and energy (Jiménez-Carmona et al., 1999; Tongnuanchan, Benjakul, 2014).

The previously mentioned deficiencies of conventional methods (hydrodiffusion and solvent extraction) have led to the consideration of the use of solvent-free microwave. It is a quick method to extract essential oils from dry seeds, aromatic herbs and spices. The advantages of this practice involves shorter time, higher selectivity and yield and being environmental friendly (Tomaniová et al., 1998; Lopez-Avila et al., 2002). Solvent-free microwave is a combination of dry distillation and microwave heating, performed under atmospheric pressure without water or any solvent. Concentration of volatile compounds and isolation are carried out by a single stage (Lucchesi et al., 2004; Bayramoglu et al., 2008; Tongnuanchan, Benjakul, 2014).

### *2.4.* Artemisia *genus*

*Artemisia* is one of the largest genera of the biggest flowering plant family, the family *Asteraceae*. The genus presents a huge ecological plasticity: species occur from high mountains to sea level, from wetlands to arid zones. There are cosmopolitan species, such as landscape-dominating plants over large areas, and there are endemics, which have a restricted distribution area. Regarding the economic uses, many species of this genus have been and are used at folk and industrial level as well. Some are widely cultivated and submitted to breeding programmes as crops (Vallès et al., 2011; Abad et al., 2012).

The genus *Artemisia* includes important medicinal plants, which are currently the subject of the phytochemical attention because of their chemical and biological diversity. This diverse genus is distributed in the temperate and cold regions of North America and Eurasia (Wang, 2004; Nguyen, Németh, 2016). The genus includes more than 350 species and produces terpenoids, coumarins, flavonoids, sterols, glycosides and polyacetylenes, which are biologically active against malaria, cancer, viruses, hemorrhagy, sudoresis, free radicals, angina, ulcer, hepatitis and other diseases that might be treated by such compounds (Tan et al., 1998; Ferreira, Janick, 2004).

***Artemisia absinthium*** L., commonly known as wormwood/bitter wormwood (Figure 1), is a perennial medicinal and aromatic plant distributed throughout various parts of Europe, temperate Asia northwards to Southern Siberia, Karelia and Lapland, but it can be found in New Zealand and America (both North and South) as an introduced species since it has escaped from domestication (Maw et al., 1985). The morphology of the plant is characterized by small and globular flowers of yellow colour; silvery hoary leaf twigs on both surfaces; high, branching stem covered by white hairs; woody, hardy rosette; and height of 40-150 cm (Maw et al., 1985; Nguyen, Németh, 2016).

**Figure 1:** *Artemisia absinthium* L. *(Source: A. Dorottya Frank, 2022)*

It has been used in traditional medicine as a cardiac stimulant, stomachic, antispasmodic and anthelmintic agents, preparations were also used for its antiparasitic effects, anorexia treatment, indigestion, inflammation conditions of the liver, and restoring declining mental function (Guarrera, 2005). *A. absinthium* is a rich source of antioxidants which may support healing of skin wounds (Bora, Sharma, 2011a; Craciunescu et al., 2012). Pharmacological and clinical

investigations have indicated the significance of this species in neuroprotection strategies (Omer et al., 2007; Bora, Sharma, 2010; Krebs et al., 2010; Nguyen et al., 2018). *A. absinthium* aerial parts are ingredients of dietary supplements, many gastric herbal preparations, and alcoholic beverages (Lachenmeier, 2010), for example absinthe, which was the most popular spirit drink in Europe in the late nineteenth century (Lachenmeier et al., 2006). Numerous secondary metabolites and other compounds have been isolated from wormwood, the essential oil obtained from glands on the aerial parts being the most important (Nguyen, Németh, 2016).

***Artemisia alba*** Turra (syn. *A. camphorata* Vill., *A. lobelii* All.; incl. *A. incanescens* Jordan, *A. suavis* Jordan) is an aromatic, perennial, sub-Mediterranean species (Figure 2), widespread in southern Europe and north-western Africa, but restricted to dry, calcareous habitats in central Europe (Tutin, 1964; Coassini Lokar et al., 1987).

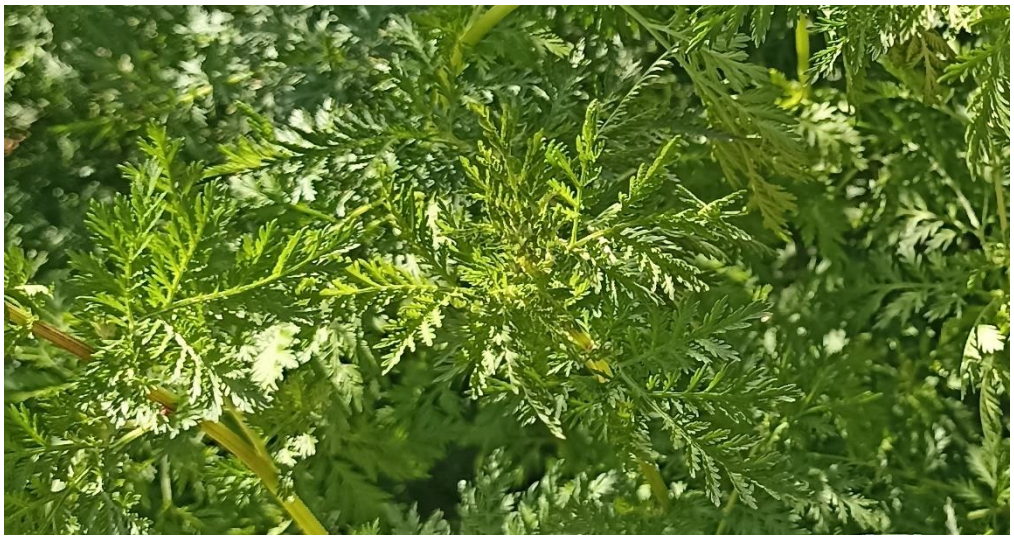
**Figure 2:** *Artemisia alba* Turra *(Source: Péter Radácsi, 2024)*

This plant is known for its "water-conservation" strategy that might be regarded as an adaptation to dry grassland conditions (Erschbamer et al., 1983; Coldea et al., 2023). It grows in xerophytic associations on calcareous soils in northwest Yugoslavia and in northeast Italy. The plant is an indicator of the most xerophytic grassland communities, mainly composed of Mediterranean and Illyrian species, along the edge of the South Eastern Alps and in the north Adriatic area (Karst). This species may occur at the edge of light forests or on rock outcrops (Coassini Lokar et al., 1987). The plant is glabrous to white-tomentose with a stout, branched stock and 30-100 cm long stems, which is woody below (Tutin, 1964). Due to the diverse environments in which *A. alba* grows, it indicates great differences in smell. Specimens found at the edge of the South Eastern Alps have a very light, herbaceous smell, while the ones

growing in the Karst are characterized by a strong, camphorated scent (Coassini Lokar et al., 1987). Peron et al. (2017) pointed out that *Artemisia alba* is a taxonomically problematic species characterized by common polymorphism, which leads to a quite high variability in secondary metabolite content (Peron et al., 2017). The study of Coassini Lokar et al. (1987) indicated that this species produces mainly oxygenated monoterpenes when growing in mesophytic plant communities and mostly sesquiterpene hydrocarbons in thermophytic plant communities (Coassini Lokar et al., 1987).

***Artemisia annua*** L., commonly known as annual wormwood (Figure 3), is an herbaceous annual medicinal plant. It is cultivated in Asia, Central and Eastern Europe, temperate regions of Australia, Africa, America, and tropical areas (Willcox, 2009; Alesaeidi, Miraj, 2016; Septembre-Malaterre et al., 2020). The morphology of *A. annua* is characterized by globular flowers of yellow colour; green or yellow-green leaves with dotted glands on the surface; erect, ridged stem of green, yellow-green or violet-green colour (brown or violet-brown in the late growth stage); with a height of 30-150 cm (up to 300 cm when cultivated); the plant is either hairless or covered with scattered, dense patches of fine hair (Guoqiao et al., 2018).

**Figure 3:** *Artemisia annua* L. *(Source: A. Dorottya Frank, 2022)*



The plant is mentioned in numerous ancient texts for its efficacy in treating a variety of diseases, including jaundice, consumptive fever, wounds from summer heat, lice, tuberculosis, dysentery, hemorrhoids, scabies, as well as for pain relief. In Iran it is utilized as a carminative, antispasmodic, or sedative remedy for children (Sadiq et al., 2013; Feng et al., 2020; Septembre-Malaterre et al., 2020; Trendafilova et al., 2020). *A. annua* has a long history in traditional medicine in Asia and Africa, where it had been employed for treating malaria and

fever in traditional medicine, typically consumed as a tea or pressed juice (Mueller et al., 2000; Ćavar et al., 2012). The dried herb of *A. annua* is officially recognized by the current pharmacopoeia of the People's Republic of China as a treatment for malaria and fever (Gupta et al., 2009), and has been utilized for clinical trials. The anti-plasmodial, anti-hyperlipidemic, anti-convulsant, anti-microbial, antiviral, anti-cholesterolemic (Abad et al., 2012; Lubbe et al., 2012; Wang et al., 2020), antioxidant (Brisibe et al., 2009) and antifungal (Li et al., 2019) effects of this plant were also determined, as well as important pharmacological activities, including antitumor, anti-obesity and anti-inflammatory activities (Ho et al., 2014; Kim et al., 2014; Septembre-Malaterre et al., 2020) .

***Artemisia pontica*** L., known as roman wormwood (Figure 4) is a rather common Eurasian species (Kirpicznikov, 1969) distributed in Middle Asia, western Siberia, the Caucuses, the European part of Russia and Crimea (Talzhanov et al., 2005).

**Figure 4:** *Artemisia pontica* L. *(Source: © RBG Kew, Dr Henry Oakeley's RCP Medicinal Plants, https://creativecommons.org/licenses/by/3.0/)*

Its aerial parts are traditionally used as sedatives, bitter tonic and anthelmintics (Bos et al., 2005), while studies have reported analgesic, anti-inflammatory (Ivanescu et al., 2021), antioxidant (Nikolova et al., 2010), and insecticidal effects (Derwich et al., 2009). This herb presents repellent activity against the yellow fewer mosquito, *Aedes aegypti*, transmitter of several viruses (Tabanca et al., 2011).

***Artemisia scoparia*** Waldst. & Kit., commonly known as redstem wormwood (Figure 5), is a faintly scented annual herb, widely distributed throughout the world, particularly in central Europe and southwest Asia (Abad et al., 2012).

**Figure 5:** *Artemisia scoparia* Waldst. & Kit. *(Source: Mokkie, CC BY-SA 4.0, Wikimedia Commons, 2016)*

Phytotoxins, volatile essential oil and other non-volatile secondary compounds are present in *A. scoparia*, which account for its success. It has been detected that the aerial parts of this plant contain a volatile essential oil, which has medicinal value. It has antibacterial, antipyretic, insecticidal, anticholesterolemic, cholagogue, vasodilatatory, purgative, antiseptic and diuretic activity, and is also used for the treatment of hepatitis, jaundice and gall bladder (Brendler et al., 2004; Abad et al., 2012). The monoterpenoid-rich essential oil of *A. scoparia* exhibited a strong antioxidant and radical scavenging activity (Singh et al., 2008; Bora, Sharma, 2011b).

**Figure 6:** *Artemisia vulgaris* L. *(Source: A. Dorottya Frank, 2022)*

***Artemisia vulgaris*** L., commonly known as mugwort, is a perennial plant (Figure 6), which is native to Asia and Europe, but largely distributed in North America, Africa, Australia and the

Himalayas (Ekiert et al., 2020; Trendafilova et al., 2020). In the Philippines, it is widely used as an antihypertensive. Other medicinal activities of the plant have also been suggested, such as antispasmodic, anti-inflammatory, anthelmintic and carminative properties, and it has been used to ease severe menstrual pain (dysmenorrhoea) and induce labour or miscarriage (Quisumbing, 1951; Abad et al., 2012). In the Iranian traditional medicine, this species is used in the treatment of cervicitis (Nabimeybodi et al., 2019), while it is reported as a drug with various utilizations, such as gonorrheal sore, steam bath for pleurisy, rheumatism, cold, headache, an anthelmintic, in childbirth and pains of afterbirth (Vestal, Schultes, 1939). *A. vulgaris* extract is widely known to have anti-allergenic and anti-histaminic effects, therefore, mugwort lotion is used clinically for treating itch in icteric and dialytic patiens, and has also showed good results in patiens with post-burn hypertrophic scars (Ogawa et al., 2008; Trendafilova et al., 2020).

### *2.4.1. Chemical composition of* Artemisia *species*

***Artemisia absinthium*** ethanolic extract was examined in India, resulting in the identification of rutin, scopoletin, salicylic acid, luteolin, kaempferol, dihydroartemisinin, artemisinin and artemether (Singh et al., 2021). The composition of the *A. absinthium* essential oil exhibits a large intraspecific variability. Bicyclic monoterpene thujone may be considered as the most characteristic constituent of *A. absinthium* essential oil (Meschler, Howlett, 1999; Juteau et al., 2003). α- and β-thujones, both isomeric forms were reported in the essential oil of the plant, β-thujone usually being present in higher concentration than α-thujone, but the actual proportion may change on a large scale (Nguyen, Németh, 2016). In a study on Serbian natural *A. absinthium* populations, β-thujone was detected in 63.4% of the total oil isolated from the aerial parts of the plant, thus being the absolute major component, while α-thujone was present in only 0.4% (Blagojević et al., 2006). In wild collected Iranian plants, α-thujone was described in 18.6%, while β-thujone in 23.8% (Rezaeinode, Khangholi, 2008). Besides the well-known β-thujone, at least 17 other major compounds were described in the oil, such as myrcene, epoxyocimene, sabinene, sabinyl acetate, chrysanthenol, chrysanthenyl acetate (Nguyen, Németh, 2016), β-pinene, hydrocarbon monoterpenes, polyphenolic compounds (Kordali et al., 2005), flavonoids, sesquiterpene lactones, lingans and tannins (Bora, Sharma, 2011b).

In the study of Coassini Lokar et al. (1987) on essential oil of ***Artemisia alba*** originating from different associations, 21 components were identified. The most abundant oxygenated terpenes were borneol (association A, B, C, D); camphor, myrtenol, bornyl acetate (B); trans-pinocarveol

(B, C) and terpinen-4-ol (C), while the most abundant sesquiterpene hydrocarbons were δ-cadinene (A, C, D) and β-cubenene (B) (Coassini Lokar et al., 1987). The results of Stanisabljevi et al. (2013) showed that the major constituents of *A. alba* essential oil were camphor (23.7%), artemisia ketone (15.2%) and 1,8-cineole (14.1%), and in the volatile fraction, scopoletin (14.0%) and corymbolone (10.3%) (Stanisavljevi et al., 2013).

Among the numerous bioactive metabolites that have been identified in ***Artemisia annua***, artemisinin (a lactone sesquiterpene endoperoxide) is the most extensively studied due to its antimalarial activity (Castilho et al., 2008). The nutritional profile of this plant is also remarkable, containing minerals, amino acids, vitamins and essential elements for health (Brisibe et al., 2009). Since the discovery of *A. annua*, over 600 secondary metabolites have been found within the whole plant (Van der Kooy, Sullivan, 2013), such as monoterpenoids, triterpenoids, sesquiterpenoids, flavonoids, steroids, alkaloids, coumarins, flavonoids and benzenoids (Bhakuni et al., 2001; Yi-wu et al., 2014; Li et al., 2019; Septembre-Malaterre et al., 2020). From *A. annua* plant parts, originating from northern areas of Pakistan, artemisinin was found in the material extracted by sonication, with the highest concentration found in the leaves (0.44 ± 0.03%) and flowers (0.42 ± 0.03%), followed by the stems (0.08%) (Mannan et al., 2010; Anibogwu et al., 2021). During a study conducted in India, rutin, salicylic acid, luteolin, kaempferol, eugenol, isoeugenol, dihydroartemisinin, and artemisinin were identified in the ethanolic extract of *A. annua* (Singh et al., 2021). Deoxyartemisinin and dihydro-epideoxyarteannuin B were isolated from the sesquiterpene lactone-enriched fraction obtained from the crude ethanol extract of A. annua (Foglio et al., 2002; Bora, Sharma, 2011b).

Sesquiterpene lactones have been isolated from the aerial parts of ***Artemisia pontica*** growing in Bulgaria (Todorova et al., 1996; Trendafilova et al., 1996), while in a Kazakhstan population rarely encountered flavonoids 7-O-methyl- and 4′,7-di-O-methyl-esters of apigenin were identified (Talzhanov et al., 2005). As root components, polyene and coumarin derivatives were reported (Bohlmann et al., 1974; Todorova et al., 1996). An investigation on the main volatile components of aerial parts of *A. pontica* grown in Bulgaria revealed that oxygen-containing monoterpenes dominated (36.7%) the essential composition, 1,8-cineole and camphor (14.1%, 13.9%) found to be the main compounds. Other relatively abundant components reported were terpinen-4-ol, sabina ketone, dehydro sabina ketone (3.0%, 1.5%, 1.3%) (Bos et al., 2005). In a Kazakh *A. pontica* population, the principal essential oil constituents were 1,8-cineol, α-thujone, camphor and borneol (25.2%, 23.2%, 16.0%, 7.7%) (Talzhanov et al., 2005).

***Artemisia scoparia*** has been reported to contain artemisinin in small amounts (Singh, Sarin, 2010; Ivanescu et al., 2015). The investigation of Joshi et al. (2010) on the composition of *A. scoparia* essential oil showed the dominant presence of phenyl alkynes (61.2%, 85.5%), γ-terpinene (11.1%), p-cymene (4.5%) and (E)-β-ocimene (4.4%) (Joshi et al., 2010). In the essential oil from *A. scoparia* from Tajikistan a total of 32 compounds were identified, representing 98% of the total oil composition. The diacetylenes 1-phenyl-2,4-pentadiyne (34.2%) and capillene (4.9%) dominated the oil, while other major compounds were β-pinene (21.3%), methyl eugenol (5.5%), α-pinene (5.4%), mycrene (5.2%), limonene (5%) and β-ocimene (3.8%) (Sharopov, Setzer, 2011; Abad et al., 2012).

In ***Artemisia vulgaris*** flowers and leaves collected in Pakistan, artemisinin was detected at concentrations ranging from 0.05% to 0.15%. The extraction was carried out using sonication (Mannan et al., 2010). In a study conducted in the Philippines, yomogin and 1,2,3,4-diepoxy-11(13) eudesmen-12,8-olide were isolated from *A. vulgaris* leaves, which were extracted through silica gel column chromatography using gradient elution (Natividad et al., 2011; Ivanescu et al., 2015; Anibogwu et al., 2021). In ethanolic extracts of *A. vulgaris* originating from India, rutin, scopoletin, luteolin, naringenin, kaempferol, eugenol, isoeugenol, dihydroartemisinin, chrysin, and artemisinin were determined (Singh et al., 2021). Various flavonoids were isolated from the entire plant, categorized into classes such as flavones (jaceosidine, tricine, chrysoeriol, eupafolin, apigenin, luteolin, diosmetin), flavone glycosides (vitexin and luteolin 7-glucoside), flavanones (eriodictyol and homoeriodictyol), flavonols (isorhamnetin) and flavonol glycosides (kaempferol 3-rhamnoside, kaempferol 3-glucoside, kaempferol 7-glucoside, kaempferol 3-rutinoside, quercetin, quercetin 3-galactoside, quercetin 3-glucoside and rutin). Among these compounds, eriodictyol and luteolin were found to be the most abundant (Lee et al., 1998; Abiri et al., 2018). The extracted oil of *A. vulgaris* grown under greenhouse conditions, was found to be rich in camphor, α-thujone, germacrene D, camphene, 1,8-cineole and β-caryophyllene (16.8%, 11.3%, 7.2%, 6.5%, 5.8%, 5.4%), according to the research conducted by Govindaraj et al. (2008). The essential oil was extracted from leaf samples, mainly characterized by monoterpenes (72%) and sesquiterpenes (26%) (Govindaraj et al., 2008). Blagojević et al. (2006) examined the essential oil chemical composition of wild-growing *A. vulgaris* populations from Serbia. The principal components of the oil isolated from aerial parts were 1,8-cineole, sabinene, β-thujone and β-caryophyllene oxide (28.9%, 13.7%, 13.5%, 6.5%) (Blagojević et al., 2006).

*2.4.2. Allelopathic effects of* Artemisia *species*

The investigation of Kapoor et al. (2019) revealed that the aqueous leaf extracts of ***Artemisia absinthium*** significantly affects the germination and seedling growth (shoot and root length) of *Parthenium hysterophorus.* The aqueous extract had an impact on the photosynthetic pigments (chlorophylls and carotenoids) of the treated plants. The level of non-enzymatic antioxidants (ascorbic acid, GSH) were significantly reduced, while the antioxidant enzymes (CAT, DHAR, APOX, SOD) and total phenolic content were increased in *Parthenium* plants treated. In conclusion, aqueous extracts of *A. absinthium* can be utilized as botanical herbicides to control the spread of *P. hysterophorus* (Kapoor et al., 2019). Chirca and Fabian (1973) studied some allelopathic effects caused by *Artemisia absinthium.* According to their results, volatile emanations from the leaves did not affect the germination of *Linum usitatissimum, Lepidium draba* or *Sinapis alba,* but completely inhibited it in wheat. Seedling growth of *S. alba* was markedly stimulated, while inhibited that of *L. usitatissimum, L. dabra* and wheat, especially. Root extract inhibited germination in wheat, and reduced shoot and root elongation in the other three species by 53-85%. Aqueous extract of the leaves prevented germination of all four species (Chirca, Fabian, 1973).

***Artemisia alba*** essential oil has been registered for specified uses in agriculture (in particular biocidal effects) (Raveau et al., 2020). Schulz et al. (2007) investigated the interference of allelopathic monoterpenes with *Arabidopsis thaliana* cuticular waxes and their effect on transpiration. According to their findings, exposure to camphor (100 mg / 10 L) and menthol (50 mg / 10 L) for 24 h increased transpiration of *A. thaliana* completely developed rosette leaves similar to de-waxing. Long term exposure (more than 48 h) to 100 mg / 50 mg killed the plants by desiccation. Investigations of the stomatal apertures indicated that stomatal opening induced by monoterpenes is followed by extreme swelling and a final breakdown of the protoplasts. Exposure of *A. thaliana* to volatiles of *Artemisia camphorata* (syn. *Artemisia alba), Lavandula latifolia* and *Mentha piperita* resulted in a significant enhance of stomata aperture but swelling of the protoplasts was less exhibited (Schulz et al., 2007).

Artemisinin is probably the primary compound in ***Artemisia annua*** to be assessed as a potential herbicide. Allelopathic effect of artemisinin have been examined in several studies (Chen, Leather, 1990; Maurya et al., 2022). The study of Duke et al. (1987) revealed that artemisinin is a selective phytotoxin. Their results show that artemisinin inhibited the germination and growth of shoots and roots of *Lactuca sativa,* and shoot and root growth of *Amaranthus*

*retroflexus, Ipomoea lacunose* and *Portulaca oleracea* at 33 μM, while no effect was observed at 33 μM on growth of *Sorghum bicolor* (Duke et al., 1987). Lydon et al. (1997) examined the effects of leaf tissue and leaf-tissue extracts of *A. annua* and pure artemisinin on plant growth *(Amaranthus retroflexus, Chenopodium album, Glycine max, Zea mays)*. The treatment with leaf tissue and leaf-tissue extract resulted in species-specific inhibition of growth. While the $MeCl_2$ extract containing artemisinin showed similar effecs to leaf tissue on *Amaranthus retroflexus*, the aqueous extract without artemisinin and the extract residue also exhibited allelopathic effects, suggesting that the allelopathy of *A. annua* cannot be solely attributed to artemisinin (Lydon et al., 1997).

Allelochemicals indirectly affect the other plants through the inhibition of microorganisms (Mei et al., 2010). Kiplimo et al. (2016) investigated the larvicidal activity of ***Artemisia pontica*** growing in Kenya. According to their results, the essential oil may be used in the treatment of diseases caused by *Quinque fasciatus* (Kiplimo et al., 2016). The insecticide effect of *Artemisia pontica, A. herba-alba* and *A. absinthium* essential oils was studied on *Acanthoscelides obtectus* (responsible for green beans rot) by Derwich et al. (2009). The results indicated that all tested insects were sensitive to the essential oils of examined species(Derwich et al., 2009). Marchant and Cooper (1987) discovered that a phototoxin, specifically an acetylenic epoxide from *A. pontica* (ponticaepoxide) exhibits an $LC_{50}$ of 1.47 ppm against mosquito larvae when exposed to UV light (Marchant, Cooper, 1987; Souto et al., 2021).

The allelopathic potential of ***Artemisia scoparia*** was studied by Kaur and Batish (2010), who examined the effect of volatile oil of *A. scoparia* leaves against *Zea mays, Triticum aestivum, Chenopodium murale, Bidens pilosa* and *Amaranthus viridis.* Seed germination of all the test plants was inhibited at each concentrations (0.5, 1.0, 2.5, 5.0 μL/Petri dish). The oil treatment also severely affected the cellular respiration and chlorophyll content. As a general rule, the inhibitory effect was greater on weeds than on crops (Kaur, Batish, 2010). According to the study of Kaur et al. (2010) the volatile oil hydrodistilled from *A. scoparia* significantly reduced the emergence and seedling growth (in terms of shoot and root length) of *Achyranthes aspera, Parthenium hysterophorus, Cassia occidentalis, Ageratum conyzoides* and *Echinochloa crus-galli.* Generally, the root length was inhibited more as compared to the shoot length (Kaur et al., 2010). Both studies conclude that *A. scoparia* oil could be used as a bioherbicide due to its strong phytotoxicity against weeds.

Phenolics released by ***Artemisia vulgaris*** were determined by Inderjit and Foy (1999) to play an important role in the growth inhibition of *Trifolium pratense* (red clover). Meanwhile, change in soil characteristics due to the addition of *A. vulgaris* probably affected the allelopathic effects. Soils amended with *A. vulgaris* had higher levels of phenolics, lower nitrate and higher pH levels, higher levels of organic matter and soluble salts, $PO_4$, Zn, K, Fe, Mn, Cu, Al and B (Inderjit, Foy, 1999). According to Inderjit et al. (2001) soil-mediated chemical interference significantly affects allelopathy (Inderjit et al., 2001). The allelopathic effect of aqueous extract from *A. vulgaris* on seed germination and growth of *Sorghum vulgare, Triticum aestivum, Raphanus sativus, Brassica campestris* and *Cucumis sativus* was examined by Mei et al. (2010). Their study revealed that *A. vulgaris* aqueous extract strongly inhibited the germination, speed of germination and seedling growth of all tested crops. The results indicate that the allelopathy of mugwort could break cell membranes by promoting MDA (Mei et al., 2010).

# 3. Materials and methods

## *3.1. Plant materials*

The current experiments were carried out in the framework of Hungarian-Serbian contribution. This research was supported by the National Research Development and Innovation Office (2019-2.1.11-TÉT-2020-00245). The examined *Artemisia* plant material was collected and extracted in Serbia in 2022 and 2023.

This study includes two experiments, in which leaf extracts and essential oils of different *Artemisia* species were used to evaluate the allelopathic effects of these plants on seed germination and seedling growth of white mustard (Sinapis alba).

The experiment with the leaf extracts included the following species: *Artemisia absinthium*, *Artemisia alba*, *Artemisia annua*, *Artemisia pontica*, *Artemisia scoparia* and *Artemisia vulgaris.* The crude drugs were extracted by a solvent of methanol and methylene chloride in a 1:1 ratio.

**Figure 7:** Ethanol leaf extracts of *Artemisia alba* (1), *A. annua* (2), *A. absinthium* (3), *A. scoparia* (4), *A. pontica* (5), *A. vulgaris* (6) *(Source: A. Dorottya Frank, 2023)*

The dried plant extracts (weights ranging from 0,20 g to 0,29 g) were soaked in 7 mL of 96% pure ethanol, then put in an ultrasonic water bath for proper mixing. Subsequently, the extracts were diluted with 96% pure ethanol until a 0,01% solution was obtained (results on Figure 7).

The experiment with the essential oils included the following species: *Artemisia absinthium, Artemisia alba, Artemisia pontica, Artemisia scoparia* and *Artemisia vulgaris.*

The essential oil vials (and later the Petri dishes) were labelled as follows: AABSZEU stands for *A. absinthium;* AALBSEU for *A. alba*; APONZEU for *A. pontica;* ACAMNBGEU for *A. scoparia;* AVCSEU for *A. vulgaris* (Figure 8).

**Figure 8:** Essential oil of *Artemisia pontica, A. absinthium, A. scoparia, A. vulgaris, A. alba* (from left to right) *(Source: A. Dorottya Frank, 2022)*


As a first step in the preparation of the test species *(Sinapis alba)*, the weight of 25 white mustard seeds were measured, since 25 seeds were put in each Petri dishes. The 25 seeds weighed 60.01 mg. Thousand seed weight (TSW) of *Sinapis alba* is 4 – 8 g.

After the measurement, all seeds were sterilised in a 120 mL solution of 60% ethanol, for 30 seconds. Then the seeds were washed with distilled water and then left to dry.

### *3.2. Chemical composition of plant material*

Hereby presented some of the study results of Radulović et al. (2024) regarding phenolic compounds identified in the leaf extracts of *A. alba, A. annua, A. pontica* and *A. vulgaris* originating from Serbia, the same area where the *Artemisia* species examined in the current study came from. Although it cannot be stated with certainty, it is possible that the plants presented in this study contain similar substances as those investigated in the research of Radulović et al. (2024).

Radulović et al. (2024) determined 13 phenolic compounds and quinic acid in the examined *Artemisia* samples. The dominant compound was chlorogenic acid in all extracts, in highest amount in *A. alba*, and lowest in *A. vulgaris* extract. Rutin was found in extracts of *A. alba* and *A. pontica,* vitexin in *A. annua,* and esculin in extracts of *A. vulgaris* as the second dominant compound. Caffeic and quinic acid were detected in highest concentration in *A. alba*, and was

also found in *A. vulgaris* extract. Flavonoids were identified, namely apigenin and naringenin, in the leaf extract of *A. annua;* and rutin, luteolin, apigenin and hispidulin in *A. alba* leaf extract (Radulović et al., 2024).

### *3.3. Methods*

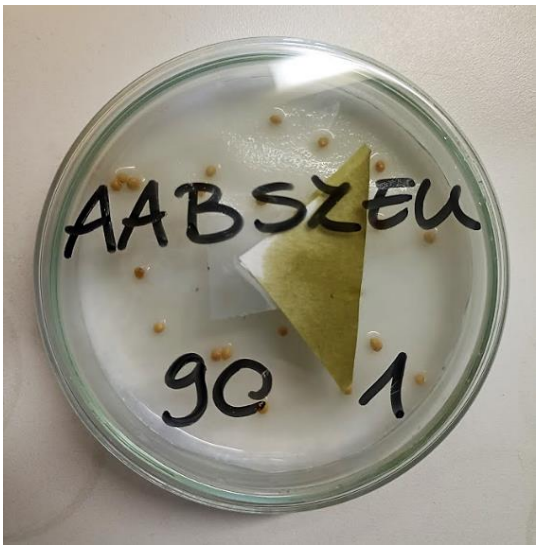
#### *3.3.1. Seed germination and measurement*

In both experiments, 2 layers of filter was placed at the bottom in each Petri dish, as were 25 mustard seeds (distance between seeds quite equidistant). For every examined *Artemisia* species, 3 samples were prepared for each amount of the given plant extract and essential oil.

In the case of the examination of plant extracts' effect, seeds were treated with 100 μL, 300 μL or 1000 μL ethanol plant extract. After injecting the extracts, the solvent was evaporated. Untreated (C1) and positive controls (C2) were applied. In the case of C1 no ethanol or extract was injected at all while in C2 1 mL ethanol (96%) was injected and then the Petri dishes were sealed.

For the essential oil treatment, the seeds in all Petri dishes were watered with 5 mL distilled water. Small, double layered filter triangles were sticked to the upper piece of the Petri dishes, above the seeds, where the essential oils were dripped onto. Effects of the following essential oil amounts were observed: 10 μL, 30 μL, and 90 μL. See an example of a Petri dish prepared with white mustard seeds and the given essential oil on Figure 9.

**Figure 9:** Petri dish with *Sinapis alba* seeds and 90 μL *Artemisia absinthium* essential oil, sample No. 1 *(Source: A. Dorottya Frank, 2022)*



Petri dishes were taped around for the purpose of hermetic seal, then placed into a Sanyo MLR-351H incubator (Figure 10) with a program set for 8 hours of light (~12 klux) at 30 °C and 16 hours dark at 20 °C. The whole incubation period was 7 days, seedlings were measured on the 3rd and 7th days. On the 3rd day, the seedlings were quantified, while on the 7th day this quantification was supplemented by the measurement of seedling height and root length. For seedling height and root length measurement ImageJ software was used.

**Figure 10:** Samples in the incubator *(Source: A. Dorottya Frank, 2022)*

*3.3.2. Data analysis*

SPSS software was used for statistical analysis of the study's results, including descriptive data analysis and two-way analysis of variance (ANOVA). For the analysis of seedling height and root length Post Hoc test was also used.

# 4. Results and evaluation

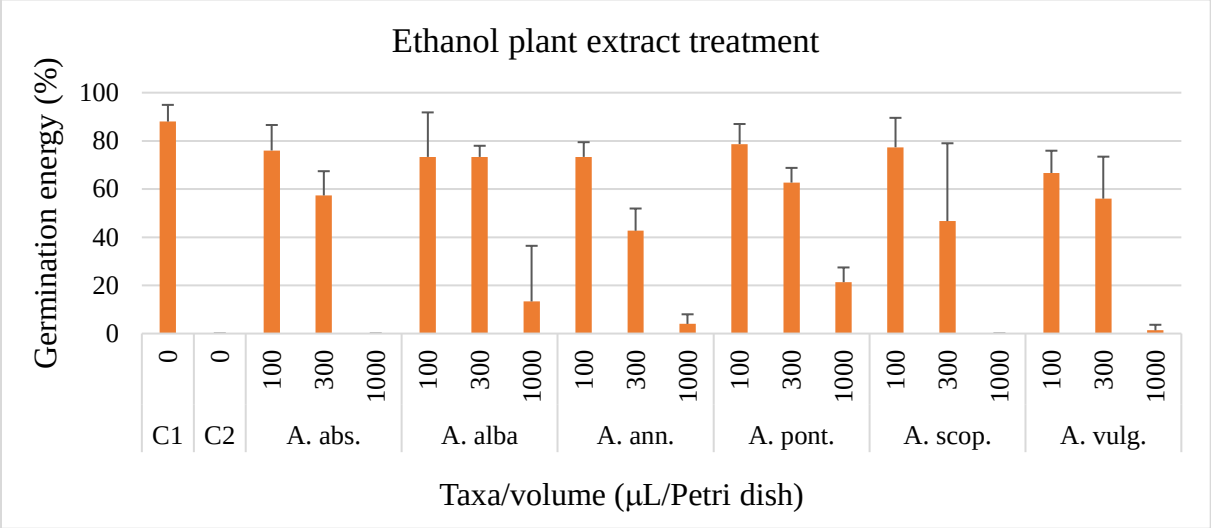
## *4.1. Germination energy and germination capacity*

Germination energy expresses the percentage of germinating seeds on the 3rd day, while germination capacity is the percentage of total seeds germinating on the 7th days.

### *4.1.1. Ethanol plant extract treatment*

Examination of the **germination energy** of white mustard *(Sinapis alba)* seeds treated with *Artemisia* ethanol plant extracts revealed that both the taxa ($p \leq 0{,}001$) and volume ($p \leq 0{,}001$) had a significant effect. Additionally, interaction between the taxa and volume was observed ($p \leq 0{,}001$).

**Figure 11:** Germination energy of *Sinapis alba* treated with *Artemisia* ethanol leaf extracts (C1 = untreated control; C2 = positive control treated with 96% pure ethanol)
*(Source: A. Dorottya Frank, 2024)*

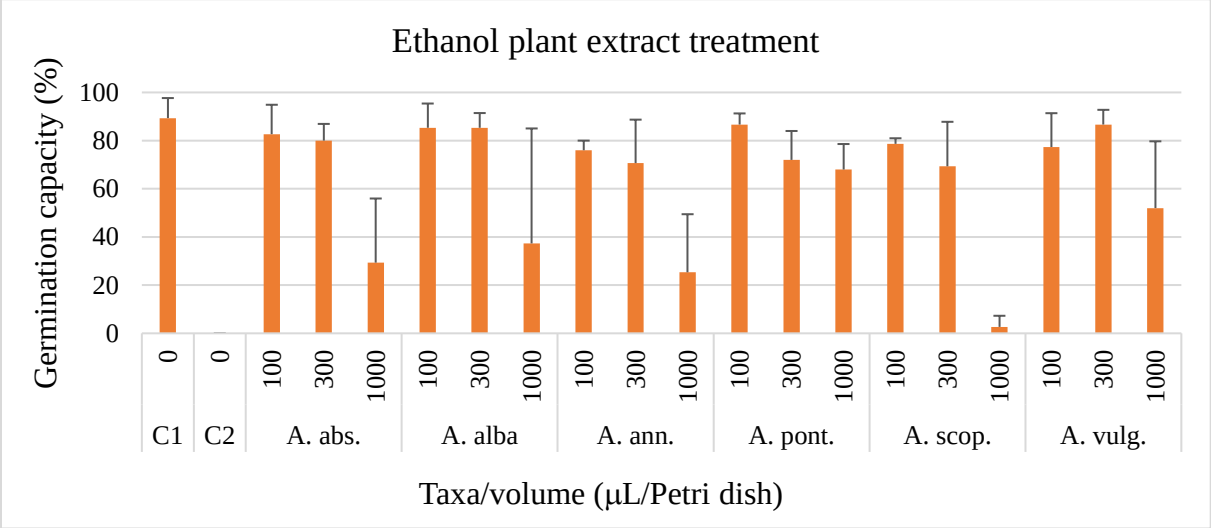



All ethanol plant extracts reduced the germination energy compared to the untreated control (C1), 100 µL having slight effect (mostly 10-15% difference compared to C1) and 1000 µL having the most significant (Figure 11). It can be established that the increase in the volume of ethanol plant extract gradually and significantly reduced the germination energy of white mustard, with the exception of *A. alba* where the 100 µL and 300 µL treatments had the same results. Apart from that, the volume definitely affected the germination energy: at 1000 µL there were only a few or no germinating seeds. Consequently, 1000 µL showed the strongest inhibitory effect in all cases.

Considering all volumes, the ethanol plant extract of *A. annua* showed the strongest inhibitory effect on germination energy, followed by: *A. scoparia* = *A. vulgaris* > *A. absinthium* > *A. alba* > *A. pontica.*

Analysis of the **germination capacity** of white mustard seeds treated with *Artemisia* ethanol plant extracts indicated that both the taxa ($p \leq 0{,}001$) and volume ($p \leq 0{,}001$) had a significant effect, but showed no interaction between these two variables ($p \leq 0{,}147$). No interaction between the taxa and volume means that all examined species can be described by the same tendencies.

**Figure 12:** Germination capacity of *Sinapis alba* treated with *Artemisia* ethanol leaf extracts (C1 = untreated control; C2 = positive control treated with pure ethanol)
*(Source: A. Dorottya Frank, 2024)*

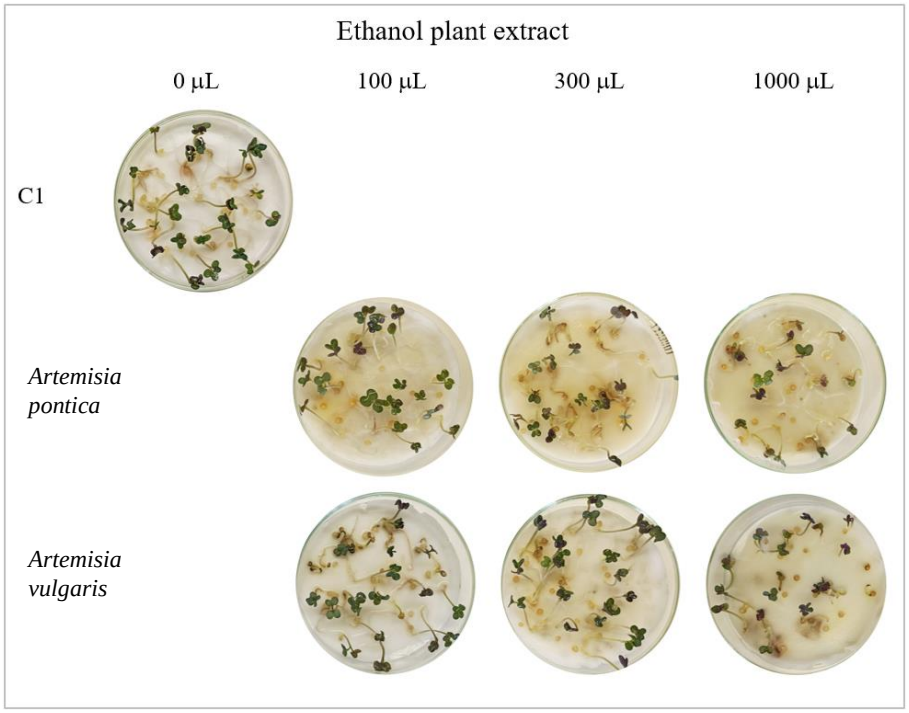



Compared to the untreated control (C1) all ethanol plant extracts had some reducing effect on the germination capacity, 100 µL and 300 µL causing slight reduction (4-20%) and 1000 µL being the most effective, with a reduction rate mostly above 50% compared to C1, except *A. vulgaris* and *A. pontica* (Figure 12). It can be determined that in most of the cases, the increase in the volume of ethanol plant extract gradually reduced the germination energy of white mustard, with the exception of *A. alba* (100 µL and 300 µL had the same results) and *A. vulgaris* (100 µL caused a greater reduction than 300 µL). Significant reduction was induced by the volume of 1000 µL.

Despite the results of germination capacity, which is based on the number of germinated seeds, indicating that the 1000 µL volume of *A. pontica* and *A. vulgaris* were not as effective as the

same volume of other *Artemisia* species, Figure 13 shows that those seeds that did germinate were degraded and small. It can be concluded that 1000 µL had the strongest inhibitory effect in all cases.

**Figure 13:** Allelopathic effect of *A. pontica* and *A. vulgaris* ethanol plant extracts on *Sinapis alba* seeds (C1 = untreated control) *(Source: A. Dorottya Frank, 2024)*



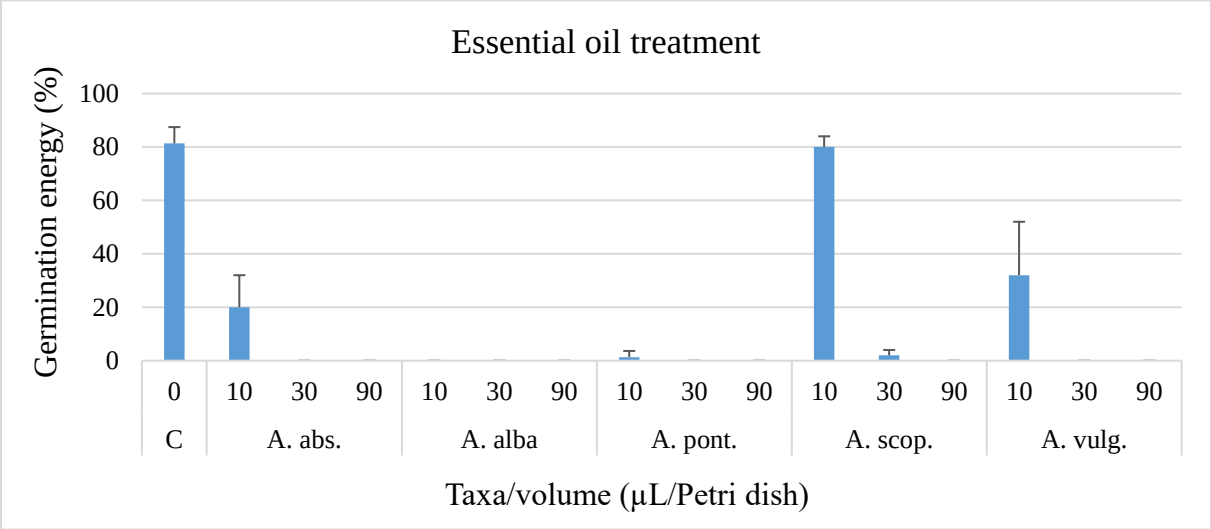

Considering all volumes, the ethanol plant extract of *A. scoparia* had the strongest inhibitory effect on germination capacity, followed by: *A. annua* > *A. absinthium* > *A. alba* > *A. vulgaris* > *A. pontica.*

*4.1.2. Essential oil treatment*

During the examination of the **germination energy** of white mustard *(Sinapis alba)* seeds treated with *Artemisia* essential oils it was determined that both the taxa ($p \leq 0{,}001$) and volume ($p \leq 0{,}001$) had a significant effect. Furthermore, interaction between the taxa and volume was observed ($p \leq 0{,}001$).

All essential oils reduced the germination energy compared to the control (C), with the exception of the 10 µL volume of *A. scoparia* (Figure 14). It can be stated that the increase in the volume of injected essential oil gradually and significantly reduced the germination energy of white mustard. The volume considerably affected the germination energy: at 90 µL there were no germinating seeds. Accordingly, 90 µL had the strongest inhibitory effect in all cases.

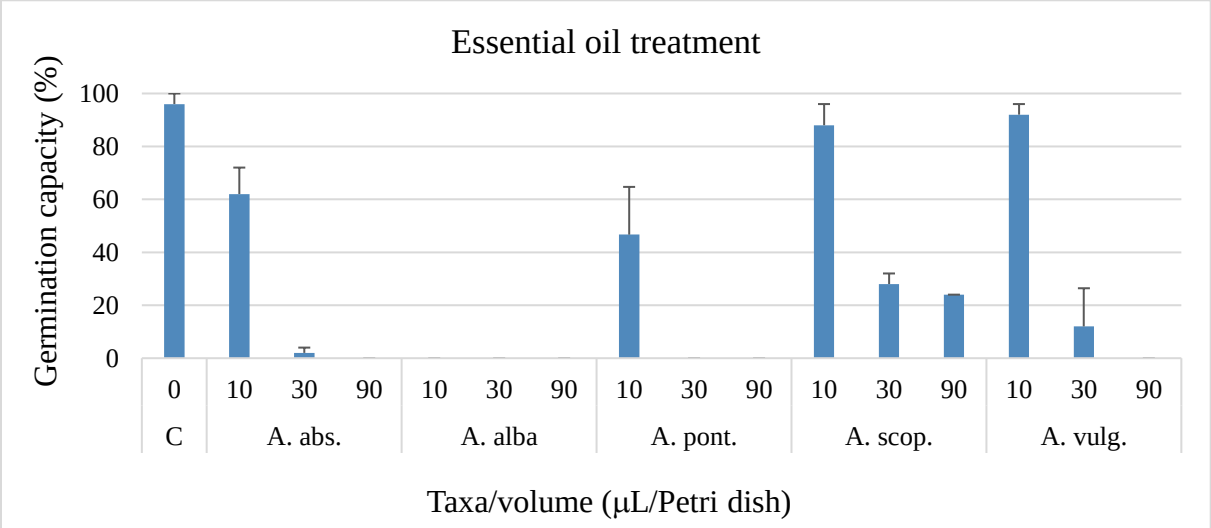
**Figure 14:** Germination energy of *Sinapis alba* treated with *Artemisia* essential oils (C = untreated control) *(Source: A. Dorottya Frank, 2024)*




Considering all volumes, the essential oil of *A. alba* showed the strongest inhibitory effect on germination energy, followed by: *A. pontica* > *A. absinthium* > *A. vulgaris* > *A. scoparia*.

Examination of the **germination capacity** of white mustard seeds treated with *Artemisia* essential oils showed that both the taxa ($p \leq 0{,}001$) and volume ($p \leq 0{,}001$) had a significant effect. In addition, interaction between the taxa and volume was observed ($p \leq 0{,}001$).

**Figure 15:** Germination capacity of *Sinapis alba* treated with *Artemisia* essential oils (C = untreated control) *(Source: A. Dorottya Frank, 2024)*




Compared to the control (C) all essential oils evidently reduced the germination capacity with the exception of the 10 µL volume of *A. vulgaris* and *A. scoparia*, which caused a slight

reduction (4 and 8% difference compared to the control, Figure 15). It can be stated that the increase in the volume of injected essential oil gradually and significantly reduced the germination capacity of white mustard. The volume considerably affected the germination capacity: at 90 µL there were no germinating seeds except in the case of *A. scoparia* (90 µL still showed the greatest reducing effect). Accordingly, 90 µL had the strongest inhibitory effect in all cases.

Although only a slight reducing effect had been observed of the 10 µL essential oil of *A. vulgaris* and *A. scoparia* on the germination capacity, which is based on the number of germinated seeds, Figure 16 demonstrates that while 10 µL of *A. vulgaris* reduced the size of some seedlings, 10 µL of *A. scoparia* caused significant chlorosis and damage as well.

**Figure 16:** Allelopathic effect of *A. scoparia* and *A. vulgaris* essential oils on germination of *Sinapis alba* (C = control, "X" = no germination) *(Source: A. Dorottya Frank, 2024)*

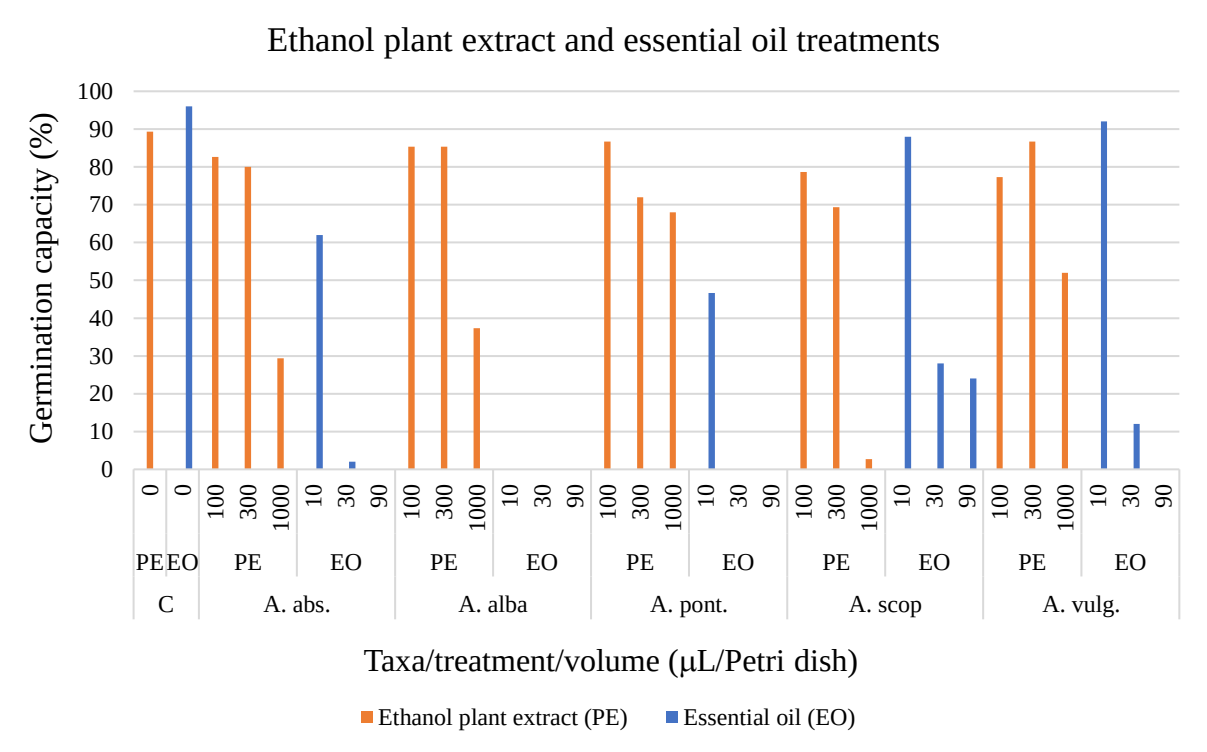

Essential oil treatment of the other examined *Artemisia* species resulted in similar degradations on germinated seeds: 10 µL of *A. pontica* resulted in small-sized seedlings, while *A. absinthium* caused great reduction in size as well as chlorotic leaves.

Considering all volumes, the essential oil of *A. alba* showed the strongest inhibitory effect on germination capacity, followed by: *A. pontica* > *A. absinthium* > *A. vulgaris* > *A. scoparia.*

*4.1.3. Comparison of ethanol plant extract and essential oil treatment*

In comparison, the examined *Artemisia* essential oils (10, 30, 90 µL) were found to be more effective in inhibiting the germination of *Sinapis alba* seeds than the ethanol plant extracts (100, 300, 1000 µL) of the same species (Figure 17). Only those species were compared where both plant extract and essential oil samples were available.

**Figure 17:** Comparison of ethanol leaf extracts and essential oils of *Artemisia* species regarding their allelopathic effect on the germination capacity of *Sinapis alba* seeds (C = untreated control) *(Source: A. Dorottya Frank, 2024)*




*Artemisia alba* essential oil had the most significant reducing effect on germination capacity, showing total inhibition at all volumes. The essential oil of *A. pontica* appeared to have similar results, since even 10 µL volume caused stronger inhibition than any volume of the ethanol *A. pontica* extracts. 1000 µL of *A. absinthium* ethanol plant extract induced lower germination capacity than 10 µL essential oil of the plant, but it was still less effective than the 30 and 90 µL volumes of the essential oil treatment. *A. scoparia* was the only examined species whose 1000 µL ethanol plant extract treatment resulted in greater inhibition of germination than any

essential oil volumes of the same plant species. *A. vulgaris* was an exception in both of the experiments. During the plant extract treatment its 300 µL volume showed less effectiveness in reducing the number of germinating seeds than the 100 µL, and although its essential oil in 10 µL had a very slight effect, *A. vulgaris* was a great example of the significance of increasing the volume. Considering all taxa, the essential oil treatment (10, 30, 90 µL) showed stronger inhibitory effect on the germination capacity of *Sinapis alba* than the ethanol plant extract treatment (100, 300, 1000 µL).

**Figure 18:** Combined results of *Artemisia* ethanol leaf extract and essential oil treatments on the germination capacity of *Sinapis alba* seeds *(Source: A. Dorottya Frank, 2024)*

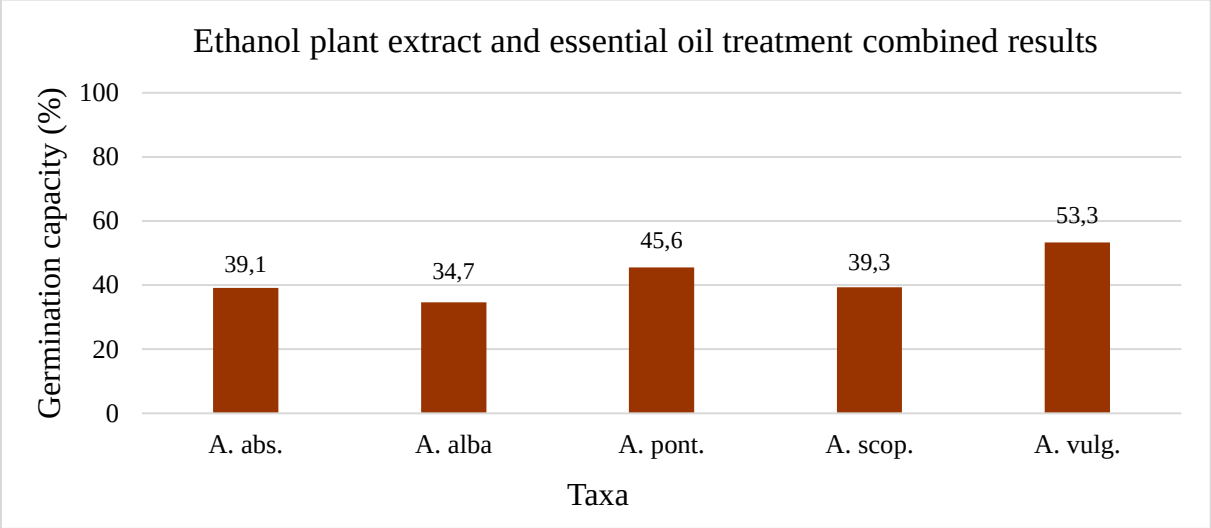



Figure 18 describes the allelopathic potential of the compared species, including all volumes of both ethanol plant extract and essential oil treatments. Including both experiments, *Artemisia alba* showed the strongest inhibitory potential, followed by: *A. absinthium* > *A. scoparia* > *A. pontica* > *A. vulgaris.*

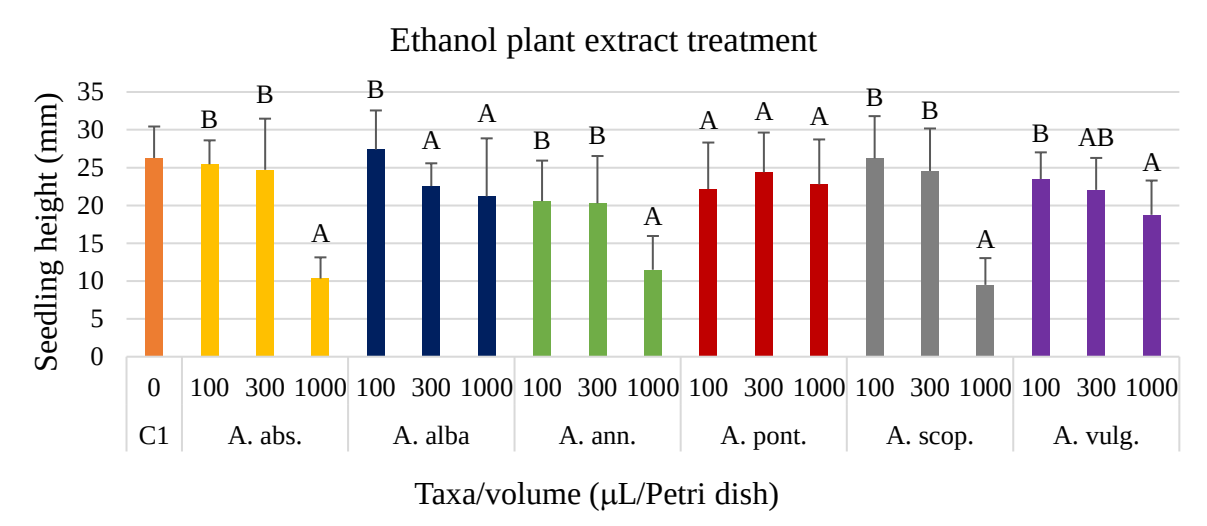
### *4.2. Seedling height and root length*

Seedling height and root length were measured on the 7$^{th}$ day of the experiments. It is important to note that seedling height and root length could have been measured of germinated seeds only, consequently the evaluation of this chapter's figures (Figure 19, 20, 21, 22) is recommended alongside the results of the ethanol plant extract and essential oil treatments on germination energy and germination capacity.

*4.2.1. Ethanol plant extract treatment*

During the evaluation of the **seedling height** of germinated white mustard *(Sinapis alba)* seeds treated with *Artemisia* ethanol plant extracts it was determined that both the taxa ($p \leq 0{,}001$) and volume ($p \leq 0{,}001$) had a significant effect. In addition, interaction was observed between the two variables ($p \leq 0{,}001$). In comparison to the untreated control (C1) all ethanol plant extracts reduced the seedling height, except the 100 µL of *A. alba* and *A. scoparia* (Figure 19).

**Figure 19:** Seedling height of germinated *Sinapis alba* seeds treated with *Artemisia* ethanol leaf extracts (different letters above the columns mean statistically different groups based on fixed species) *(Source: A. Dorottya Frank, 2024)*

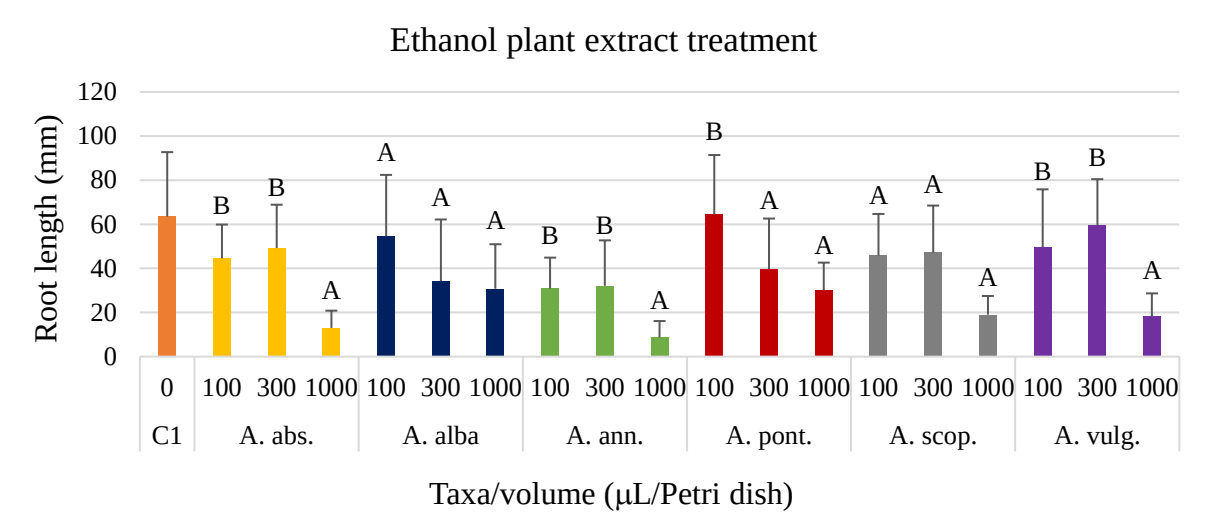



The results of Post Hoc test showed if there was significant difference between the effect of volumes within each taxon (A, B and AB groups, Figure 19). Accordingly, *A. vulgaris* was described by three groups (A, AB, B) demonstrating gradual reducing effect, while in the case of *A. absinthium*, *A. alba*, *A. annua* and *A. scoparia* two groups (A, B) were present that shows significant difference induced by increasing the volume. *A. pontica* was an exception with only one group (A), therefore the volume did not affected significantly the seedling height in this case (at least up to 1000 µL).

In conclusion, the volume of ethanol extracts was a significant factor in reducing the seedling height of the germinating seeds of white mustard. Considering all volumes, the ethanol extract of *A. annua* showed the strongest reducing effect on seedling height, followed by: *A. scoparia* > *A. absinthium* > *A. vulgaris* > *A. pontica* > *A. alba.*

Examination of the **root length** of germinated white mustard *(Sinapis alba)* seeds treated with *Artemisia* ethanol plant extracts showed that both the taxa ($p \leq 0{,}001$) and volume ($p \leq 0{,}001$) had a significant effect, and there was interaction between the two variables ($p \leq 0{,}002$). Compared to the untreated control (C1) all ethanol plant extracts showed reducing effect on the root length, except the 100 µL of *A pontica.* The results of Post Hoc test revelaed if there was significant difference between the effect of volumes within each taxon (A, B and AB groups, Figure 20).

**Figure 20:** Root length of germinated *Sinapis alba* seeds treated with *Artemisia* ethanol leaf extracts (different letters above the columns mean statistically different groups based on fixed species) *(Source: A. Dorottya Frank, 2024)*




According to the test results, *A. absinthium, A. annua, A. pontica* and *A. vulgaris* were described by two groups (A, B) that shows significant difference induced by increasing the volume. *A. alba* and *A. scoparia* were exceptions with only one group (A) present, consequently, the volume did not affected significantly the root length in these cases (at least up to 1000 µL), still differences between volumes can be observed.

In conclusion, the volume of ethanol extracts was a significant factor in reducing the root length of the germinating seeds of white mustard. Considering all volumes, the ethanol plant extract of *A. annua* showed the strongest reducing effect on root length, followed by: *A. absinthium* > *A. scoparia* > *A. alba* > *A. vulgaris* > *A. pontica.*

*4. 2. 2. Essential oil treatment*

Evaluation of the **seedling height** of germinated white mustard *(Sinapis alba)* seeds treated with *Artemisia* essential oils indicated that only the volume ($p \leq 0{,}001$) had a significant effect, however, it showed some interaction between the taxa and volume ($p \leq 0{,}032$). Since the essential oil treatment was greatly effective in reducing germination, there were no seedlings to measure in the case of *A. alba* (at all), the 30 and 90 µL of *A. absinthium* and *A. pontica*, and the 90 µL of *A. vulgaris* essential oils. Therefore only *A. scoparia* could have been analysed by Post Hoc test to see if there was significant difference between the effect of volumes within one taxon (A, B and AB groups, Figure 21).

**Figure 21:** Seedling height of germinated *Sinapis alba* seeds treated with *Artemisia* essential oils (different letters above the columns mean statistically different groups based on fixed species) *(Source: A. Dorottya Frank, 2024)*

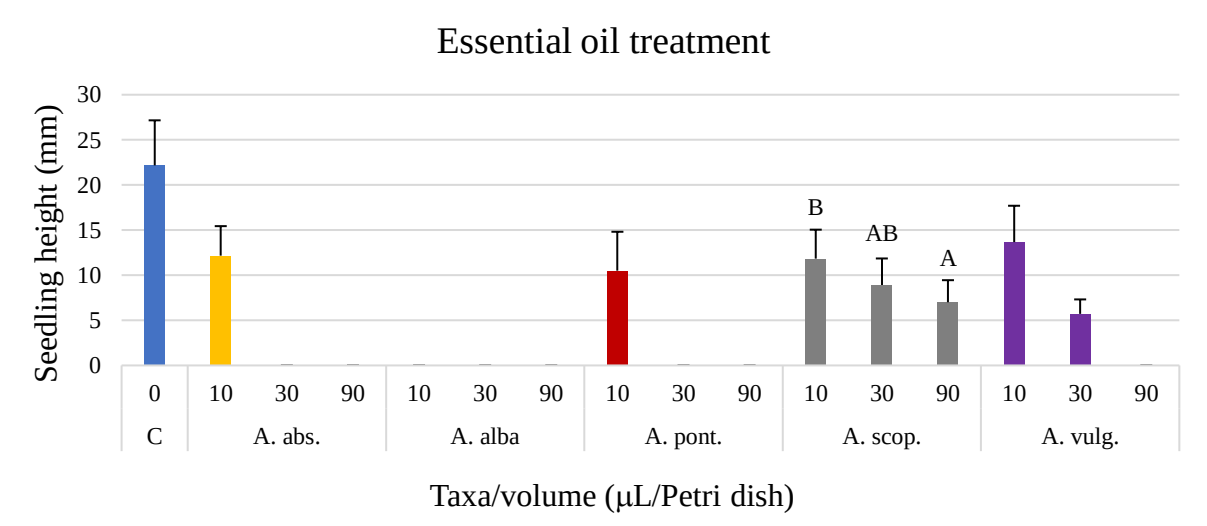
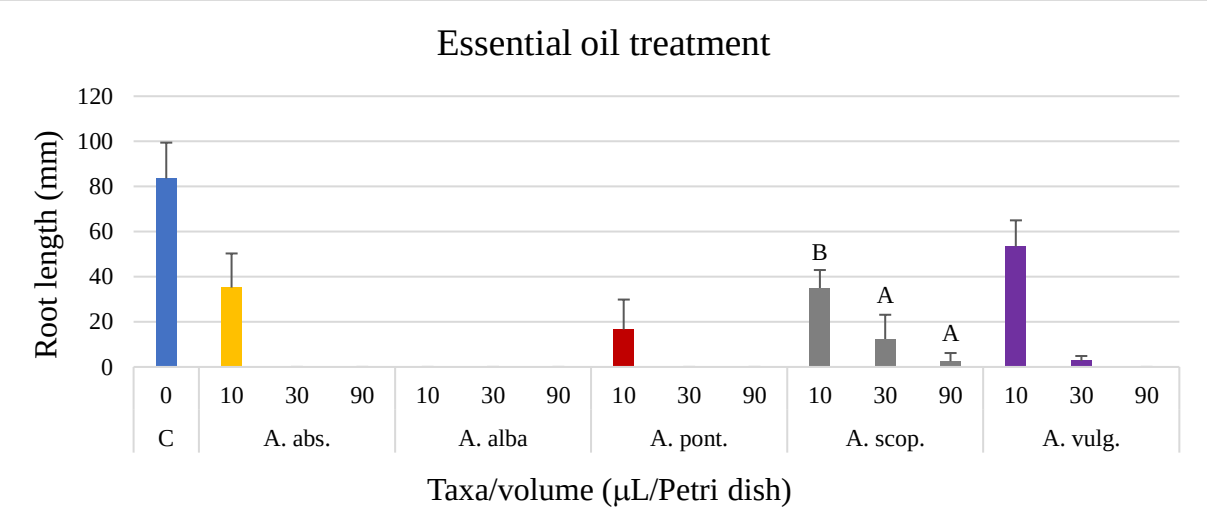



Figure 21 shows that in the case of *A. scoparia* and *A. vulgaris* gradual reduction can be observed, while *A. absinthium* and *A. pontica* caused significant inhibition, and the treatment with *A. alba* essential oil resulted in complete inhibition of germination, even in 10 µL volume.

In conclusion, the volume of essential oils was a significant factor in reducing the seedling height of the germinating seeds of white mustard, meanwhile the taxon as a factor resulted in notable differences in the inhibitory effect as well. Considering all volumes, the essential oil of *A. alba* showed the strongest reducing effect on seedling height, followed by: *A. pontica* > *A. absinthium* > *A. vulgaris* > *A. scoparia.*

During the analysis of the **root length** of germinated white mustard *(Sinapis alba)* seeds treated with *Artemisia* essential oils it was determined that both the taxa ($p \leq 0{,}001$) and volume ($p \leq 0{,}001$) had a significant effect, additionally, interaction between the two variables ($p \leq 0{,}001$) was observed. The essential oil treatment was greatly effective in reducing germination. Consequently, there were no seedlings to measure in the case of *A. alba* (at all), the 30 and 90 µL of *A. absinthium* and *A. pontica,* and the 90 µL of *A. vulgaris*. Therefore only *A. scoparia* could have been analysed by Post Hoc test if there was significant difference between the effect of volumes within one taxon (A, B and AB groups, Figure 22).

**Figure 22:** Root length of germinated *Sinapis alba* seeds treated with *Artemisia* essential oils (different letters above the columns mean statistically different groups based on fixed species) *(Source: A. Dorottya Frank, 2024)*




According to the results presented on Figure 22, all essential oils significantly reduced the root length compared to the control (C). It can be concluded that the volume of essential oils was a significant factor in reducing the root length of the germinating seeds of white mustard, meanwhile the taxon as a factor resulted in notable differences in the inhibitory effect as well. Considering all volumes, the essential oil of *A. alba* showed the strongest reducing effect on root length, followed by: *A. pontica* > *A. absinthium* > *A. scoparia* > *A. vulgaris.*

## 5. Conclusions and suggestions

During the study, inhibitory effect of *Artemisia absinthium, A. alba, A. annua, A. pontica, A. scoparia* and *A. vulgaris* was observed on seed germination and seedling growth of white mustard *(Sinapis alba),* as well as different extents of allelopathic potential of ethanol leaf extracts and essential oils of the examined species. Volume was a significant reducing factor in both of the experiments.

This study is in correlation with many other papers that investigated the allelopathic effect of some of these *Artemisia* species. Kapoor et al. (2019) investigated the effect of aqueous leaf extracts of *A. absinthium* on germination and seedling growth of *Parthenium hysterophorus,* and it resulted in significant reduction and chlorosis (Kapoor et al., 2019). Chirca and Fabian (1973) studied some allelopathic effects caused by *A. absinthium* on germination and seedling growth of *Linum usitatissimum, Lepidium draba, Sinapis alba* and wheat. They revealed that the root extract inhibited germination in wheat, and reduced shoot and root elongation in the other three species by 53-85%, while aqueous extract of the leaves prevented germination of all four species (Chirca, Fabian, 1973). Artemisinin is probably the main compound in *A. annua,* which has been examined for its allelopathic effect in several studies (Chen, Leather, 1990; Maurya et al., 2022). The study of Duke et al. (1987) revealed that artemisinin is a selective phytotoxin. Their results show that artemisinin inhibited the germination and growth of shoots and roots of *Lactuca sativa,* and shoot and root growth of *Amaranthus retroflexus, Ipomoea lacunose* and *Portulaca oleracea* (Duke et al., 1987). Lydon et al. (1997) examined the effects of leaf tissue and leaf-tissue extracts of *A. annua* and pure artemisinin on plant growth *(Amaranthus retroflexus, Chenopodium album, Glycine max, Zea mays),* which resulted in species-specific inhibition of growth (Lydon et al., 1997). Kaur and Batish (2010) investigated the effect of volatile oil of *A. scoparia* leaves against *Zea mays, Triticum aestivum, Chenopodium murale, Bidens pilosa* and *Amaranthus viridis.* Their results showed that seed germination of all the test plants was inhibited at each concentrations (0.5, 1.0, 2.5, 5.0 μL/Petri dish), and the oil treatment also severely affected the chlorophyll content (Kaur, Batish, 2010). According to Kaur et al. (2010), the volatile oil hydrodistilled from *A. scoparia* significantly reduced the emergence and seedling growth (in terms of shoot and root length) of *Achyranthes aspera, Parthenium hysterophorus, Cassia occidentalis, Ageratum conyzoides* and *Echinochloa crus-galli* (Kaur et al., 2010). Phenolics released by *A. vulgaris* were determined by Inderjit and Foy (1999) to play an important role in the growth inhibition of *Trifolium*

*pratense* (Inderjit, Foy, 1999). Mei et al. (2010) investigated the allelopathic effect of aqueous extract of *A. vulgaris* on seed germination and growth of *Sorghum vulgare, Triticum aestivum, Raphanus sativus, Brassica campestris* and *Cucumis sativus,* revealing that it strongly inhibited the germination, speed of germination and seedling growth of all tested crops (Mei et al., 2010).

As the genus *Artemisia* has the potential to be used as a bioherbicide, further research is recommended. Future studies could benefit from a larger and more diverse sample size, involving other test species (especially weeds) and testing different type of extracts with differing volumes. Another area for future research is the determination of the active compounds of the plant species used in the study, as well as the isolation of active compounds from the most effective extract and discovering its potential.

## 6. Summary

The current study investigated and analysed the allelopathic effect of plant extracts and essential oils of six species of *Artemisia* genus (*A. absinthium* L., *A. alba* Turra, *A. annua* L., *A. pontica* L., *A. scoparia* Waldst. & Kit., and *A. vulgaris* L.) on seed germination and seedling growth of white mustard *(Sinapis alba).* Seeds were treated with a 0,01% solution of ethanol leaf extract in 100 μL, 300 μL or 1000 μL, while the other experiment involved 10 μL, 30 μL, and 90 μL essential oil treatment. In both of the cases, seeds were germinated in Petri dishes with a Sanyo MLR-351H incubator (program: 8 hours light (~12 klux) at 30 °C; 16 hours dark at 20 °C). The whole incubation period was 7 days, seedlings were quantified on the 3$^{rd}$ day (germination energy), and quantified and measured on the 7$^{th}$ day (germination capacity, seedling height, root length).

According to the results, it can be determined that all *Artemisia* species had allelopathic effect on seed germination and seedling growth, in which volume played a significant role. During the experiment with ethanol plant extracts, *A. annua* caused the strongest inhibitory effect, although the 1000 μL of *A. scoparia* showed a greater reducing effect on germination capacity. In the essential oil treatment, *A. alba* showed the strongest effect, completely inhibiting germination in every volume.

This study shows that the genus *Artemisia* has the potential to be used as a bioherbicide, thus, further research is recommended. Future studies could benefit from a larger and more diverse sample size, involving other test species (especially weeds) and testing different type of extracts with differing volumes. Another area for future research is the determination of the active compounds of the plant species used in the study, as well as the isolation of active compounds from the most effective extract and discovering its potential.

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## List of figures

**DECLARATION**

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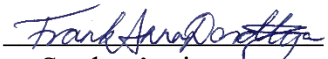

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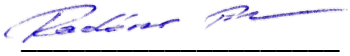

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