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Phytotoxicity of essential oil extracted from leaves of Artemisia herba-alba

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Dedication

It is with great respect that I would like to dedicate this modest work,

To our dear mothers;

For their love, support, and being a source of light in hard times. Thank you for everything, for what you have done and given for me.

to your immense contribution in shaping our future.

To our sisters,

I wish you a life full of happiness, prosperity and success.

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Introduction

Introduction

Plants are an immense source of complex chemical molecules exploited by man in perfume, agro-food, cosmetics and pharmaceutical. Most plants contain essential oils; they are then called “aromatic plants”. These essential oils are found in many parts of the plant: wood, leaves, fruits, bark, seeds and roots. These are complex mixtures made up of several tens or even more than a hundred of compounds, mainly terpenes and aromatic compounds. (Metari,2015)

Essential oils have, at all times, occupied an important place in daily life of man who used them to perfume themselves, to flavor food or even heal. The knowledge of essential oils goes back a long time when prehistoric man already practiced, in his own way, the extraction of odorous principles from plants. He immersed, in the same container filled with water, fragrant plants and stones burning (Robert, 2000).

Essential oils are therefore an interesting source of new compounds in the search for bioactive molecules.

Algeria is one of the countries known for their taxonomic diversity given its privileged biogeographical position and its extent between the Mediterranean and sub-Saharan Dahmani (Pereira *et al* ., 2003).

Artemisia herba-alba Asso. (syn: A. inculta Del.), known as the desert wormwood (Sheeh in arabic), is a medicinal and aromatic dwarf shrub belonging to the genus Artemisia, Asteraceae family (Tilaoui *et al*., 2011), that grows wild in the arid areas, steppes and Sahara (-Hamzaoui and Baaliouamer, 2010).

The essential oil of *A. herba-alba* (known as armoise oil) is historically known in traditional and herbal medicine. Numerous scientists have showed its various biological and pharmacological effects, especially the antimicrobial ones (bacteria and fungi) (Hudaib and Aburjai, 2006).

Essential oils are generally known as non-phytotoxic compounds with potential activity against microorganisms except when they are inappropriately used. In that case, they can have harmful effects on the human health (Mizanur-Rahman *et al*., 2013).

The aim of the present study is to investigate the phytotoxic activity of *Artemisia herba-alba* essential oil. To achieve our aim, we followed three steps: first, plants were collected; second, essential oils and germination bioassays were extracted for test the phytotoxicity of *A. herba-alba* essential oils.

The study was divided into two parts: a bibliographical part and a practical part, in which the methodology of the study and the results of the discussion were successively studied.

Chapter I:

Bibliographic review

1. Essential oils

1.1.History

The name essential oil is believed to had been coined in the 16th century by the Swiss medical reformer Paracelsus von Hohenheim, **(Guenther, 1948).** Today, about three thousand essential oils are known, about 300 of which are used commercially **(Braak and Leijten, 1999).** At the same time, these plants are being used in the healing practices of different civilizations according to the different evolutionary stages associated with their use **(Franchomme and Pénoël, 1990) .**

1.2. Definition

The definition given by AFNOR in 2000 is that "essential oils are products obtained from the raw material of plant origin, either by steam distillation, by mechanical processes from 'epicarp of Citrus, or by dry distillation. Essential oils are substances of plant origin with a complex composition characterized by its volatility, oil and fragrance, present in aromatic plants in several places (in flowers, leaves, fruits, seeds, bark and roots) **(Fernandez and Chemat, 2012).**

1.3. Location of essential oils

The synthesis and accumulation of essential oils are generally associated with specialized histological structures, most often on or near the surface of plants **(Bruton, 1987).** It can be stored in all the organs of the plant such as flowers (bergamot, tuberose), but also in the leaves (eucalyptus, lemongrass) and less typically in bark (cinnamon), wood (rosewood, sandalwood), rootq (vetiver), rhizomes (turmeric, ginger), fruits (fennel), seeds (nutmeg) **(Bruneton, 1993).**

Oils may exist in different organs at the same time. The chemical composition may vary from one organ to another. These aromatic essences are produced by secretory glands found in almost all parts of the plant **(Dugo and Giacomo, 2002).**

1.4. Physiological role of the essential oil in the plant

Many plants produce essential oils in the form of secondary metabolites. Their exact role in the vital processes of plants is still poorly understood. EOs can have several "useful" effects on plants such as attracting insects to facilitate pollination, acting as an energy source, and promoting certain chemical reactions that protect desert plants from humidity during their operation and they may also act as insect repellents **(Benayache, 2013).**

For some authors, essential oils constitute the "waste" of plant cell metabolism (Salle, 1991). For others, they will serve to attract insects to fertilize them or keep them away. The attraction of insects to pollinate flowering plants is also attributed to the essential oils these plants contain. Ultimately, essential oils will constitute a means of defense for plants against predators such as microorganisms (bacteria and fungi) and herbivores (Bruneton, 1999). In nature, essential oils play an important role in plant protection as antibacterial, antiviral, antifungal and insecticidal substances, and can also combat herbivores by reducing their appetite for plants.

1.5. Chemical composition of essential oils

The chemical composition of an EO is very complex **(Bruneton, 1995; Arnaud, 1985).** Indeed, the number of isolated compounds is about a thousand with many left to discover **(Belaiche, 1979).** EOs are mainly made up of two groups of distinct odorous compounds, these are terpenes (mono and sesquiterpenes), predominant in most types of gasoline and aromatic compounds derived from phenylpropane.

1.5.1. Terpenes

Are natural hydrocarbons, of cyclic structure or open chain. (Figure 01)Their most important structural particularity is the presence of isoprene units with 5 carbon atoms ($C5H8$) in their skeleton. They are subdivided according to the number of isoprene entities into monoterpene ($(C5H8)2$), sesquiterpenes ($(C5H8)3$), diterpenes ($(C5H8)4$), tetra terpenes ($(C5H8)8$), polyterpenes ($(C5H8)$ n) where n can be 9 to 30**(Bessedik, and Bahri, 2018).**

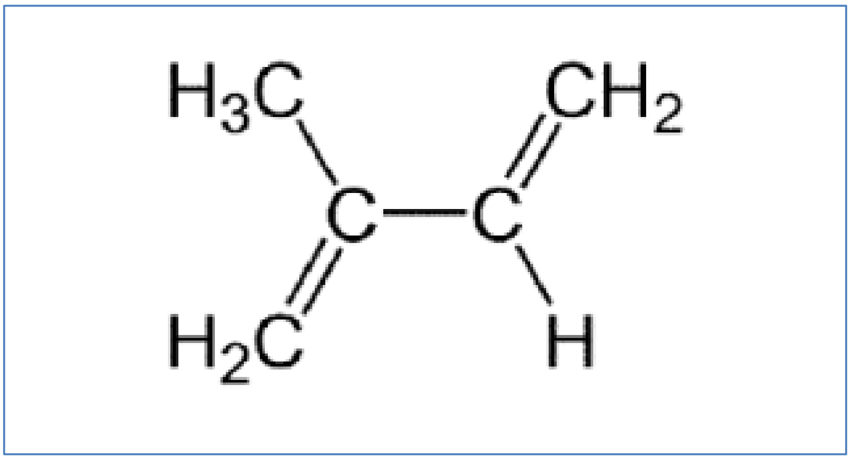


Figure 1: Structure of isoprene (C5H8) **(Bakkali *et al*., 2008)**.

1.5.2. Aromatic compound

Aromatic compounds are another class of volatile compounds frequently encountered in aromatic compounds derived from phenylpropane. (Figure 02) This class includes odorous compounds whose biogenesis is different from that of terpenes (Bruneton, 1999). Among these various aromatic compounds, mention may be made of aldehydes (anisic, cuminic, cinnamic), phenols and ethers (thymol, eugenol, anethole) and coumarins (bergapten, umbelliferone). Acyclic compounds such as low molecular weight organic acids (acetic, formic, valeric) can also be encountered.

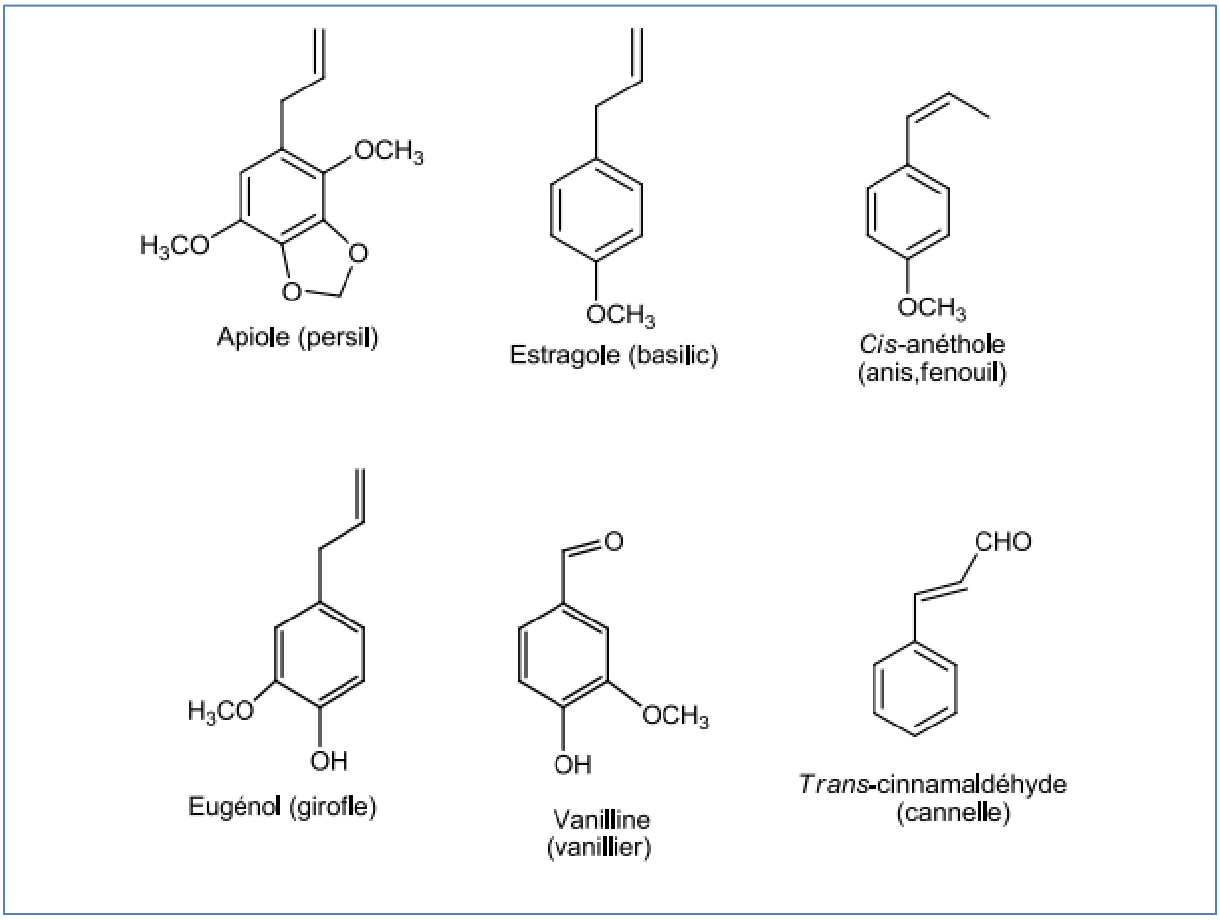


Figure 2: Examples of structures of compounds derived from phenylpropane **(Guignard, 2009).**

1.5.3. Aliphatic compounds

Some oils contain small amounts of aliphatic compounds, generally of low molecular mass, which can be carried away with the essential oil during hydrodistillation. They are linear or branched hydrocarbons or their oxygenated derivatives (acids, alcohols or aldehydes). Examples include (Z)-hex-3-enol (green note of cut grass) or often-3-ol (characteristic note of button mushroom: Agaricus sports), and Olea europaea, Cannabis sativa. **(Bruneton, 1999).**

1.6. Biological activities

Essential oils are known to have antiseptic and antimicrobial properties. Many of them have antitoxic, anti-venomous, antiviral, antioxidant, and antiparasitic properties and they are also known to have anticarcinogenic properties **(Baser and Buchbauer, 2015).**

1.6.1. Antibacterial activity

The antibacterial activity of essential oils is mainly related to their chemical composition, in particular their major volatile compounds. So far, no study can give a clear and precise idea of the mode of action of essential oils. Given the complexity of their chemical composition, everything suggests that this mode of action is quite complex and difficult to identify from a molecular perspective. It is likely that each of the constituents of EOs has its own unique mechanism of action **(Guinoiseau, 2010).**

The characteristics of essential oils are attributed to the terpenoid and phenylpropanoid derivatives of which they are made. The activity of these bioactive molecules depends both on the lipophilic character of their hydrocarbon skeleton and on the hydrophilic characteristic of their functional groups. Oxygenated molecules are generally more active than hydrocarbon molecules **(Wendakoon, and Sakaguchi, 1995).**

Terpenes as well as flavonoids can penetrate the double phospholipid layer of the membrane of the bacterial cell and induce its rupture. The cytoplasmic content is discharged outside the cell involving its destruction **(Tsuchiya, 1996).** Additionally, chemo-osmotic disruption and intracytoplasmic potassium leakage may occur, followed by the release of nucleic acids, ATP, and inorganic phosphate **(Daroui, 2011).**

1.6.2. Antifungal activity

In the phytosanitary and Agro-food field, essential oils or their active compounds could also be used as protective agents against phytopathogenic fungi and microorganisms invading foodstuffs **(Balchin, 2003).**

The fungicidal and fungistatic effect of essential oils against pathogenic fungi has been the subject of several studies **(Karaman, 2001; Duarte, 2005).** As for the antibacterial activity, the antifungal power is attributed to the presence of certain chemical functions in the composition of essential oils. The antifungal action of these compounds is due to an increase in the permeability of the plasma membrane followed by a rupture of the latter, leading to leakage of the cytoplasmic content and therefore the death of the yeast **(Cox, 2000).**

Indeed, the terpenic compounds of essential oils, and more specifically their functional groups such as phenols and aldehydes react with membrane enzymes, degrade the plasma membrane of yeasts **(Knobloch, 1989).**

1.6.3. Pesticide activity

Despite the many synthetic molecules likely to be used, plant protection can also be done with certain natural essences. Pesticides are very diverse and are classified according to their activity as insecticides, molluscicides, nematicides, and germicides. Some oils are recognized for their effectiveness on phytopathogenic fungi such as the case of Citrus essences on the mycelial growth of Phaeoramularia angolensis **(Cheng *et al*., 2004).**

1.6.4. Antiseptic activity and respiratory diseases.

Some essential oils have a marked antiseptic power. The latter is exerted on various bacterial strains, including those usually resistant to antibiotics (tetracycline, oxolinic acid) **(Burt, 2004).** Indeed, they are "eubiotic" **(Ngassapa *et al*., 2003),** i.e. they destroy parasites without interference with the host organism unlike antibiotics which very often interact with parasites by denaturing them with side effects on the treated subjects. They generally act in low doses. Savoury, cinnamon, thyme, clove, lavender, and eucalyptus essences are the most antiseptic. Some of their compounds such as citral, geraniol, linalool and thymol are on average 7 to 10 times more antiseptic than phenol **(Bruneton, 1999; Ngassapa *et al*., 2003).** Some essential oils are used in the treatment of certain diseases of the respiratory tract: cough, bronchitis, and angina **(Milijaona *et al*., 2003).**

1.6.5. Antioxidant activities

Some constituents of essential oils have a very marked antioxidant power and are now marketed: this is the case of eugenol, thymol, carvacrol, etc. The literature on the subject show that essential oils are a good source of natural antioxidants sought after for their relative harmlessness **(Burits and Bucar, 2000; Candan *et al*., 2003; Tepe *et al*., 2005).**

1.7. Techniques for extracting essential oils

There are several different methods for extracting plant essences; this diversity is due to the variety of materials and the considerable sensitivity of their constituents. The most suitable possible method is made according to the nature of the plant material studied, the Physico-chemical characteristics of the essence to be extracted and the use of the extract. The worldwide market for essential oil grows rapidly and nowadays a lot of scientific research is presently focused on the industrial development together with environmental preservation of essential oils through using different techniques as Hydrodistillation (HD), Supercritical Fluid Extraction (SFE), Microwave-Assisted Hydrodistillation (MAHD) and Ultrasound-assisted extraction (UAE).

2. Saharan plant species

2.1. Sahara desert definition

The Sahara is the largest of deserts (07 million km2) and it is also most expressive and typical by its extreme aridity, where conditions reach their greatest harshness. (Figure 03)

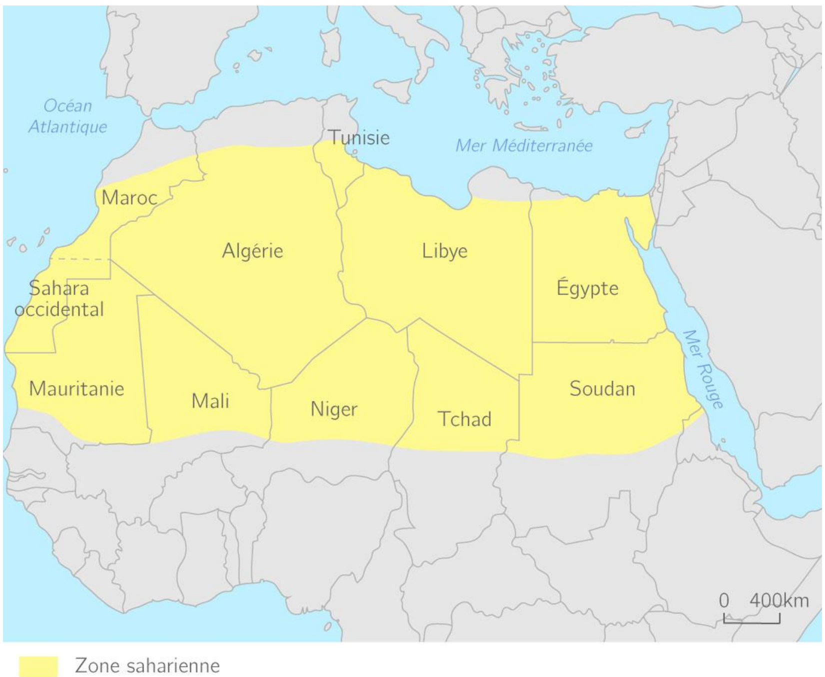


Figure 3: Location of the Great Sahara **(Cohen-Solal J., 2018).**

2.2. Saharan spontaneous plant species

The plants' cover is discontinuous and very irregular, the plants mainly use the sites where the water supply is a little less favorable than elsewhere **(Ozenda, 1991; Le Houerou, 1990).**

Vegetation of the Sahara is very sparse: trees are as rare as they are scattered and grass appears only for a very short period of the year when the conditions become favorable **(Schiffers, 1971).**

2.3. Use of spontaneous Saharan plants

2.3.1. Food plants

The importance of spontaneous plants in human food is negligible. Various Saharan plants provide edible fruits that are quite mediocre (*Zizyphs lotus*, *Ficus salcifolia*, etc.) with edible parts including seeds, leaves, shoots and/or tubers **(Derouiche N and Benhamed K, 2012).**

2.3.2. Medicinal plants

According to the World Health Organization (WHO) statistics, about 80% of African populations use traditional medicine for their primary health care. Ethnomedicines have been practiced in Algeria at a large scale, **(Fatiha, H.-B *et al*, 2017)** investigating 65 species, covering 36 families from Saharan area that treat several diseases.

2.3.3. Poisonous plants

The toxicity of Saharan plants has been demonstrated by many authors. In the Algerian Sahara, the toxic plant species are not consumed spontaneously by animals **(Djennane, 2016).**

3. Chemicals herbicides

3.1. Definition

Herbicides are products belonging to the family of pesticides, substances developed for the purpose of controlling or destroying organisms deemed undesirable **(Gouvernement du Québec, 2002a**). Whether natural or produced by humans (synthetic molecules), the absorption and metabolization of herbicides by plants cause the death of these organisms **(Agra-Ost, 2006).** According to Agriculture and Agri-Food Canada, weed control is a key step for successful agriculture **(Government of Canada, 2012).**

3.2. Characteristic of herbicides

Pesticides were segregated into five categories according to the risks associated with their use. Herbicides are counted as class three (3) when used for agricultural, commercial or industrial purposes. It is possible to group herbicides into different classes according to their selectivity, chemical family, mode of penetration and absorption by plants as well as their period of application (preventive and curative use) **(Agra -Ost, 2006).**

Table 1: Characteristics of the classification of herbicides by selectivity

Nature of the category	Sub-category	Characteristics	Source
herbicide selectivity	Absolute	"absolute" herbicides act on all plant species present at the place of application. The mode of action of the herbicide is therefore not distinctive between nuisance (weed) and the species to be protected (crop).	**(Beckert, 2011).**
	selective	selective herbicides only act on plants presenting the specific properties and necessary for the mode of action of the herbicide. By example, some herbicides will only act on roots present on the surface, thus protecting the cultures with deeper roots; it is about specificity.	**(Agra - Ost, 2006).**
	of use	"of use" herbicides are only used for specific applications. For example, some herbicides will be used as a growth inhibitor. In this case, the weeds are not destroyed, but their development is restricted, thus reducing the impact on crops.	**(Beckert, 2011).**

3.3. Mode of action

there are several types herbicides, It was possible to group them into three (3) families according to their selectivity.

The differences between herbicides are not limited to their formulation, but also in terms of their mode of action. In other words, once the active molecule is released in the plant, its mechanisms of action will differ from one herbicide to another. In order to describe the different mechanisms of action, it is necessary to carry out another classification for the herbicides. The new classification comes from the Herbicide Resistance Action Committee (HRAC), an international organization whose objective is coordinating actions in the field of herbicide resistance management **(Beckert, 2011).**

Class A herbicide

The synthesis of fatty acids, which ensures the integrity of plant cell membranes, is a two-step process. First of all, the CO2 captured by the plants is fixed on abiotin molecule under the action of an enzyme: biotin carboxylase. The CO2 is then transferred to acetylcoenzyme a carboxylase that is the catalyst necessary for the synthesis of fatty acids. Transfer of CO2 from biotin to acetylcoenzyme,A carboxylase is achieved under the action of the enzyme carboxyl transferase. Class A herbicides act on the site active in carboxyl transferase, thus preventing the transfer of CO2 to acetylcoenzyme A carboxylase. As acetylcoenzyme A carboxylase is no longer supplied with CO2, fatty acids can no longer be synthesized, resulting in the death of the plant. Class herbicides A are therefore acetylcoenzyme A carboxylase inhibitors **(Beckert, 2011).**

Class B herbicide

In plants, the biosynthesis of amino acids (leucine, isoleucine and valine) is catalyzed by acetolactate synthase. Like class A herbicides, class B herbicides will also intervene at the level of the enzyme in order to inhibit its effectiveness. However, the class B herbicides will not act directly on the active site of the enzyme, but on a separate site. Although Class B herbicides do not act directly on the active site, the interaction of herbicides with the enzyme will result in either the modification of the structure of the enzyme, thus modifying the nature of the active site; or providing spatial clutter, making access to the active site impossible; this is allosteric inhibition. The herbicides of class B are therefore acetolactate synthase inhibitors **(Beckert, 2011).**

Class C herbicide

Plant life relies on photosynthesis. Plants convert water and CO2 from the air captured in protein and oxygen **(UQAM, 2002).** Photosynthesis is only possible when the plant is subjected to light radiation. It is the energy received from the radiation that is used for the photolysis of water, thus forming dioxygen. During photolysis, electrons are released and there is a transfer of electrons in the plant until the restoration of nicotinamide adenine dinucleotide phosphate (NADP+) **(Beckert, 2011).** Platosquinone plays the role of mobile electron carrier in the plant (UQAM, 2002). Herbicides of class C act as an inhibitor at the level of electronic transfer so that the NADP+ is no longer reduced. However, it is not the lack of reduction in NADP+ that causes cell death because NADP+ no longer captures electrons, these are captured by the dioxygen molecules formed during the photolysis of water **(Beckert, 2011).**

The excited dioxygen molecules then become very reactive and carry out the peroxidation of lipids and leads to the destruction of the photosynthetic pigments of the plant.

However, lipids or fatty acids ensure the integrity of plant cell membranes (ibid.), Lipid peroxidation break down cell membranes causing the death of the plant.

Class G herbicide

Chorismate, which leads to the formation of ubiquinone, folic acid and acids aromatic plant amino acids (phenylalanine, tyrosine and tryptophan), is produced by shikimic acid. The enzyme enoyl-pyruvyl-shikimate-3-phosphate (EPSP) synthase catalyzes the formation of chorismate in plants. Glyphosate, which is the only herbicide in the class G, acts as an inhibitor in competition with EPSP synthase by binding to the active site of the phosphoenolpyruvate. Consequently, the presence of glyphosate in the plant leads to stopping the synthesis of EPSP synthase and therefore of shikimic acid (Beckert, 2011). Non- synthesis of shikimic acid is fatal for the plant because it results in a depletion of carbon resources of the plant **(Hopkins, 2003).** In fact, approximately 30% of carbon substances in a plant come from shikimic acid (ibid.). Glyphosate is therefore a enoyl-pyruvyl-shikimate-3-phosphate synthase inhibitor.

Class H herbicide

Glutamine is synthesized by the condensation of ammonium on glutamate. Glutamine is essential to the life of a plant since it allows the degradation of glyoxylate, toxic, produced during photorespiration (Hopkins, 2003). Class H herbicides act by competition with glutamate by binding to the active site of glutamine synthase **(Beckert,2011).**

Under the effect of class H herbicides, glyoxylate is no longer degraded and accumulates in the plant. The latter inhibits ribulose di-phosphate carboxylase which has a direct impact on electronic transfers as elaborated for class C herbicides (ibid.). The plant dies due to lipid peroxidation which destroys the integrity of membranes cellular.

Class O herbicide

Plant growth is regulated by indoleacetic acid **(Hopkins, 2003).** Class O herbicides have the same effects on plants as indolacetic acid. In low concentration, the application of these herbicides to the body is not harmful.

On the other hand, at high concentrations, auxin herbicides (class O) deregulate the growth of plant tissues. It is observed in plants treated with herbicides of class O for curvatures of the growing tissues (symptom of epinasty) as well as a thickening of the rods (phenomenon of fasciation) resulting from cell proliferation in the cambiums of the plant. Curvatures of plant tissues as well as magnification rods compress the vessels which leads to blocking the flow of sap and causing the death of the plant (ibid.).

3.4. Herbicides impacts

3.4.1. On the human health

The herbicide glyphosate, N-(phosphonomethyl) glycine, has been used extensively in the past 40 years. In recent years; concerns have increased worldwide about the potential wide ranging direct and indirect health effects of the large scale use of glyphosate. In 2015, the World Health Organization reclassified glyphosate as probably carcinogenic to humans **(Bruggen *et al*, 2018).**

3.4.2. Environmental impact

The purpose of using herbicides is to improve agricultural yields by destroying undesirable species, weeds. In order to be effective, herbicides must possess toxicity towards the target species while protecting crops and the environment. Nevertheless, the massive use of herbicides has been a source of contamination for the various spheres of the environment (water, air and soil). In the next section, modes of contamination as well as the resulting consequences on the environment and the use of herbicides in agricultural practices are discussed.

Chapter II:
Material and methods

1. Plant material

1.1. Description of plant species

Artemisia herba-alba is a species of steppe plants growing in arid or semi-arid lands of North Africa, the Middle East and Spain **(Mohamed *et al*., 2010).** Several names are attributed to this plant; steppe thyme, desert wormwood, etc. In North Africa and the Middle East, it is commonly called "Shih".

Artemisia herba alba from the French name "Armoise blanche" **(Figure 4)** is a spontaneous, aromatic plant, vivacious and hermaphroditic. It is a Mediterranean species and Saharo-Indian. It is very common in North Africa and the Middle East, and can be found in the Algerian steppe, in the Canary Islands and in South Africa. This species of steppe plants grows in arid or semi-arid lands **(Mohamed and al., 2010).**

Figure 4: Photograph of white sagebrush in the desert. **(Original, 2022)**

Several names are attributed to this plant; Steppe Thyme, Desert Wormwood, and others. In North Africa (Algeria and others) and the Middle East, the plant is commonly called Shiḥ. The English name Tor, attributed to all sagebrush, alludes to its beneficial vermifuge power for humans and livestock **(Messai *et al*., 2011).**

1.2. Chemical composition

The plant has a much lower cellulose rate than its appearance would suggest (17 to 33%). Dry matter (DM) provides between 6 and 11% of raw protein matter, 72% of which is made up of amino acids. The rate of β-carotene varies between 1.3 and 7 mg/kg depending on the season. The molecules identified from different extract of the *Artemisia herba alba* are sesquiterpene lactones, coumarins and acetylenic hydrocarbons. **(Da Silva J A, 2004).**

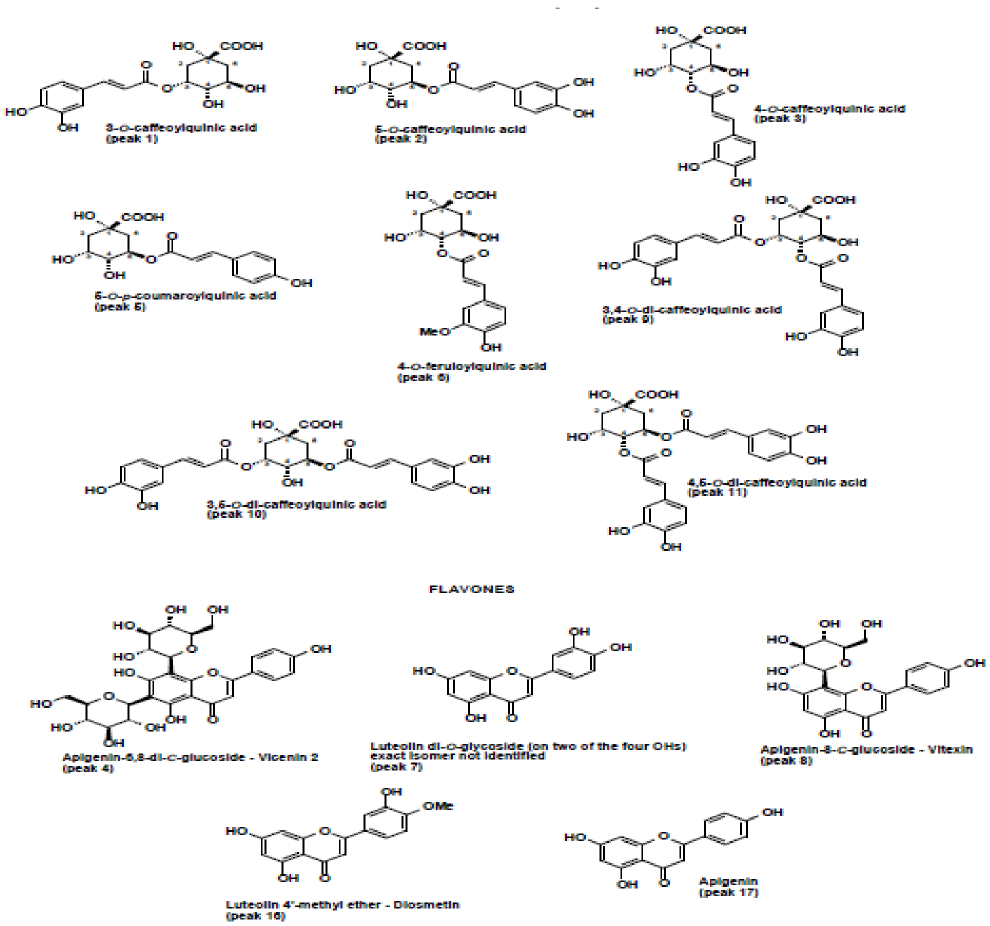
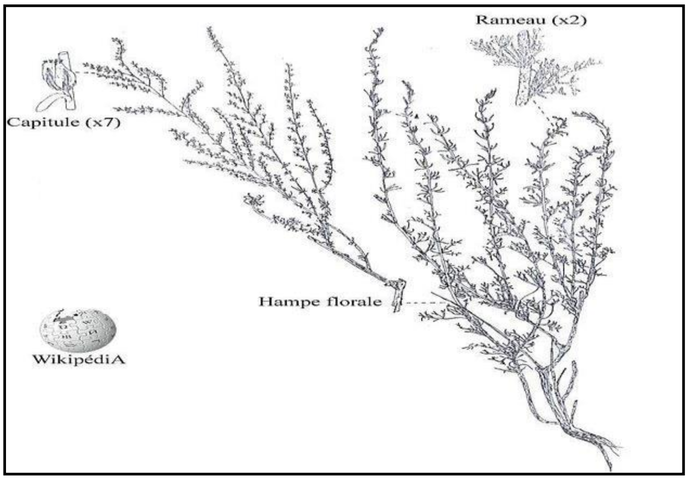


Figure 5: Structure of the compounds identified in the extract of *Artemisia herba* alba. **(Original, 2022)**

1.3. Botanical description

Artemisia herba alba is a woody plant in the form of bushes woolly whites 30-80 cm tall. The stem bears lateral expansions of branchlets and leaves. Sheets are very small in size with 3 to 5 leaflets per leaf; they are white, woolly, short and pubescent. The flowers are yellow, grouped in heads; the fruit contains only one seed. The roots are very thick, woolly, deep set and hold firmly to the ground.



a. Detail drawing after POTTER, 1981 by *A. Herba alba.*

b. **:** photograph of the flowers of the species of *A. Herba alba*

c. photograph of the leaves and branches of the species of *A. Herba alba*

Figure 6: a.b.c – photograph of *A. Herba* alba**(Original, 2022)**

1.4.Botanical classification

The genus Artemisia is represented by four species, three of which are localized in the Sahara: *Artemisia-compestris* L, *Artemisia herba alba* and *Artemisia judaica*, *Artemisia arborescence* is generally found in the north of the country.

(Deysson ,1967) classified *Artemisia herba alba* as follows:

- **Phylum:** Spermatophytes or phanerogams

- **Sub-Phylum:** Dicotyledons

- **Subclass:** Gamopetals

- **Order:** Asterales

- Family: Asteraceae

- Subfamily: Radiated

- Genus: Artemisia

-**Species:** *Artemisia herba alba*

1.5. Plant harvest

Aerial parts of *A. herba-alba* Asso. were collected at the vegetative stage in April 2022 from a population located at Guentis in **Tebessa** province, east of Algeria. The species was identified by the botanist of the department of Biology, University of Eloued , Algeria.

a. geographical location of the wilaya b.Map of Guentis region

Figure 7: a.b-geographical location of harvest region (Tebessa-Algeria) **(Original, 2022)**

2. Methods

2.1. Plant materiel preparation

A.herba-alba aerial parts were dried in the dark, at room temperature and inert atmosphere, after cleaning and grounded at small fragments.

Figure 8: plant *A. herba* hlba grinding stage**. (Original, 2022)**

2.2. Essentials oils isolation

Samples of 100 g for each, of *A. herba-alba* were hydrodistilled for 3 h in a Clevenger-type apparatus to obtain the essential oil. Each sample was stored in sealed vials protected from light at 4 °C until analysis. The experiment was replicated several times until the required quantity was reached.

Figure 9: hydro-distillation Clevenger apparatus **(Original, 2022)**

2.3. Yield measure

To measure yield results, a quantity of 100g of the Artemisia was introduced to 600ml of distilled water in a hydro-distillation Clevenger apparatus for a period of 3 hours. When the required time has elapsed, yield result was measured using this equation:

$$\mathbf{R\ \% = (mh/\ mv) \times 100}$$

R% : yield of EO.

mh : mass of EO obtained in grams.

mv: mass of plant material used in grams (100 g).

2.4. Oil density measure

The general formula for calculating relative density at 20°C is as follows:

$$\mathbf{d20/20 = (\rho EO/\rho water\ \grave{a}\ 20°C) + (0.00073\ (stm - 20))}$$

ρEO volumetric mass of EO

ρwater : volumetric mass of water at 20°C

stm : sample temperature during measurement

2.5. Dose preparation

Acetone (analytical grade, Sigma-Aldrich, Germany) was used for the dilution of oil samples. 5% acetone solution was prepared by mixing 5ml of acetone with 100ml of distilled water. Four solutions of different oil concentrations were created with concentrations of 0.5%, 1%, 1.5%, and 2%, respectively. The solutions were realized by replacing a specific quantity of the acetone solution with a proportionate quantity of the Artemisia oil.

Figure 10: solutions obtained by diluting oil. **(Original, 2022)**

2.6. Germination bioassays

To begin, seeds of *Zea mays* were distributed among 18 petri dishes tapissed with 90mm diameter Whatman filter paper N°1. The petri dishes were divided into six groups with each group made of two additional duplicates of the sample. 10 mays seeds were placed in each petri dish. The first group was put in distilled water. Zea mays was put it an acetone solution in the second group. In the third group, the zea mays was put in an acetone solution containing 0.5 percent Artemisia oil. In the fourth group, the zea mays was put in an acetone solution containing 1% Artemisia oil. In the fifth group, the zea mays was put in an acetone solution containing 1.5 percent Artemisia oil. In the sixth group, the zea mays was put in an acetone solution containing 2% Artemisia oil.

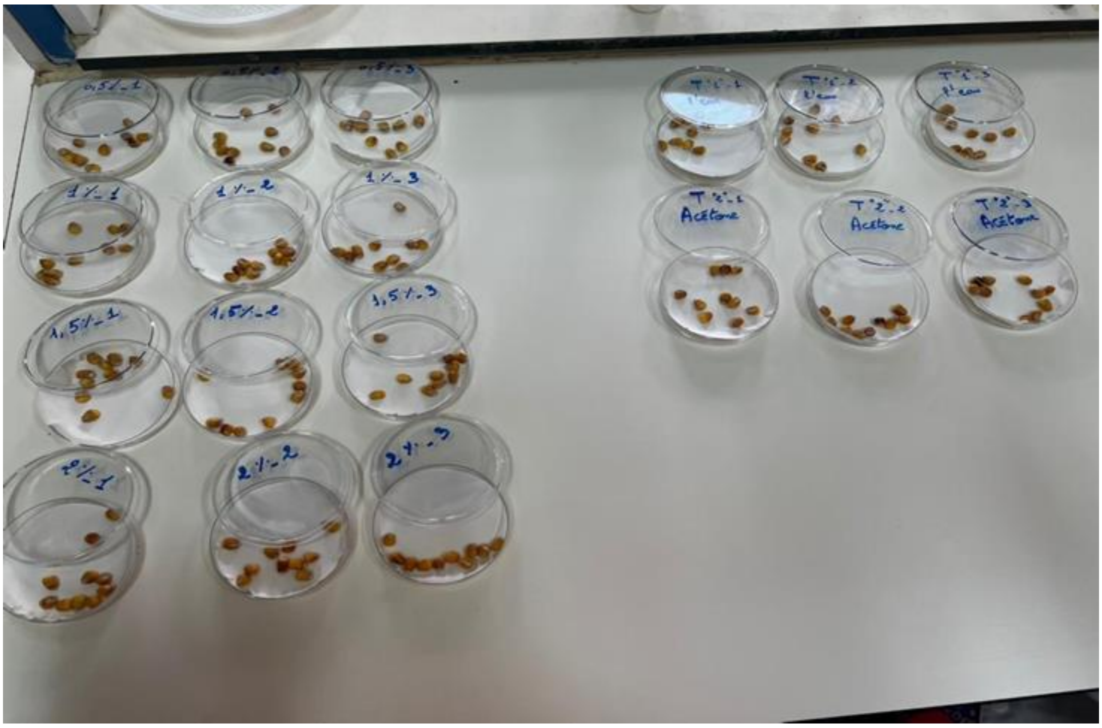


Figure 11: germination bioassays. **(Original, 2022)**

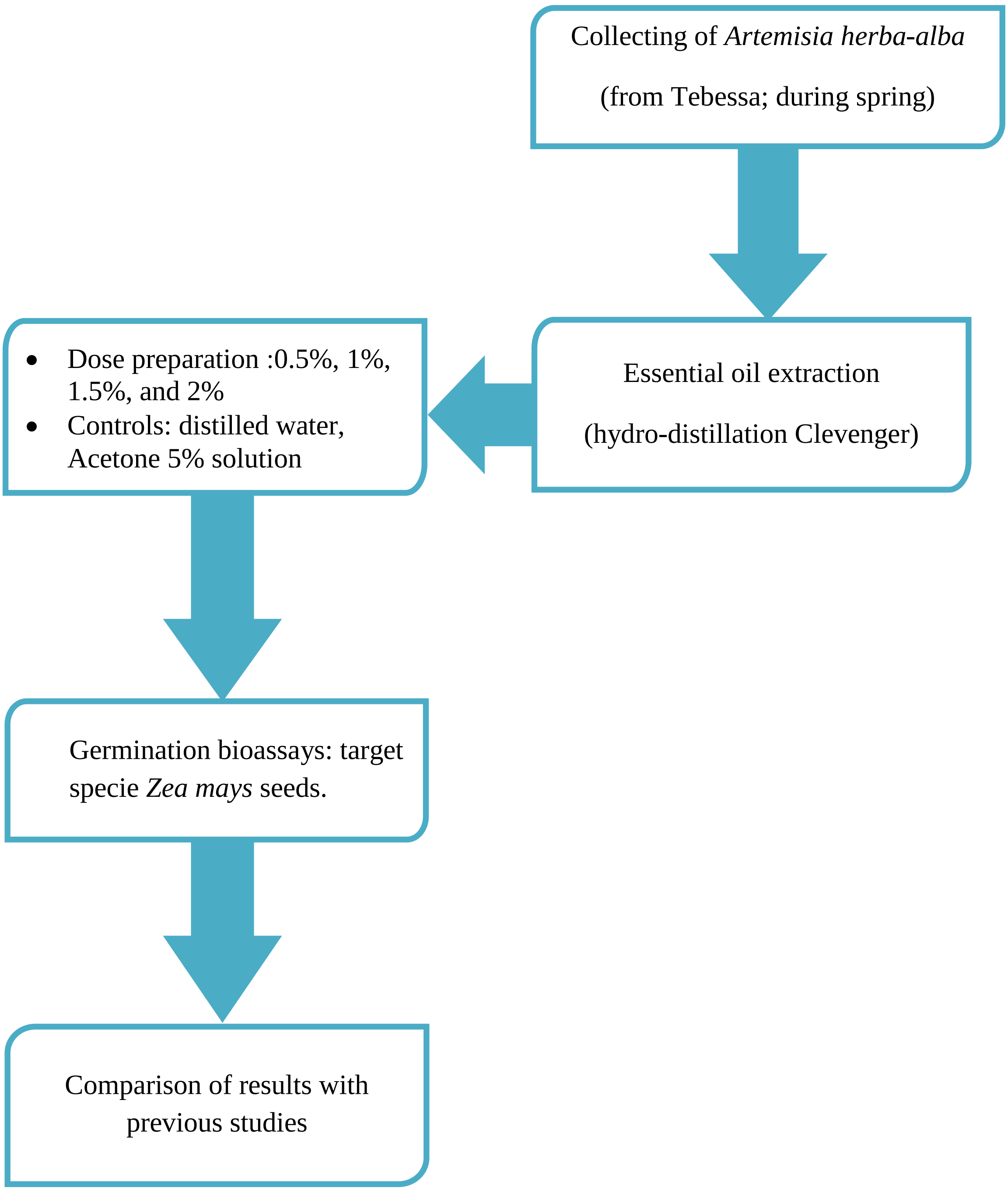


Figure 12: Methodology flowchart

Chapter III:
Results and discussion

1. Measuring yields

In this study, hydrodistillation of the aerial part of *A. herba alba* resulted in 0.76% yield on a dry mass basis. Among *A. herba-alba* phytochemical constituents, essential oils have been extensively studied. The variability of essential oils yield isolated from the same specie is due to collecting samples in different regions and seasons. A review of the existing literature on *A. herba-alba* essential oils afforded a large number of studies. Although the isolation procedure was similar in most cases such as the plant parts, physiological stage, status (fresh or dry), except for the geographical origin (harvest area) that was diverse (Table2).

Table 2: Yield of essential oils extracted from A.herba alba

Yield extraction	Harvest area	Plant part	References
0.76	Tebessa- Algeria	Aerial part	This study
2	Moroco	Aerial part	Ouachikh *et al*. (2009)
2.2	Tunisia	Aerial part	Mighri *et al*. (2009
1.5-3.3	Algeria	Aerial part	Boutemak *et al*. (2009)

2. Oil relative density

Regarding the physical parameter, the relative density at 20°C of EO is evaluated to be 0.768. In comparison with previous studies, a quite difference in density of EO of the plant collected in the same season from two similar areas (Tebessa in our study and Batna from study of Mohcen (2021).

Table 3 : Relative density of essential oils

EO density	Harvest area	collected season	References
0.768	Tebessa- Algeria	Spring	Aour study
0.9367	Batna- Algeria	Spring	Mohcen (2021)

3. Dose determination

Table (4) presented concentrations of A. *herba-alb*a essential oil in percentage (0.5 ml of essential oil in 100 ml of aceton solution) with corresponding value calculated in g/ml.

Table 4 : Concentration of A. herba-alba essential oil in (%) and (g/ml)

Dose (%)	**Dose (g/ml)**
0.5	0.38
1	0.76
1.5	1.15
2	1.53

4. Germination rate and inhibition percentage of germination

The essential oil was evaluated for its phytotoxic activity against germination of *Zea mays* seeds. Figure (13) represents the germination rate of *Zea mays* seeds treated by essentials oils of *A.herba-alb*a at different concentrations (0.5, 1, 1.5 and 2 %). Controls 1, 2 distillated water and acetone 5% solution respectively represent a maximum germination rate (100%) in figure (13) and null inhibition percentage (0%) in figure (14). On the other hand, no germination occurred in the petri-dishes treated by oils at different doses with a maximum inhibition percentage of germination (100%) figure (14) and null germination rate (0%) figure (13).

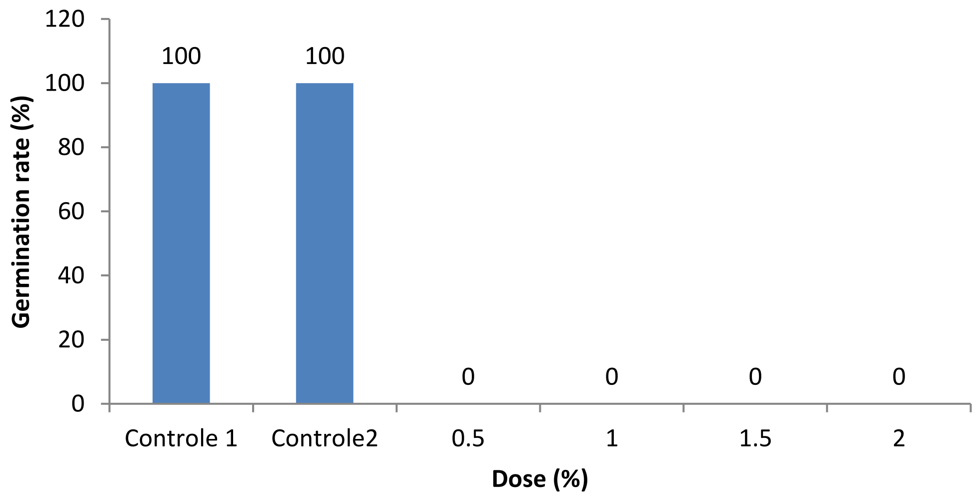


Figure13 : Germination rate of Zea mays seed treated by different dose of *A.herba alba* essential oils.

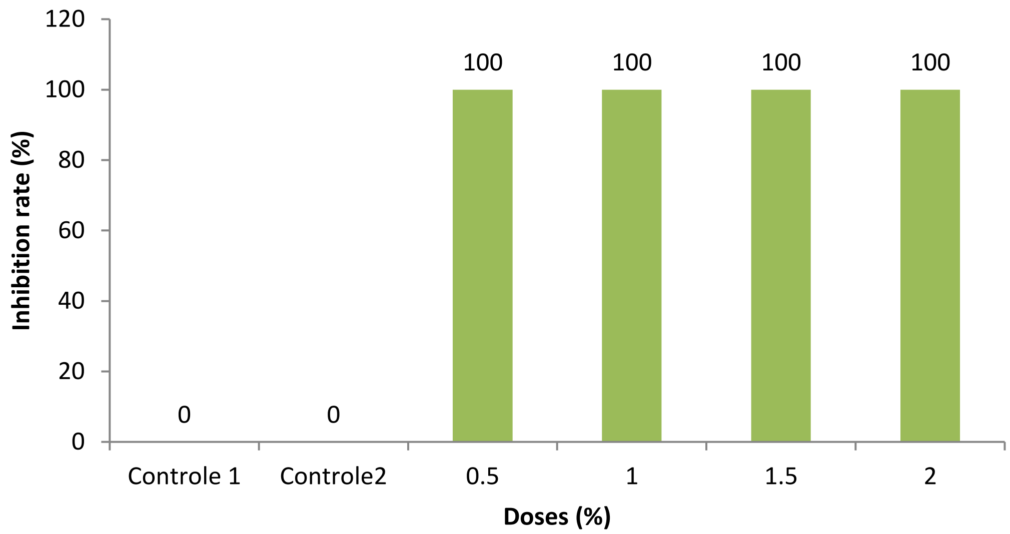


Figure 14: Inhibition percentage of germination of Zea mays seed treated by different dose of *A.herba* alba essential oils.

5. Phytotoxicity comparison of *A.herba-alba* essential oil

Plant essential oils are mixtures of bioactive, fragrant, volatile, and lipophilic secondary metabolites that are also synthesized to defend against various plant pathogens; they have been scientifically studied worldwide to control weed species **(Satyal *et al*., 2012)**

Artemisia herba-alba belongs to the Asteraceae family; its essential oil is known for its antimicrobial, antioxidant, insecticidal, and antispasmodic activity. It is also used in traditional medicine as an antispasmodic and in treatment of diabetes mellitus **(Mohamed *et al*, 2019).**

A.herba-alba contains phenolic compounds (quinic acid derivatives, flavonols, coumarins, dihydrochalcones), sesquiterpene lactones and flavonoid alkaloids.

Table (5) presents the important effect of inhibition of germination of different target species at different concentrations mediated by the essential oils of *A. herba-alba*.

The phytotoxic activity observed in this study may be linked to the presence of active compounds which can inhibit the seeds germination. Certainly, the effect of some monoterpenes such as chrysanthenone, *trans*-thujone, *cis*-thujone, camphor and α-pinene presented in *A. herba alba* EOs on plant seeds was already studied table(6).

Table 5 : Allelopathic effect of essential oil

Dose (%)	Results of inhibition (%)	Effect
Control1	0	Any effect
Control2	0	Any effect
0.5	100	Strong effect
1	100	Strong effect
1.5	100	Strong effect
2	100	Strong effect

Table 6 : Comparison of germination inhibition

EO doses	**Target species**	**inhibition of germination**	**References**
0.5, 1, 1.5 and 2 %	*Zea mays*	100%	this study
0, 100, 500, 1000, 1500 and 2000 ppm	*Agropyron desertorum*	47, 25, 16, 10, 5.4, and 1.4 %	**Tilaki *et al*, 2013**
2 and 1mg/mL	*Medicago sativa L*	100, 38.70%	**Aljaiyash *et al*, 2018**

Conclusion

Conclusion

The main objective of this work was to extract an essential oil from *Artemisia herba-alba* plant collected from the region of Tebessa north-est of Algeria, and subsequently test its phytotoxicity.

The phytotoxic effect of *Artemisia herba-alba* essential oils was tested under laboratory conditions at different concentrations (0.5, 1, 1.5, and 2%) on germination of model specie *Zea mays* seeds.

Hydrodistillation of the aerial part of *A. herba alba* in a 0.76% yield on a dry mass basis, and the obtained essential oil has a relative density 0.768 at 20°C.

The results of germination bioassays show that the essential oil tested at different doses totally inhibits the germination of the *Zea mays* seeds (inhibition percentage =100%).

In comparison with previous studies, essential oil of *Artemisia herba-alba* collected from Tebessa province, has a strong phytotoxic effect tbat may be caused by the richness of plant species in allelochemicals compounds. This richness is due to the specific nature of the region (climate, soil).

In the perspective, the phytotoxic activity of *Artemisia herba-alba* essential oil should be tested at lower doses to find the minimum inhibitory concentration, and analyze their chemical composition to discover the bioactive molecule.

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Abstract

This work aimed to evaluating the phytotoxicity of the scented oils of the white artemesia. To this end, essential oils were extracted from the top/surface of the plant by means of distillation. the extract yield was estimated at 0.76% while the relative density of the oil was determined to be 0.768. The phytotoxic activity of the obtained scented oil was tested on zea mays seeds. It was diluted and divided into four acetone solution concentrations (0.5, 01, 1.5, 2) respecitvely.

The germination test results revealed a potent toxicity with a complete inhibition rate of 100% for the four concentration compared to the control (distilled water and 5% concentrated acetone solution) where a maximum rate of germination observed was 100%.

Keyword : phytotoxicity . aromatic oils . white artemesia. zea mays seeds. germination test

Résumé

L'objectif de ce travail est l'évaluation de l'activité phytotoxique de l'huile essentielle de l'armoise blanche artimesia herba alba. pour atteindre cet objectif l'extraction des huiles essentielles de la partie aérienne de cette plante à était faite par hydrodistilation. le rendement d'extraction est évalué de0.76% , alors que la densité relative de l'huile est calculé de 0.768

L'activité phytotoxique de l'huile essentielle obtenue est testé sur des graines d'une espèce modèle "Zea mays". L'huile essentielle est diluée dans une solution acétone 5% en 4 concentrations 0.5,1,1.5 et 2 %. Les résultats des tests de germination ont montré une forte phytotoxicité avec un taux d'inhibition total (100%) pour les 4 concentrations par rapport aux témoins (eau distillé et solution acétone 5%) où un taux de germination maximal est observé (100%).

Mot-clé : l'activité phytotoxique . huile essentielle . armoise blanche artimesia herba alba. Zea mays . tests de germination

الملخص

الهدف من هذا العمل هو تقييم نشاط السُّمية النباتية للزيت العطري لنبات الشيح الأبيض . لتحقيق هذا الهدف ، تم استخراج الزيوت الأساسية من الجزء العلوي/ السطحي لهذا النبات عن طريق التقطير المائي. تم تقدير إنتاجية الاستخلاص ب 0.76٪ ، بينما وُجدت الكثافة النسبية للزيت 0.768

تم اختبار نشاط السُّمية النباتية للزيت العطري الذي تم الحصول عليه على بذور نموذج نبات الذرة ، يخفف الزيت العطري في محلول أسيتون 5٪ في 4 تركيزات 0.5 ، 1 ، 1.5 و 2٪.

أظهرت نتائج اختبارات الإنبات سمية نباتية قوية مع معدل تثبيط كلي (100٪) للتركيزات الأربعة مقارنة بعناصر التحكم (ماء مقطر و 5٪ محلول أسيتون) حيث لوحظ أقصى معدل إنبات (100٪).

كلمات المفتاحية: نشاط السمية.الزيت العطري. نبات الشيح الأبيض .نبات الذرة .اختبار نشاط السمية