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ALLELOPATHIC POTENTIAL OF VOLATILE/ESSENTIAL OILS AND HYDROSOLS OBTAINED FROM CULTURED MEDICINAL PLANTS

Cătălina STAN (TUDORA)[1, 2], Adriana MUSCALU[2], Valentin Nicolae VLĂDUȚ[2], Florentina ISRAEL-ROMING[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Mărăști Blvd., District 1, Bucharest, Romania
[2]National Institute of Research-Development for Machines and Installations Designed to Agriculture and Food Industry - INMA, 6 Ion Ionescu de la Brad Avenue, District 1, Bucharest, Romania

Corresponding author email: cmc_tudora@yahoo.com

Abstract

Using the chemical substances in weed control, diseases and pests, without discernment, has been responsible for environmental damage and human health. For these reasons, in the last years research has intensified its efforts to find alternative agriculture strategies. One of these is represented by the Integrated Weed Management System (IWMS), so that the capacity to combat by natural ways could have a wider and more valuable application. From the multidisciplinary and interdisciplinary point of view approach, some species of agricultural interest are already known for their allelopathic effects and can be used as instruments for weed management, disease and pests. A feasible alternative is represented by the identification of natural substances with allelopathic effects for the production of natural bio pesticides. Research done so far has highlighted the possibility of using volatile / essential oils and hydrosols extracted from medicinal and aromatic plants disproof in controlled environment, such as horticulture (in greenhouses and solariums). The advantage of using such natural compounds is the fast decomposing process in the environment and thus is less harmful and can be applied in organic farming.

***Key words**: allelopathy, medicinal plants, essential oil.*

INTRODUCTION

In the production of medicinal and aromatic plants, the quality of the products is given by the content in active compounds.

When selecting a specie for a particular crop area, consideration is given to the complexity of the interaction of the different vegetation factors, so as to ensure an optimal ratio between the pedoclimatic conditions and the biological requirements of the plants.

There are avoided natural conditions which can increase vegetable biomass production to the detriment of the active principles.

Today, about 3,000 plants are used to obtain volatile oils, out of which 300 are world marketed (CBI, 2009a, Lelieveld, 2017).

The largest consumer of volatile oils is the United States, followed by Western European countries (France, Germany, Great Britain) and Japan (Holmes, 2005).

It is difficult to make a more accurate estimation of global production of volatile/essential oils, but in 2009 an estimate of the first 20 volatile oils was reported, which is much higher, over

104,000 tonnes (CBI, 2009b). Volatile oils were classified into 3 large groups based on volumes globally produced.

Production of the first group exceeds 100 tons / year (e.g.: *Mentha piperita, Lavandula hybrida, Citrus sinensis, C. aurantium*). The second group is between 50-100 tons / year (e.g.: *Salvia sclarea, Ocimum basilicum, Thymus vulgaris*). The third group is between 1-50 tons / year (e.g.: *Hyssopus officialis, Artemisia dracunculus, Carum carvi*) (Shrinivas and Kudli, 2008).

Data on the evolution of the surfaces and the production of medicinal and aromatic plants cultivated in Romania are shown in Figure 1 (Source: 2016 RSY-Statistical Yearbook of Romania, 2016).

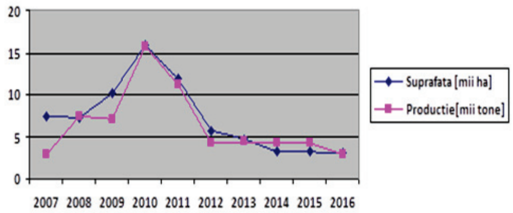

Figure 1. Correlation between aromatic plants surfaces and productions obtained during 2007-2016

Processing of medicinal plants can lead to: essential oils and hydrosols (floral waters), which are very concentrated mixtures of volatile chemicals; pharmaceutical products for pharmaceutical companies that produce drugs based on medicinal herbs extracted, or use plant derived compounds as raw material; herbal products such as extracts, teas, tinctures, capsules, etc.; nutraceuticals/functional foods that represent a group of products called health products or dietary supplements that are classified as fortified foods with health benefits other than basic diets; natural dyes. They are also increasingly used in other industries as: paints and varnishes, food, ecological industry and painting restoration; cosmetic and personal care products for cosmetic companies that produce a wide range of beauty and personal care products, hair care, perfumes and floral waters; recent group of cosmetics that contain products containing one or more bioactive compounds and which are used to enhance health and beauty. Some important ingredients used in the cosmetics industry are: oils, fats and waxes, essential oils and oleoresins, plant extracts and dyes; plant protection products in the form of extracts or compounds which can be directly used and others serve as precursors for the production of protective agents used against weeds, insects, pathogens (Vinod et al., 2014; Muthe et al., 2016).

EXTRACTION TECHNIQUES OF VOLATILE / ESSENTIAL OILS FROM MEDICINAL PLANTS

Volatile/essential oils are aromatic oily liquids obtained from various parts of medicinal plants, soluble in organic solvents and lipids, having a generally lower density than water. Among extraction techniques for obtaining volatile/essential oil, the most commonly used are:

Steam distillation - volatile substances are entrained by water vapours, even if they have high boiling points. A diffusion process of the volatile oil from the plant cells takes place before, depending on its location in the plant or on the chemical composition. When oil diffusion becomes more difficult, or when components have high viscosity and remain on the vessel walls, organic solvents (2-3%) such as benzene, hexane, etc. are also used. The laboratory uses Clevenger installations type, where the water recirculates, the plant being in constant contact with water. In industrial distillation plants there are fixed boilers installed on special platforms or mobile boilers so that planting can be done directly from the harvesting site. After mixing the volatile oil, it is collected in the Florentine vessels where the oil is decanting and separating (Tamas et al., 1996; Handa et. al., 2008).

The hydro diffusion - water vapours is forced to make a reverse circuit, which leads to saving time and energy, thus avoiding the degradation of the obtained oil quality (Tamas et al., 1996; Handa et. al., 2008).

Rotary Cones Column - distillation is done at lower temperatures, the plant material is loaded through the top, flows along the cones and it is spread over the surface of the rotating cones in a thin layer. The bottom-feed water vapours flows counter currently to the plant product and drives the volatile components into the condenser and then into the Florentine vessel (Tamas et. al., 1996; Huie C., 2002).

Vacuum microwave hydro distillation (VHMD) - azeotropic entrainment of volatile oil with vapours obtained only from the existing water in the vegetal material, is carried out in the extraction vessel and the distillation temperature is lower than 100°C. This technique is highly efficient, leading to high quality oil obtained in shorter extraction time (up to 10 time faster than classic hydro distillation) and saving energy, water, time and labour too (Tamas et al., 1996; Filly et. al., 2014).

Extraction with supercritical fluids - the most used supercritical fluid is considered to be CO_2, free of toxicity and with high dissolving power of volatile components in oils. Extraction occurs spontaneously, without other solvents, at low temperature (Majdalani et al., 1993; Tamas et al., 1996). This extraction method protects very well the volatile oil constituents against thermal distortion.

Extraction with organic solvents involves the use of hexane, petroleum, petroleum ether or ethanol at about 50°C. Since some volatiles (pigments, waxes) are extracted too with volatile components, further steps are required to remove them. This technique is used on delicate plants with lower content of aromatic compounds and has the disadvantage of longer extraction period and high solvent consumption.

The quality of the oil and the yield of extraction are determined by a number of factors: the quality of the plant material, the available installations and the rigorous control of the extraction process parameters. The quality of the plant product is also influenced by a number of factors (Paun, 1988; Fleurentin et al., 1990; Tamas et al., 1996). Among these can be mentioned: pedoclimatic factors, applied crop technologies, photoperiodicity, the part of the plant where volatile oil accumulates, the used chemistries, the traumas produced by some pathogens and pests, the harvesting period, the process of processing the vegetal material etc.

CHEMICAL COMPOSITION OF VOLATILE/ESSENTIAL OILS

These very complex natural mixtures can contain about 20-60 compounds in different concentrations, mainly monoterpenes, sesquiterpenes and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides). Some volatile compounds include phenylpropanes and sulfur-specific or nitrogen-specific substances. Saturated or sulphuris compounds (e.g.: glucosinolates or isothiocyanate derivatives found in garlic and mustard oils) are also secondary metabolites of various plants. Generally, the volatile/essential oil composition is a balance between the different contained compounds, although in many species a constituent can prevail over all others. They are characterized by two or three main components in fairly high concentrations (20-70%), compared to other components present in small quantities. These important components determine the biological properties of the essential oils. The compounds include two groups of distinct biosynthetic origin (Croteau et al., 2000; Betts, 2001; Bowles, 2003). The main group is terpenes: monoterpenes such as monocyclic carbides (e.g.: cimenels, sabinens, etc.), bicyclic carbides (e.g.: alpha and beta pinen), acyclic alcohols (e.g.: citronellol, geraniol), phenols (e.g.: carvacrol, thymol) and terpenoids (e.g., ascaridol, menthol, etc.) and the other of the aromatic and aliphatic constituents (e.g., cinemaldehyde, chavicol, eugenol, estragole, anethol, etc.), characterised by low molecular weights. The main botanical families containing these compounds are: *Apiaceae* (e.g., *Parsley* with 7.71-10.56% apiol, *Anise* with 80-90% anethol, *Fennel* with 50-70% trans-anethol), *Lauraceae* (e.g. *Cinnamon* with 65-80% cinnamaldehyde), *Lamiaceae* (e.g. *Lavender* with more than 25% linalool). According to Chen et al. (2012), secondary metabolites of medicinal plants are predisposed to qualitative and quantitative variations, depending on several factors such as: genetic deviation, physiological conditions, season and harvest period and phenological development stage of the plant. Farming practices (spacing and harvesting period) critically influence quantitative characteristics of many medicinal and aromatic plants, which ultimately lead to general plant growth and increased yields. Long time, the optimum period for harvesting plant raw material was considered to be one of the most important factors influencing the accumulation of volatile oil in plants (Lee and Ding, 2016). Specialty literature shows that there is a direct correlation between the ontogeny of medicinal and aromatic plants and the temperature, daylight (Sangwan et al., 2001). In the study by Halva et al. (1992) it was reported that the dill growth and accumulation of essential oils is increased with light levels and the highest accumulation level was recorded under full sunlight. It is believed that the production of secondary metabolites is stimulated by the stressful environment. Meteorological parameters (temperature and precipitation) influence the quantity and composition of volatile oils in several medicinal and aromatic plants. Temperature and humidity are the most important factors influencing the oil content of the *Lamiaceae* family (Verzea M., 2002). Cooler nights and hotter days have a negative effect on the oil content of several medicinal and aromatic plants. The hyssop (*Hyssopus officinalis* L.) achieves the maximum increase in leaf oil content under hot, sunny days. Poor clouds or low temperatures adversely affect both parameters. The variability of morphogenetic, ontogenetic, diurnal and ecological factors affects secondary metabolites of plants, especially essential oils and their compounds. In addition, the highest essential oil content is obtained during the warm period, between plant growth and complete blossoming (Süleyman et al., 2016). The question is whether the biological effects of volatile / essential oils are the result of the synergism of all competing molecules, or reflect only the action of the main molecules present at the highest levels according to chromatographic analyses. Most of the research papers report, only the concentration of the main constituents of essential oils such as: *terpineol, eugenol, timol, carvacrol, carvone, geraniol, linalool, citronelol, nerol, safrole, eucalyptol, limonene, cinnamaldehyde*. It is generally observed that those major compounds sufficiently reflect the biophysical and biological characteristics of the volatile oils from which they were isolated (Ipek et al., 2005), the magnitude of the effects depending only on their concentration when tested alone or contained in the oils they come from. The synergistic action of the various chemical compounds contained in a volatile oil, compared to the action of one or two main components of the volatile oil, seems to be questionable. However, it is possible that the activity of the main components to be modulated by other minor molecules (Santana-Rios et al., 2001; Hoet et al., 2006). Probably many components of essential oils play a role in defining perfume, density, texture, colour, cell penetration (Cal, 2006), lipophilic or hydrophilic attraction, and cell wall, membrane and cellular distribution. This latter feature is very important because the oil distribution in the cell determines the different types of reactions produced, depending on the place it occupies in the cell. For biological purposes, it is more appropriate to study the whole oil than a few components, because the concept of synergy seems to be significant.

ALLELOPATHIC POTENTIAL OF MEDICINAL PLANTS AND VOLATILE/ESSENTIAL OILS

The ability of plants to inhibit or stimulate the growth of other plants by releasing chemicals into the environment has been called allelopathy. So the beneficial or harmful effect of a plant on another plant by producing chemical compounds that it releases directly or indirectly to the environment is called allelopathy (Siddiqui et al., 2009). The allelopathic phenomenon has long been noticed, but its intensive, scientific research to identify the chemical compounds involved (allelopathic substances-secondary metabolites of low molecular weight plants present in different plant parts) is done since a few years ago. The allelopathic interactions between plants are of practical importance, especially in the case of plants of economic interest (such as medicinal plants), when other species are successively cultured on the same field. In this way, the allelopathic compounds derived from the remaining vegetable residues from the previous culture, have inhibitory/stimulating effects on the growth and development of other plant species (Netzly and Butler, 1986). Understanding and controlling such phenomena in the future creates the possibility of using these allelopathic compounds either as growth regulators or as natural bio-pesticides / pesticides. Recent studies have shown the allelopathic capacity of volatile oils and hydrosols (floral waters) obtained by various methods from medicinal plants. Volatile oils are spread in the plant, some families being very rich in such substances, both in number and quantity (e.g.: *Pinaceae, Lamiaceae, Umbeliferae, Myrtaceae, Lauraceae, Rutaceae, Caryophylaceae*, *Asteraceae* etc.). These allelochimic substances indirectly influence plant growth, inhibit the activity of microorganisms such as nitrifying bacteria and ectomycorrhizers that help to fix nitrogen (Hunter and Menges, 2002). Allelopathy is one of the factors that help a plant to settle in an ecosystem (Moktar Hossain et al., 2012). The use of synthetic herbicides everywhere in the world has led to the emergence of weed species that are resistant to herbicides. Environmental concerns about herbicide safety have led to the finding of weed management systems that are not so dependent on herbicides. Considering these, allelopathy was recognized as a new approach, a new ecological weed management system (Pukclai and Kato-Noguchi, 2011). Allelopathy has a considerable role in agricultural ecosystems, so that the growth and development of crops, weeds and trees maybe influenced. Research papers has shown that the allelopathic effects are positive or negative, depending on the applied dose and the body. Several authors (Ruszkowski D., 2004; Arouiee H., 2010; Gahukar R.T., 2012) argued that allochimic substances released into the environment affect the plants closed to them, by reducing cell membranes, their permeability, interrupting the absorption of substances minerals and cause damage to the genetic material. In Asian and African countries, due to growing demands for wild native plants as herbal remedies, medicinal plants have become a component of agricultural ecosystems (Badmus and Afolayan, 2012). Many plant species have been investigated for their allelopathic potential, especially aromatic plants capable of producing a large amount of chemical substances (Campiglia et al., 2007). Different secondary metabolites known as allelochimic susceptors such as monoterpenes, sesquiterpenes and alpha-pines from essential oils of specific plants prevent germination of seeds and cause morphological and physiological changes in plant growth (Badmus and Afolayan, 2012). Many plant species have been investigated for their allelopathic potential, especially aromatic plants capable of producing a large amount of chemical substances (Campiglia et al., 2007). Different secondary metabolites known as allochimic susceptors such as monoterpenes, sesquiterpenes and alpha-pines from essential oils of specific plants prevent germination of seeds and cause morphological and physiological changes in plant growth (Badmus and Afolayan, 2012). Mahboobi and Heidarian (2016) have studied the allelopathic effects of medicinal plants on the germination and growth of seedlings of various weed species. Three different weed species (*Peganum harmala, Alyssum* spp.*, Amaranthus retroflexus*), which were applied in five dry matter concentrations from four medicinal plants (*Menta, Rosmarin, Lavandula* and *Achilea*) were used. The effect of different concentrations of dry substances on germination, plumula length, root length, vigor and weight of plantule was studied. The results obtained indicated that the weeds show different sensitivities to the allelochimic substances present in the medicinal plants. Also, the inhibitory effects of the medicinal plants were different, which could be due to the type, quantity and characteristics of the allelophathic substances produced by these plants. The conclusions of this study have shown that at higher concentrations of medicinal herbs, more inhibitory effects on germination and growth of weed seedlings were observed and can be successfully used in organic crops and for the production of natural herbicides. Many studies (Piyo et al., 2009, Saad et al., 2012) investigated antifungal, antibacterial and antioxidant activity of volatile/essential oil obtained from basil-*Ocimum* spp. Seven fungi species have been isolated from plants: *Alternaria* sp*., Aspergillus flavus, Botrytis cinerea, Cladosporium herbarum, Eurotium amstelodami* and *Eurotium chevalieri. E. Chevalieri* was most sensitive to basil oil, while *A. flavus* was the most resistant (Jakowienko et al., 2011). Basil oil proved a strong antifungal activity against fungi, such as: *Penicillium aurantiogriseum, Penicillium glabrum, Penicillium chrys ogenum* and *Penicillium brevicompactum* (Kocić-Tanackov et al., 2012). The greatest sensitivity was recorded by the *P. chrysogenum* with complete inhibition of growth, at a concentration of basil oil extract of 1.5%. Basil oil at a concentration of 0.6% v / v had a 100% inhibition of mycelial growth on pathogenic fungi: *Fusarium moniliforme and Pyricularia arisea* and of 50% inhibitionat *Fusarium proliferatum*, but was not efficient against *Rhizoctonia solani* fungi. Volatile basil oil at a concentration of 0.8% v / v may inhibit germination of *F. monoliformespores* (91%) and *Alternaria brassicicola* (100%) (Piyo et al., 2009). Eugenol (a compound present in volatile essential oil) has been shown to have a very strong antifungal activity against *Absida glauca, Aspergillus nidulans, Aspergillus niger, Colletotrium chamus, F. monoliforme, Pestoltia psidi* and *Rhizopus nadssus* (Soković et al., 2013). Linalool and anethol showed good antifungal activity at doses of 0.03-0.3 mg / mL and 1.3-2.8 μL / mL respectively in microdilution tests (Soković et al., 2013). Pest management is confronted with safety and efficacy challenges due to human and environmental hazards caused by the widespread use of synthetic insecticides. Insecticides obtained from plants are of great interest and developing (Patruica et al., 2017). Basil oil has shown increased toxicity to locusts (*Acrida exaltata*) and fruit flies (Chang et al., 2009). The LC50 average lethal concentration in basil oil against *Planococcus ficus* ranged from 44 to 47 mg/ml (Karamaouna et al., 2013). Basil oil is a potential control agent against mites (*Tetranychus urticae*) and white mussel (*Bemisla tabaci*) in greenhouse conditions. Basil oil has shown antifungal and insecticidal activity against *Aphis craccivora* Koch. Successful adoption of basil oil in the protecttion of food promises an ecological option compatible with international regulations on biosecurity. Extract and essential oils obtained from Hyssop-*Hyssopus* spp., show insecticidal properties against flies, beetles, green caterpillars, mites, larvae of worms, cabbage moths, mushrooms, etc. The plant attracts hummingbirds, butterflies, bees, etc. which are good pollinators for plants growing in the vicinity of hyssop. Hyssop oil has toxic, antibacterial and antifungal properties and is used against many plant diseases (e.g. *Phytophtora infestans*). The allelophatic activity of hyssop oil at different concentrations was evaluated against seed germination and root growth in crop species: radish (*Raphanus sativus* L. c. Saxa), garden mustard (*Lepidium sativum* L.) and salad (*Lactuca sativa* L.) (Almeida et al., 2010). In general, volatile oils obtained from medicinal plants containing low molecular weight compounds and lipophilic properties can easily penetrate through cell membranes to induce biological reactions. The hyssop plant - *Hyssopus officinalis*, possess antimicrobial activity *in vitro* against *Klebsiella* sp. and *Erwinia amylovora*, two important pathogens and it can be used in the treatment of plant diseases (Dehghanzadeh et al., 2012). In addition, *in vitro* and *in vivo* antifungal activity of hyssop oil, was evaluated against two phytopathogenic fungi, *Pyrenophora avenae and Pyricularia oryzae* - mycelial growth of fungi was completely inhibited by hyssop oil at a concentration of 0.4%. Also hyssop oil determined reduced germination of *Botrytis fabae* and *Uromyces viciae-fabae* (Letessier et al., 2001). Two volatile/essential hyssop oils demonstrated a significant antifungal activity against 13 strains of phytopathogens. Experimental reports have shown that 100% inhibition for all pathogens has been achieved when using 1600 ppm hyssop oil (Fraternale et al., 2004). The hyssop extracts have a higher antioxidant activity than the volatile/essential oils because the latter are mainly rich in oxygennated monoterpenes, which are known to have low activity against free radicals (Baj et al., 2010).

CONCLUSIONS

Due to the increased interest for natural products such as volatile/essential oils, it is important to know biological activity for developing new applications in human health, agriculture and environmental protection. The biological effects of volatile/essential oils could be the result of a synergy of all molecules, or could only reflect the activity of the main ones. Almost all literature cases analyze only the main constituents of volatile / essential oils obtained from medicinal herbs. In this sense, it may be better that oils to be studied as a whole rather than for some components, because the allelopathic interactions are of practical importance, especially in the case of plants of economic interest, such as medicinal plants. The use of volatile/essential oils to control weeds in organic farming seems promising, but these natural herbs react quickly, and their effectiveness is limited by their higher volatility. Alternative formulation such as microencapsulation can be developed in order to reduce the applied amounts and to increase the effectiveness in time by reducing volatility, reducing material handling, and slowing down the decomposing rate within the environment.

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