

Essential Oils and Their Characteristics

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1.1 Introduction

Essential oils are the main raw material for the aroma and fragrance, food and pharmaceutical industries. They have important biological activities that have been disclosed often in recent years. However, as the industry seeks its practical application and the development of new natural drugs containing active compounds from essential oils, there is an urgent need to standardise the plant material source. For this to become achievable, it is necessary to know the different factors that affect the production of essential oils by plants, in terms of its quantity as well as its quality.

It is known that plants produce essential oils as secondary metabolites in response to a physiological stress, pathogen attack and ecological factors. Also, in nature, the essential oils are recognised as defence compounds and attractors of pollinators, facilitating the reproduction of the vegetal species. The environmental variations, in turn, are also important in a plant's ability to produce these compounds. Considering all of these factors, the main problems related to the cultivation of aromatic plants are due to variations that occur in quantitative and qualitative changes in the essential oils production. The main factors involved in the biosynthesis of essential oils by medicinal and aromatic plants are discussed in this chapter.

In order to optimise its commercial exploitation, the different factors involved in the production of essential oils must be taken into account, since the induction into its substance synthesis could affect the specific compounds of interest and their economic applications, as well as affecting the standard amount of produced oil.

1.1.1 Chemical Characteristics of Essential Oils

The designation *essential oil* originated from Aristotle's era, because the of the idea of life-essential elements — fire, air, earth and water. In this case, the fifth element was

considered to be the soul or the spirit of life. Distillation and evaporation were the processes of removing the soul from the plant or essential oils. Nowadays, these oils are also known as volatile oils, but far from being soul, essential oils are a complexity of aroma's composition. Those constituents of essential oils are generally derived from phenylpropanoid routes (Thayumanavan & Sadasivam, 2003).

The studies of those routes have disclosed the relevance of the aspect of physiology regulation, but certainly the isoprenoid exemplifies the major group of secondary metabolites in herbs, which exhibit extremely vast varieties of chemical structures and biochemical functions. Since primary metabolites exist in all plant cells that are qualified by division, secondary metabolites are there exclusive by accident, and are not essential for that herb. In contrariety to primary metabolites, secondary compounds vary extensively in their occurrence in those herbs and some may appear only in a unique or a few species (Krings & Berger, 1998).

Due to the connection of terpenoids in many pharmacological properties and their great value added specially for pharmaceutical, cosmetic and food industries, the isoprenoid route has been a spotlight for most related articles. Essential oils are nearly always rotational and have a high refractory index; they are sparingly soluble in water, usually less dense than water and liquid at room temperature, but there is some exception, as trans-anethole (anise camphor) from the oil of anise (*Pimpinella anisum* L.), and they may be classified using different criteria: consistency, origin and chemical nature. As stated by their consistency, essential oils are classified as essences, balsams or resins. Depending on their origin, essential oils are natural, artificial or synthetic. Essential oils are aromatic chemical compounds that came from plant's glands. Due to their volatility, flavour and toxicity, this class of compounds also plays significant aspects in the defence's herbs, communication between plants and pollinator attractiveness (Muñoz-Bertomeu *et al.*, 2007; Thayumanavan & Sadasivam, 2003).

A lot of herbs can be view as being composed of a basic unit called isoprene or isopentane. Terms such as isoprenoid or terpenoid are employed concurrently. Many terpenoids are assemble of carbon atoms from acyclic disposition to a cyclic disposition by different chemical reactions, like, condensation, addition, cyclisation, deletion or rearrangements to be transformed in a basic unit, and generally, are very extensively diffused throughout the total plant kingdom. These compounds comprise a structurally varied class that can be splitted into the main and the minor terpenoids (Daviet & Schalk, 2010; Muñoz-Bertomeu *et al.*, 2006; Daniel, 2006; Thayumanavan & Sadasivam, 2003).

Biosynthesis of terpenes can occur in distinct sector of the herb, such as bark, flowers, fruits, leaves, roots, ryzomes, seeds and wood, and have all been described to concentrate them in different herbs. Terpenes that are main metabolites include carotenes, regulators of growth, proteins, quinones, polyprenols and the sterol, substitutes of terpenes with an alcohol functional group (Daviet & Schalk, 2010). These constituents are indispensable for preserving the membrane to keep the entirety of its structure, also to protection against light, and securing the maintenance of its biological functionality. Terpenes are a large class of chemical compounds, classified by the molecular weight, being monoterpenes, sesquiterpenes, diterpenes, seterterpenes, triterpenes, tetraterpenes and phytosterols amongst others (Thayumanavan & Sadasivam, 2003).

Monoterpenes are the major contributor of most important essential oils in nature. Since the monoterpenes ($C_{10}H_{16}$) are small molecules with two isoprene units, such as menthol and linalool, and they are lipophilic; they are promptly consumed through the

skin. Synthetic compounds can be used to break down the problems come across with herbal products by creating actions for the construction of such molecules, regardless of the original species. Indeed, ways have been developed for most of the natural molecules, but, given their commonly complex spatial arrangements, the industrial production is not practicable for the majority of examples (Daviet & Schalk, 2010; Muñoz-Bertomeu *et al.*, 2006; Daniel, 2006; Thayumanavan & Sadasivam, 2003).

Characteristically, plant's secondary metabolites are cumulated and stored in relatively huge quantities, which can be explained by their role as chemical signals or defence compounds. Terpenes are built up from the union of the two carbon units with five members each by condensation, isopentenyl diphosphate synonym isopentenyl pyrophosphate (IPP) and dimethylallyldiphosphate synonym dimethylallyl pyrophosphate (DMAPP), with different modes of structure formation, number of unsaturated bonds and type of linker groups. Not all terpenoids have a composition of their structures in the repetition of five carbon atoms, as can be habitual of them, considering that they are formed usually from isoprene as forming matrix (Daniel, 2006; Thayumanavan & Sadasivam, 2003).

Terpenes consisting of more than five isoprene structures appear in all herbs, and simpler terpenes (C_{10} – C_{25}) are mainly restricted in the phylogeny classification to the vascular plants/higher plants or, synonym Tracheophyta, while sesquiterpenes have been found broadly in division Bryophyta and in the kingdom Fungi. Monoterpenoids are colourless, distilled by steam, liquids insoluble in water with a typical scent, with a range of boiling points of 140 until 180 °C. Some of them have shown potentiality as insect plague management because they simply provide herbs with defences against insects that feed from it. Many terpenes also operate as insect captivate, being green and innocuous to humans and other animals (Daniel, 2006; Thayumanavan & Sadasivam, 2003).

More than a thousand sesquiterpenes are known today. Sesquiterpenes are the biggest category of terpenoids with a broad molecular structures and are constitutes of three isoprene matrixes, that is, they are composed with 15 carbon atoms, such as farnesol, guaiazulene, bisabolol and become from medicinal plants in distilleries equipments, in the bitter-tasting substances and essential oils in a lot of herbs. Diterpenes are formed by 20 carbon atoms, by their condensation of four isoprene residues, such as taxol, gibberellins, phytol and fusicocsin. Like sesquiterpenes, we know a thousand or more C_{20} compounds in this category, which fit into 20 main typical skeletons. Triterpenes are categorised based on the linear composition or number of cyclic compounds actual. Triterpenes relate to an inharmonious compilation of chemical compounds, which are consider to be aquired from squalene; the C_{30} non cyclic component by types of rings and ligands. Pentacyclic triterpenes are usually distributed in vascular plants, occurring as glycosides with sugar ligands (saponins) or without sugar ligands (aglycones) (Daniel, 2006; Thayumanavan & Sadasivam, 2003).

Two routes have been extensively studied, leading to these precursors, through the mevalonate (typically known as MVA route; C_6) and the 1-deoxyxylulose-D-5-phosphate (coded as the DXP route) pathways. The DXP compound, also known as the 2-C-methyl-D-erythritol-4-phosphate or methylerythritol phosphate (coded as the MEP route), takes place in plant plastids (chloroplast) and also by bacteria, while the MVA track different sources such as fungus, in the herb's cytosol and in some animals. In the mevalonic acid route, the key enzyme is 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA

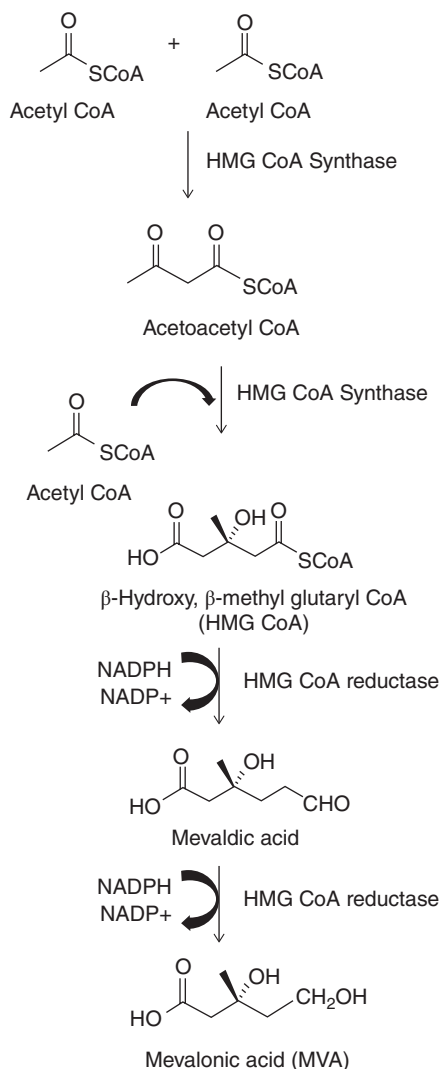


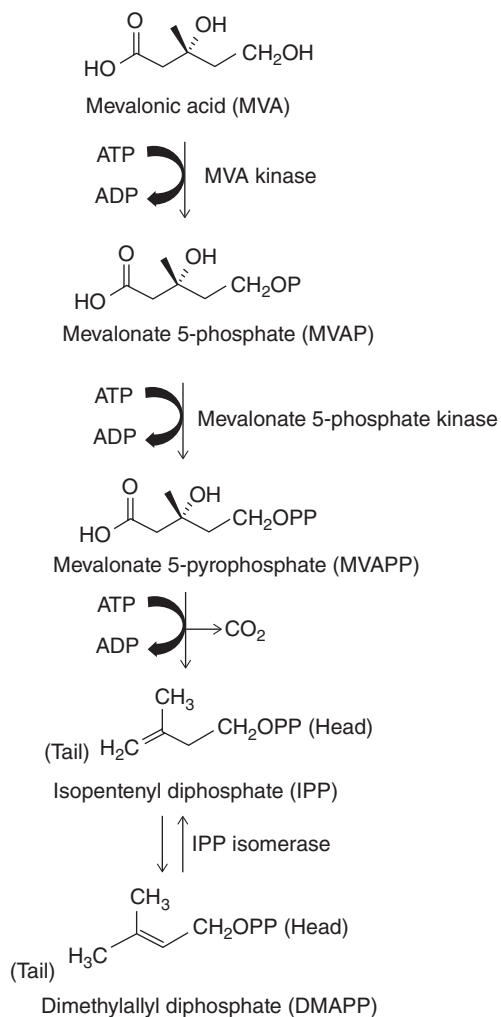
Figure 1.1 Synthesis of mevalonic acid in plants, from classical MVA pathway. HMG-CoA reductase: 3-hydroxy-3-methylglutaryl CoA reductase and HMG-CoA synthase: 3-hydroxy-3-methylglutaryl CoA synthase. From Thayumanavan & Sadasivam (2003).

reductase coded as HMGR). HMGR assemble the initial sequence of the MVA route by converting HMG-CoA to MVA using NADPH as coenzyme (Muñoz-Bertomeu *et al.*, 2007; Muñoz-Bertomeu *et al.*, 2006; Thayumanavan & Sadasivam, 2003).

The synthesis of mevalonic acid is illustrated in Figure 1.1.

In higher plants, the condensation of the basic C₅ units, isopentenyl diphosphate (coded as IPP) and dimethylallyldiphosphate (coded as DMAPP), is catalysed by the enzyme prenyltransferases (Figure 1.2), which builds the chain of prenyldiphosphate, designated as the original source for each category of terpenoids: geranyl diphosphate (coded as GPP; C₁₀), farnesyl diphosphate (coded as FPP; C₁₅) and geranylgeranyl diphosphate (coded as GGPP), respectively for the monoterpenes, sesquiterpenes and diterpenes (Figure 1.3) (Muñoz-Bertomeu *et al.*, 2007; Muñoz-Bertomeu *et al.*, 2006; Thayumanavan & Sadasivam, 2003).

Figure 1.2 Synthesis of isopentenyl diphosphate (IPP) and dimethylallyldiphosphate (DMAPP). From Thayumanavan & Sadasivam (2003).



The production of IPP and DMAPP proceeds via two alternative routes (Figure 1.4): the usual cytosolic mevalonate (MVA) route and the methylerythritol phosphate (MEP) route. The MEP route, confined in the plastids, is responsible to provide isopentenyl diphosphate and dimethylallyldiphosphate for monoterpene and sesquiterpene synthesis. The following achievement implicates the terpenoid synthases, which are presenting a vast enzyme group. Terpenoid synthases play an important function in the terpenoid synthesis, since they are responsible for the formation of farnesyl diphosphate, geranyl diphosphate and geranylgeranyl diphosphate to form the main structures of the terpenoids, and are thus at the source of the highly large number of possibilities of compounds such as squalene, generated by squalene synthase that catalyses the head-to-head reaction of two farnesyl diphosphate units, the initial important reaction in sterol category synthesis; end-product sterols (β -sitosterol and stigmasterol). Given the vast range of terpenoid configuration, many

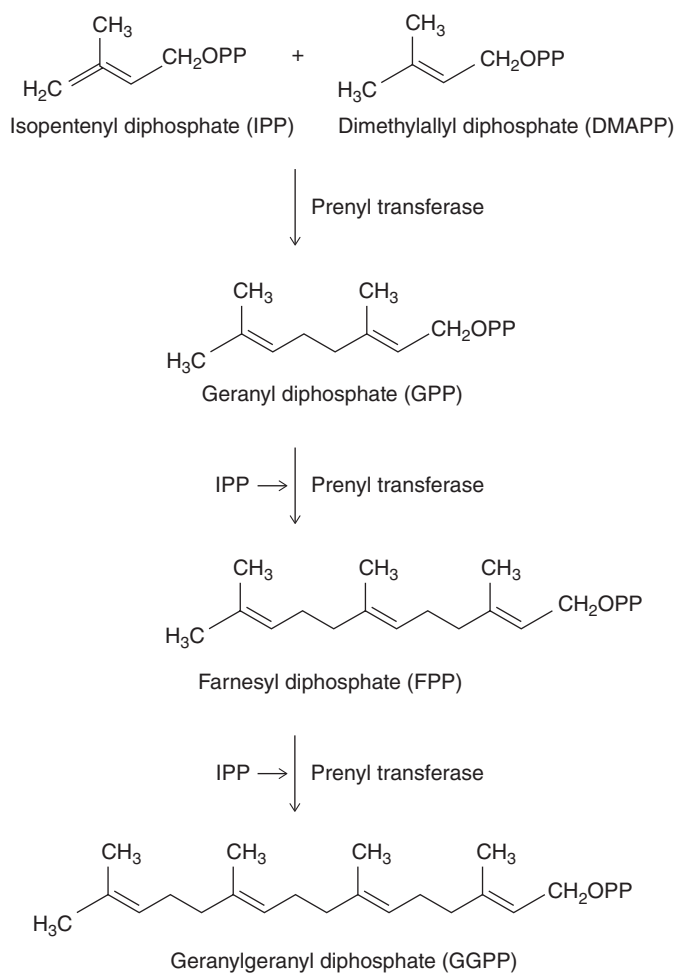


Figure 1.3 Synthesis of geranylgeranyl diphosphate (GGPP). From Thayumanavan & Sadasivam (2003).

terpenoid synthetic genes, that is, terpenoid synthases, P450, and so on, continue to be identified (Daviet & Schalk, 2010; Daniel, 2006; Muñoz-Bertomeu *et al.*, 2007; Muñoz-Bertomeu *et al.*, 2006; Wink, 1987).

The production of aroma relies on the genetic consideration and also on the growth phase of herbs. Other important factor is the environmental impact which could transform biochemically and physiologically those herbs modifying the amount and the constituents of the aroma. For this reason, the biotechnological formation of natural aroma compounds is speedily expanding although the conventional pathways of chemical reactions or removal from herbs are yet feasible. Terpenes are more costly to produce in relation to other metabolites due to the complexity of reactions. Since the advent of common food in the human life, such as beer, bread, yogurt, cheese, soy derivatives, wine and other fermented foods, microbial reactions have normally take part an important act in the production of

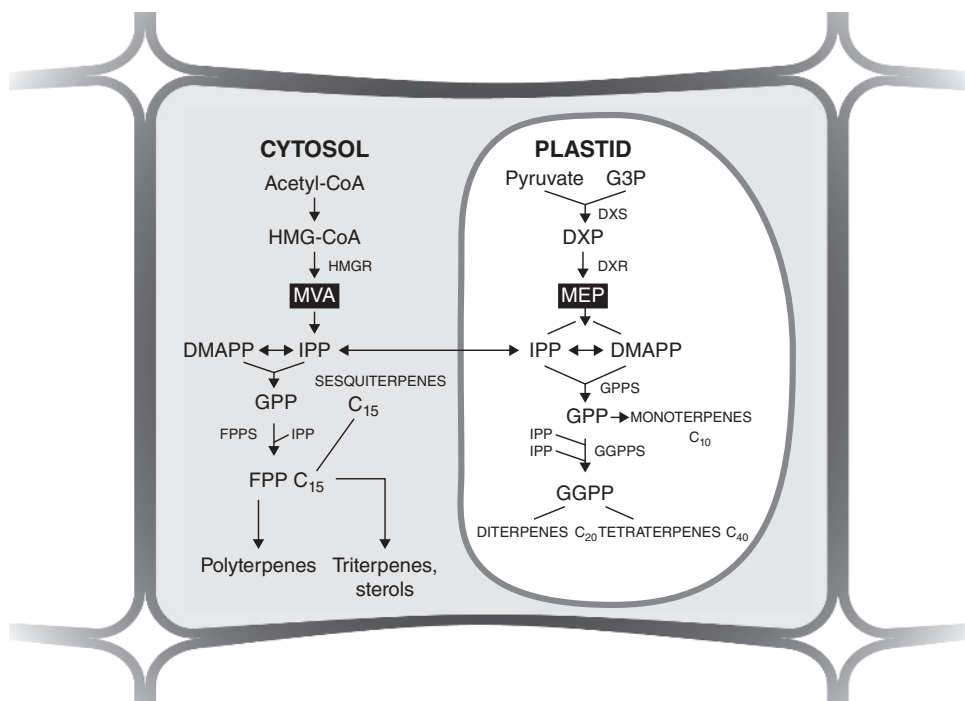


Figure 1.4 Enzymes involved in isoprenoid biosynthesis through cytosol (MVA) and plastids pathway (MEP): FPPS, farnesyl diphosphate synthase; GGPPS, geranylgeranyl diphosphate synthase; GPPS, GPP synthase; and HMGR, 3-hydroxy-3-methylglutaryl CoA reductase. FPP, farnesyl diphosphate; GGPP, geranylgeranyl diphosphate; G3P, D-glyceraldehyde 3-P; and HMG-CoA, 3-hydroxy-3-methylglutaril-CoA.

complex mixtures of food aromas. This background of biotechnology in our days has evolved from craft origins into big, attention-getting industries. Starting at the beginning of the last decade, the renewal of some techniques in the volatiles analysis area facilitated the separation and structural identification of essentials oils, such as gas chromatography. There is no doubt that the use of molecular biological strategy is helping our comprehension of some herb metabolites and how they are used in the area of physiology. Monoterpenes, which are widely distributed in nature with around 400 structures, constitutes a satisfactory precursor basis. Transformations of composition and the amount from industrial production of aroma area by genetic engineering should have an impact in the commercial sector. In recent years, substantial progress has been made in biotechnology and in the genetic area; in particular, the progress of the molecular biology apparatus has been used to discover a lot of biosynthetic routes. The combination of this expertise with the apparatus accessible for the genetic area and metabolic engineering nowadays opens a large possibility to obtain new pathways for the generation of herbal compounds. This approach has been mainly applied to the obtention of high-value pharmacological actives (Prins *et al.*, 2010; Muñoz-Bertomeu *et al.*, 2007; Krings & Berger, 1998; Rhodes, 1994; Gershenzon, 1994).

1.1.2 Factors Influencing the Quantity and Quality of Essential Oil in Plants

The essential oils market is in constant expansion, moving tens of millions of dollars annually (Marques *et al.*, 2012). The greatest producing countries are Brazil, India, China and Indonesia, and the greatest consumers of essential oils are the United States (40%), European Union (30%) and Japan (7%). In addition to its employ as the main raw material for aroma and fragrance, food and pharmaceutical industries (Prins *et al.*, 2010), essential oils have important biological activities that have been disclosed in recent years. However, as it seeks its practical application and the development of new natural drugs containing active compounds from essential oils, there is an urgent need to standardise the plant material source. For this to become achievable, it is necessary to know all factors that exert influence on the production of essential oils by plants (Prins *et al.*, 2010).

Medicinal and aromatic plants produce essential oils as secondary metabolites in response to a physiological stress, pathogen attack and ecological factors. The stress caused by physical and chemical environmental conditions, for example, may exert great influence in plant's capacity to produce these metabolites. As regards the interactions of the plant-pathogen, essential oils are recognised as defence compounds that confer protection against several natural enemies, but they also facilitate the reproduction of the vegetal species by attracting pollinators (Prins *et al.*, 2010; Marques *et al.*, 2012). Thus, there has been suggested a dual role of these compounds on alleviating both environmental and pathogen attack stresses (Tezara *et al.*, 2014).

In order to optimise its commercial exploitation, the different factors involved in the production of essential oils must be taken into account, since the induction into its substances synthesis could affect the specific compounds of interest and their economic applications, besides affecting the quality and quantity of produced oil.

The main factors related to the essential oils production are discussed in the following sections.

1.1.3 Pathogens Attack

It has been demonstrated that volatile compounds operate as plant defence against animals, microorganisms and insect herbivores (Koeduka *et al.*, 2006; Sardans *et al.*, 2010; Fürstenberg-Hägg *et al.*, 2013; Cabral *et al.*, 2013). The plant-insect interactions have led the plants to develop various defence strategies against insect feeding (Paré & Tumlinson, 1999; Fürstenberg-Hägg *et al.*, 2013). These defence mechanisms can be constitutive or inducible. Some internal signals from affected tissues of the plant include calcium signalling, enzymes phosphorylation and jasmonate signalling pathway. These affected tissues and reinforce the production of low molecular weight compounds, such as the substances found in the essential oils, which are bioactive insect repellents or intoxicants. Still, these released volatiles attract several kinds of predators, through the connection from leaves or plants and to induce protection mechanisms. Moreover, a great number of these substances are produced to avoid future attacks.

The plants can release more than 1000 volatile organic compounds mainly consisting of 6-carbon aldehydes, alcohols, esters and several terpenoids from their different parts (Gobbo-Neto & Lopes, 2007; Fürstenberg-Hägg *et al.*, 2013). A curious fact is the way

by which the plant responds to pathogen attack, producing larger amounts of a specific compound that may also vary depending on the kind or specie of the intruder, or on the plant part invaded. Valladares *et al.* (2002) analysed the alterations in composition of the essential oils and volatile release from *Minthostachys mollis* (Kunth) started by two types of herbivorous insects — a leaf miner and a gall insect. The authors observed a reduction in the pulegone concentration that was associated with both kinds of insect damage, while the menthone content significantly increased only in mint leaves. It was also verified by Huang *et al.* (2012) that the majority volatile compound emitted from *Arabidopsis thaliana* (L.) Heynh. flowers, the (E)- β -caryophyllene is synthesised as a defence against *Pseudomonas syringae* pv. tomato, a pathogenic bacterium of brassica-ceous plants. In these cases, the compound, (E)- β -caryophyllene, appears to serve as defence against pathogens that attack floral tissues and, like other floral volatile compounds, can have multiple roles in plant protection and pollinator attraction. Still, according to authors, flowers have a high risk of pathogen attack because of their rich nutrient and moisture content, and high frequency of insect visitors.

1.1.4 Environmental Factors

As mentioned before, several physical and chemical environmental factors can affect the quality and quantity of essential oils produced by medicinal and aromatic plants. Amongst these factors cited are the temperature, hydric and osmotic stress, relative humidity, photoperiod (light), nutrition (fertilisation), seasonality, soil properties (salinity, pH, chemical composition, toxins), genetic characteristics and harvest time (Abdelmajeed *et al.*, 2013). The expression of plants' secondary metabolism is also an answer to mechanical factors such as injuries, rain, hailstones, wind and sandstorms (Gobbo-Neto & Lopes, 2007; Fürstenberg-Hägg *et al.*, 2013).

Studies focusing on the effects of temperature on the production of essential oils by aromatic plants have shown that variations throughout the year, month or even day exert great influence in the plant development and hence affect the secondary metabolite production. In general, the production of essential oils increases at elevated temperatures, although it can lead to an excessive loss of these metabolites on very hot days (Lima *et al.*, 2003; Gobbo-Neto & Lopes, 2007). However, it has also been observed in a mobilisation and accumulation of certain plant metabolites in *Zea mays* L. (Christie *et al.*, 1994; Gouinguéné & Turlings, 2002), *Artemisia annua* L. (Wallaart *et al.*, 1999) and *Nicotiana tabacum* L. (Koeppel *et al.*, 1970) after submission at very low temperatures.

Although several studies approach the effects of temperature on the morphology and oil yield in plants, little information is available in literature about its effects in the volatile oil composition. Chang *et al.* (2005) observed an increase of three times on the levels of volatile oil in fresh leaves of *Ocimum basilicum* L. (basil) grown for two weeks at 25°C or 30°C, against leaves of plant cultivated at 15°C. The different temperatures also altered the chemical composition of the compounds present in the leaves. At 25°C there was an eugenol and *cis*-ocimene cumulation, whereas at 15°C a highest content of camphor and trans-farnesene was observed. In coriander, the highest oil percentage was also detected under stress temperature (Farahani *et al.*, 2008).

However, even small temperature increase of two to three degrees during the day could significantly raise the levels of essential oil in *Mentha piperita* L. (Bernáth, 1992).

The temperature effects on the production of secondary metabolites are also related to other factors, as latitude and seasonality. Seasonal fluctuations in the content of the different secondary compounds produced by medicinal and aromatic plants, including essential oils were reported. The seasonality, in turn, has a direct influence on the biomass production since vegetative development and foliar biomass are the key points for essential oil production and aromatic plant harvest (Marques *et al.*, 2012). Thus, in order to achieve highest essential oils yields, it is suggested that the harvest must be performed in the season in which higher essential oil contents are observed.

Silva *et al.* (2005) evaluated the effects of harvest season on the production and analytes present in the essential oil of *Ocimum basilicum* L. (basil) during six months. Higher yield in essential oil was obtained in January (summer – 2.26%) than in August (winter – 1.06%). However, a decrease of linalool was observed in the oil obtained in January. Alterations in the levels of the majority compounds of *Achyrocline satureioides* (Lam.) DC. (Macela), that is, trans-pinocarveol, linalyl acetate and β -pinene acetates were also observed for the oil derived from the floral capitulum originating at several harvest times (Bezerra *et al.*, 2008). Previous studies also have reported differences in the levels of essential oils obtained in different seasons. From a study about the composition and yield of the oil from *Salvia officinalis* L. grown spontaneously in Dalmatian Island, it was observed that the yield of the majority compounds, thujone, 1,8-cineole and camphor varied greatly. The amount of essential oil was higher in July, while the production of thujone was increased in October (Pitaverec *et al.*, 1984). Clones from this species on culture conditions produced maximum essential oil yield and high content of thujone in July, season of great luminosity (Putievsky *et al.*, 1992). On the other hand, in a study about the seasonal evolution in the oil composition of *Virola surinamensis* (Rol. ex Rottb.) Warb., no variation on oil yield was observed in the different seasons and times of harvest evaluated, but the relative percentage of the compounds was significantly altered (Lopes *et al.*, 1997).

The effects of day length and photon flux density on *Mentha piperita* L. oil composition have been studied by several authors in order to differentiate between photoperiodic and photosynthetic influences (Voinin *et al.*, 1990). Some authors (Grahle & Holtzel, 1963; Clark & Menary, 1979) concluded that 'the effects were caused by photoperiodic treatment rather than by differences in photosynthate between short day and long day conditions'. Also, was observed a relation between daytime photosynthesis and nighttime utilisation of photosynthate, which was directly influenced by the oil composition and the main interacting factor was the temperature (Burbott & Loomis, 1967).

In order to vouch essential oil yield and quality, it is very important to determine the environmental conditions and process factors that affect its composition. In the case of the essential oil from *O. basilicum* L. (basil), which is commonly used as flavouring and in cosmetics due to its high concentration in linalool and its value in the international market, the effects of harvesting season, temperature and drying period on its yield and chemical composition have been investigated (Carvalho-Filho *et al.*, 2006). Harvestings performed at 40 and 93 days after transplanting of seedlings showed higher essential oil yield when were carried out at 8 a.m. and at noon. After drying for a period of five days, the contents of linalool increased from 45.18% to 86.80%. These findings indicate that the ideal conditions for *O. basilicum* L. harvest is during morning and the biomass drying at 40 °C during five days aiming to get linalool-rich essential oil.

During the investigation of the daytime variation of the essential oil of four medicinal species in the central region of Iran, the yield of the essential oil from the *Eucalyptus nicholii* Maiden and Blakely leaves, *Rosmarinus officinalis* L., *Thuja occidentalis* L. and *Chamaecyparis lawsoniana* (A. Murray) Parl. also shows seasonal and diurnal variation if obtained at 7 a.m., at noon or at 6 p.m. (Ramezani *et al.*, 2009) which reinforces the conclusion that for obtaining the highest yields of essential oil and other volatile compounds, harvesting of plant material must be accomplished at special time during the day.

Sandeep *et al.* (2015) also emphasised the need for standardising and control of environmental and ecological factors for optimisation of plant compounds production. The secondary metabolites of turmeric (*Curcuma longa* L. cv. Roma) such as essential oil, oleoresin and curcumin are used for multipurposes in medicine, cosmetics food flavouring and textile industries. The curcumin content may vary from place to place as function of environment, soil and agro-climatic conditions. Plants cultivated in several agro-climatic regions showed a variation of 1.4% to 5% of curcumin. The variation in the majority compounds of the essential oil, that is, tumerone and α -phellantrene in all the areas were about 10% to 20%.

The effects of environment conditions on the content of main metabolites may improve crop yield and quality of production, as demonstrated for numerous plant species (Formisano *et al.*, 2015). *Matricaria chamomilla* L. (german chamomile) is produced by large-scale cropping due to its medicinal and industrial importance. A study about the effects of environmental conditions on crop yield and on the chemical profile of the essential oils of different chamomile genotypes, which were cultivated in Molise (Italy) in different growing environments revealed that the crop yield was strongly influenced by the soil conditions and climate, and mainly by the altitude, fertility and the water supply increasing the chamomile productivity. Still, the chemical analysis of the essential oils shows the compounds *scis*-tonghaosu, spathulenol, α -bisabolol oxide B and α -bisabolol oxide A as main constituents in all samples, but their amounts in each plant varied significantly (Formisano *et al.*, 2015).

The market of the lavender essential oils is still increasing; however, it is not well understood how the environment and growth conditions can affect the ideal harvest period of its flowers for essential oil obtainment. Hassiotisa *et al.* (2014) evaluated how the essential oil quality and quantity of *Lavandula angustifolia* Mill. cv. Etherio can be influenced during blooming by environmental conditions. The authors investigated the relationship between the lavender essential oil production and the gene expression during blooming, aiming to determine the optimum period for essential oil harvest. The authors verified that essential oil content was regulated positively by temperature and flowering stage, while it was negatively influenced by pluviosity during the flowering period. The desired profile of the essential oil with respect to the content of linalool was influenced by temperature, flower development, LaLINSgene expression (involved in the biosynthesis of essential oil) and pluviosity. Although pluviosity has significantly decreased the linalool content, the standard quality of lavender essential oil has been normalised after 10 days, suggesting that linalool production is regulated during blooming period for both environmental and growth factors. It was also proposed that the optimum harvest time to obtain rich essential oil is when the lavender reaches 60% of blooming, it is over 26°C and that it does not rain for a period of 10 days before harvesting.

1.1.5 Hydric Stress

Water is essential for life and for the metabolism of plants. So it would be logical to assume that the production of secondary metabolites would be higher in humid environments. However, this does not always occur (Morais, 2009). The hydric factor significantly impacts the growth evolution of the whole plant, and its frequency and intensity are elements of great importance for the limitation of global agricultural production (Ortolani & Camargo, 1987). Many physiological factors such as opening and closing of stomata, photosynthesis, growth and leaf expansion may change when the plant undergoes drought stress, which can cause alterations in the secondary metabolism.

According to Abdelmajeed *et al.* (2013), drought stress is induced by a restriction of the water supply that affects the leaf water potential and turgor decrease, stomata shutdown and a decrease in cell extension and growth. The hydric stress can decrease the relative plant water content from 77.7% (Rahbariana *et al.*, 2010). The decrease in plant development is a consequence of its impact on different physiological and biochemical processes, such as photosynthesis, respiratory chain, translocation, ion uptake, carbohydrates, nutrient metabolism and growth promoters.

The excess of water on the ground can change chemical and biological processes, limiting the amount of oxygen and accelerating the formation of toxic compounds to roots. On the other hand, severe percolation of water causes the removal of nutrients and inhibiting the normal growth of the plant. Water surplus, while important, causes fewer problems than the dry periods (Ortolani & Camargo, 1987). Several authors reported an increase in certain plant metabolites under hydric stress conditions, mainly the content of terpenoids (Ortolani & Camargo, 1987). The increase of terpenoids under water stress seems to occur due to a low allocation of carbon to the plant development, suggesting a trade-off between growth and defence mechanisms (Turtola *et al.*, 2003). Under moderate and several water stress, an increase in thymol content was observed in origanum (Bahreininejad *et al.*, 2013). Furthermore, it was reported for three different origanum varieties that the water deficit after early flowering (double flowers) can lead to an increase in the essential oil levels and may result in a higher quality of origanum plants and the highest water recovery effectiveness by this plant (Aziz *et al.*, 2008).

Important reductions in the growth parameters under water deficit, such as vegetative growth, lipids content and essential oil level were observed in *Salvia officinalis* L. (Belaqziz *et al.*, 2009) and *Cuminum cyminum* L. (Rajeswara, 2002). The results suggested that water shortage might act in the regulation of the production of bioactive constituents in cumin seeds, interfering in their nutritional and economic values.

In chamomile, the effects of drought stress were decreasing of plant size, number of flowers, shoot weight and apigenin level, but did not affect the essential oil quantity or its composition (Baghalian *et al.*, 2011). Also, water stress caused a significant decrease in all development parameters of *Mentha piperita* L. and in turn the essential oil productivity. Highest levels of menthol were achieved under 70% of biomass production capability (Farahani *et al.*, 2009). On the other hand, the drought influenced the essential oil content in *Nepeta cataria* L. (lemon catmint) and *Melissa officinalis* L. (lemon balm), resulting higher amount of essential oils (Iness *et al.*, 2012).

Another important factor that can change the yield and chemical composition of essential oils is the rainfall (Morais, 2009). Constant, intermittent rain can result in the loss of hydrosoluble substances mainly from leaves and flowers. It is recommended to wait about three days after the end of the rains for the plant collection, so that the essential oil levels return to normal.

Therefore, hydric stress, that is, water deficit or excess is one of the most important factors involved in the synthesis of essential oils by medicinal and aromatic plants, since they can alter significantly the plant growth, as well as to modify the composition and concentration of metabolites present in the essential oils, being the major cause of lost productivity; it is directly correlated with the secondary metabolites concentration.

1.1.6 Plant Nutrition

According to Marques *et al.* (2012), amongst all the factors influencing plant activities, nutrition requires the greatest attention since the excess or lack of nutrients may be directly related to the variations in the active substances production. This also depends on the interaction with other factors such as water availability and soil physical properties.

An increase in the synthesis of essential oils as the increase at nitrogen doses, between 3.4–13.8 kg.ha⁻¹, have been reported for three *O. basilicum* L. (basil) cultivars (Sifola & Barbieri, 2006). However, the differences in relation to the oil quality amongst the plants appear to be unrelated to the supplies containing nitrogen. On the other hand, some authors observed that formulas containing organic and inorganic N sources allowed optimal plant performance and have incurred higher yields in essential oils. Still, the mixtures altered the chemical composition of the essential oils, decreasing the amount of linalool and increasing the methyl chavicol content (Singh *et al.*, 1991).

Teles *et al.* (2014a) observed that plant nutrition did not impact the biomass and essential oil synthesis of *L. origanoides* Kunth plants. However, some changes have been observed in the essential oils composition, as well as changes in the antioxidant activity of the oils. It was concluded that for growth and essential oil production, additional fertilisation are not required for *L. origanoides* at the employed conditions; however, in order to increase the biological activities of this essential oil, organic fertilisation is suggested. After the authors studied the chemical composition, antioxidant activity and production of essential oils from *L. origanoides* Kunth plants cultivated under organic and mineral supplementation, and harvested at different development stages (Teles *et al.*, 2014b). The production and composition of essential oils were not affected by fertilisation, but their composition and antioxidant activity were influenced by the plant age, being that essential oils from grown plants showed lower antioxidant activity than young plants. In the other hand, the essential oil produced from organic fertilisation showed better antioxidant potential when compared with mineral fertilisation. In conclusion, these results show the potentialities and properties of *L. origanoides* essential oil in specific conditions as showed before indication that early harvest of and organic nutrients could be applied to increase plant activities.

Pelargonium graveolens L'Her., a significant economic crop and source of geranium essential oil have been studied as to answer to nutrient handling on culture productivity, biochemical parameters, essential oil production and profile, nutrient content and

antioxidant activity during 2012 and 2014 in Lucknow, India (Pandey & Patra, 2015). The use of different blends from poultry manure as organic fertiliser plus a chemical treatment were able to enhance plant stature, leaf area, essential oil yield and antioxidant activity when compared to control. Besides, the percentage of the majority oil compounds increased in the blend application of organic and chemical fertilisers. These results allowed to conclude that combined application of 50% of each nutrient kind (75:30:30 N:P:K kg ha⁻¹ and 2.5 kg ha⁻¹ poultry manure) incurred in an important increase of the plant growth parameters and oil yield with better flavour profile, enhanced antioxidant activities and improvement of soil characteristics.

1.1.7 Genetic Factors and Chemical Diversity

As previously mentioned, the total content as well as the relative ratios of essential oils in plants may vary depending on various factors, including the stage of plant development. Although a genetic control for the expression, this may also be influenced, according to Marques *et al.* (2012) by modifications resulting from the interactions of biochemical, physiological, ecological and evolutionary processes. Generally, the chemical diversity of secondary compounds does not only occur between and within plant families and genera but also within populations of a single species, forming chemotypes (Kleine & Müller, 2011). Thus, the synthesis of secondary metabolites in populations of the same species can vary.

In one of the pioneering works, the content and constitution of oils of 10 peppermint genotypes were analysed during three different years (Gasic *et al.*, 1987). The parameters varied both in relation to year of investigation and the genotypes assayed and the genotypes showed a specific response to environmental conditions.

More recently, several studies have focused on the geographic region and chemical variety of plant populations, but whether this structure conforms to a central-marginal model or a mosaic pattern has not been elucidated (Bravo-Monzón *et al.*, 2014). According to Bravo-Monzón *et al.* (2014), the evaluation of the chemical variety of weeds in their native habitats facilitates the knowledge of their relationships with natural predators. Thus, the authors evaluated the geographic fluctuation of diversity in Mexican populations of the bittervine weed *Mikania micrantha* Kunth and its relationship to herbivore damage. A stepwise multiple regression analysis was used to establish a relationship between geographic, climatic and chemical diversity variables and damage by herbivores. However, population-level chemical diversity was the only significant variable. The authors concluded that fluctuations in chemical diversity follow a mosaic pattern in which geographic factors or natural enemies present some effect and that is also correlated with the predators' attack.

The geographic difference had a important effect on the oil concentration in 12 genotypes or ecotypes of *Zataria multiflora* Boiss. from several regions (Sadegui *et al.*, 2015). This plant is popularly used as a spice in Iran. Three ecotypes show maximum oil yield, with a maximum difference of 1.09% between the populations. The determination of the main compounds in relation to quantity of essential oil components have allowed the recognition of three chemotypes: carvacrol, thymol and linalool of which the thymol chemotype is the most common in different parts of Iran. The chemical variability was related to genetic and environmental factors.

In accordance with Tezara *et al.* (2014), differences in terpene production amongst three chemotypes of *Lippia graveolens* Kunth. (Mexican oregano) growing wild (rainy season) and in a common garden in the Yucatán peninsula were generally unrelated to either site of origin, rainfall or chemotype, although amongst the four populations selected for physiological measurements in the field, the T (tymol) populations produced more terpene than the S (sesquiterpenoids) populations. The highest terpene content was found in plants from the high-rainfall T population with the highest photosynthetic rate. Terpene content, which was higher in plants sampled in the field than in the common garden, was determined by photosynthetic rate and water use efficiency, and only by photosynthetic rate in the garden. According to the authors, the underlying reasons for the difference between the results obtained in the field and common garden are apparently not microclimatic.

Knowing the variations in the chemical compositions of different genotypes of a plant is of commercial importance as we seek to use them in different industrial segments.

1.2 Conclusions

The present chapter shows that through knowledge of the chemical characteristics and the main factors involved in the production of essential oils by plants, it is possible to standardise the plant material, and therefore the essential oil composition. This is primordial to achieve adequate quantity and quality, mainly in terms of chemical oil composition, in order to its employment for commercial purposes and supply market demands.

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