

## Biosynthetic Factories of Essential Oils: The Aromatic Plants

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### Abstract

Essential oils are industrially important natural products of aromatic plants, which are biosynthesized in specialized cells types, such as osmophores, Glandular Trichomes, and ducts and cavities, present on different parts of these plants. In these cells, the essential oils are biosynthesized, accumulated and transferred to the atmosphere by different reported secretion mechanisms. Numerous environmental factors also affect biosynthesis of essential oils and their secretion in the atmosphere. A deep understanding of the entire process is important to improve the yield of biomass of aromatic plants and thus produce large quantities of commercial volatile oils. In this paper, the recent research on types and structure of essential oil-bearing cells, secretion mechanisms, and effect of different environmental factors on essential oil biosynthesis have been comprehensively reviewed. Therefore, this review article will provide new research directions for the researchers working in the field of aromatic plants physiology, essential oil production and many others applications.

**Keywords:** Aromatic plants cells; Cell organelles; Osmophores; Glandular trichomes; Environmental factors

### Introduction

The aromatic plants have been used by humans as valuable ethnomedicines for a long time due to the presence of the important secondary metabolites (i.e., essential oils). These essential oils are important organic molecules because they have natural essence and are biosynthesized in specialized cells of aromatic plants [1-3]. These cells have a central role in essential oil biosynthesis, accumulation, and secretion into the atmosphere; as a result, they are the natural factories for essential oil synthesis. The study of chemical composition of essential oils has revealed that these oils are comprised of highly functionalized chemical classes including monoterpenoids, sesquiterpenoids, phenylpropanoids, etc. [4].

The aromatic plant cells that secrete essential oils are very diverse in morphology, ranging from highly specialized trichomes to non-specialized cells, osmophores, and secretory cells of petal's epidermis. Previous studies have shown that ducts, cavities, secreting trichomes, conical-papillate cells, and other essential oils secretory tissues usually possess specialized cellular structures [5-10].

The pathways of biosynthesis of the essential oils and other aromatic compounds are recognized in these various types of cells. The granulocrine and eccrine mechanisms are two different mechanisms of secretion, proposed to be responsible for the secretion of essential oils. This fact is supported by studies showing that both mechanisms could exist for different compounds and different plants [11,12]. Furthermore, research shows that different environmental factors affect the yield and constituents of these essential oils [13]. In this paper, studies conducted on the specialized essential oil-secreting cells, their cellular structure, the secretion pathways, and environmental factors affecting biosynthesis of these oils have been reviewed comprehensively. Comprehensive review of the cellular structure of essential oil-secreting cells has been addressed in this paper to meet the urgent need to incorporate updated information. Taken together, this review will have a high impact on future study of aromatic plants as biosynthetic factories of essential oils, granting new opportunities in this expanding field.

### Essential Oil-secreting Cells in Aromatic Plants

Historically, essential oil plants have been valued for their medicinal, culinary, and fragrant properties and possess various biological

applications [14]. The production of essential oils in plants is generally associated with the presence of specialized secretory structures. After the formation within the plant cells, these oils are also released into the atmosphere by secreting cells such as osmophores, conical-papillate cells, glandular trichomes, ducts, cavities and occasionally non-specialized cells [11]. The structures of these cells can be characterized by state-of-the-art instruments, including light microscopy, scanning electron microscopy (SEM), and transmission electron microscopy (TEM) [15-17].

### Osmophores

The term osmophore, where "osmo-" means "odor" and "-phore" means "bearing", was established in 1962 to describe an enclosed area of floral tissue that is specialized in scent emission. Osmophores, also called floral fragrance glands, are specialized clusters of cells in flowers, and are distributed on sepals and petals to attract insect pollinators [15]. Osmophores consist of a multilayered glandular epithelium with homogeneous layers of cells, except in the 18 species of *Stanhopea* and *Sievekingia*, in which osmophores have epidermal cells that are morphologically different from the subjacent cells [16,18]. These cells contain dense cytoplasm, enormous deposits of starch, or other storage compounds within the mesophyll. These deposits are usually missing in epidermis cells. This generates a distinction between the production and the emission layer [18].

There has been success in the study of mechanisms of fragrance emission via osmophores. Osmophore cells look similar to the conical-papillate cells that can be found on the whole epidermis of petals in more than 200 species [19], including *Rosa x hybrid* (Figure 1), *Stanhopea* and *Sievekingia* [16], *Galanthus nivalis* [17], Araceae, Orchidaceae, and even the model-plant *Arabidopsis thaliana* (Brassicaceae) [5].

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This conical-papillate shape is known to offer a very large surface area for evaporation and participate in the reflection of light. The *MIXTA* gene, giving rise to the conical shape, has been cloned in *Antirrhinum majus* (*Scrophulariaceae*). Surprisingly, its overexpression in 35S::*MIXTA* *Nicotiana tabacum* (*Solanaceae*) leads to ectopically secreting trichomes on the whole plant, suggesting a relationship between conical-papillate cells and the differentiation of secreting trichomes [20].

Cytoplasmic lipid inclusions and plastoglobuli in amyloplasts are two features presumably associated with fragrance production that showed sufficient variation for inclusion here as potentially useful characters. Plastoglobuli are present in the amyloplasts of both species of *Sievekingia* and most of the species of *Stanhopea* [16]. In flowers of *Galanthus nivalis*, the osmophores have the structural features of polarization of the epidermal cell protoplasts, large cell nuclei, and large vacuoles with heterogeneous contents in the peripheral part of the cells [17].

In *Stanhopea graveolens* and *Cycnoches chlorochilon*, the osmophores are positioned in the basal part of the labellum. The area of fragrance emission increased due to the wrinkled surface of the osmophores. The remnants of secretion are noticeable on the surface of the epidermis in *S. graveolens*, but these are missing in *C. chlorochilon* [15].

### Glandular trichomes

Most plants have hairs, called trichomes, on their surface that serve a number of functions ranging from protection against insect pests to heat and moisture conservation [21]. Trichomes occur in plants in a great variety of forms and are sometimes very structurally complex [22]. Two main types of trichomes can be distinguished: non-glandular and GTs [23].

GTs (Glandular trichomes) are hairs present on the epidermis and have cells that are specific to the biosynthesis and emission of abundant quantities of specific secretory products, such as nectar, mucilage, acyl lipids, digestive enzymes, or essential oils. These secreting trichomes are very numerous and have very different morphologies in the plant kingdom [24]. GTs contain or secrete a mixture of chemicals that have been found to have an enormous array of uses in the pesticide, pharmaceutical, and flavor/fragrance industries. In addition to these industrial uses, GTs on some crop species confer resistance against insect pests [21]. Thus, today there is an increasing interest in understanding the chemistry of glandular trichome exudates and taking advantage of their potential uses [24].

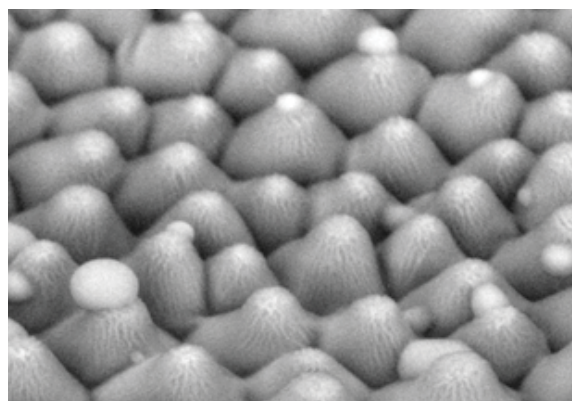
GTs are present in numerous monocotyledons plants, together with the members of the *Tradescantia* [6], *Dioscorea* [25], and *Sisyrinchium* [7]. In the eudicots, GTs are more prevalent and are unique vegetative epidermal features of many families and genera including the members of the *Lamiaceae*, *Asteraceae*, *Sphaerosepalaceae*, *Caryophyllaceae*, *Cucurbitaceae*, *Fabaceae*, *Rosaceae*, *Sapindaceae*, *Saxifragaceae* [24], and *Cannabaceae* [13]. Metcalfe and Chalk provided a more complete list of the distributions of GTs of various morphological types in dicotyledons [24].

GTs can be branched or unbranched, sessile, elongated, or short. Usually, a group of glandular cells is present at the tip of a stalk of one or more cells in length. Frequently, a thick cuticle layer on the glandular cells detaches itself from the attached cell wall to form a subcuticular pocket in which secretions accumulate [6,11,13,26-28].

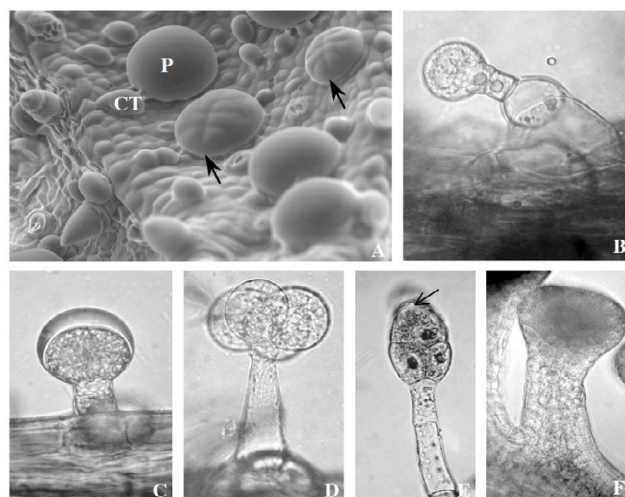
GTs are widespread in some genera of ferns [29,30] and have been

found attached to fossil seed fern leaves [31], however, appear to be absent from conifers. Within the angiosperms, apart from hydropotes and related GTs of some Nymphaeales, GTs are not present in *Amborella*, *Nymphaea*, *Illicium*, *Trimenia* and *Austrobaileya* [8,32].

GTs are often illustrated as randomly spread over surfaces of plants, although vigilant observations point out that this is usually not the case. In peppermint, for example, peltate GTs appear more or less evenly spaced and is very infrequently found as pairs or clusters. They are separated from each other by a similar number of epidermal cells, but have predictable densities within different regions of a leaf [33]. Recent studies have recognized networks of transcription factors that appear to act together as activators or inhibitors of trichome initiation and maturation in protodermal cells [24]. The diversity of secretory trichomes among the *Lamiaceae*, *Solanaceae* and *Rosaceae* is shown in Figure 2.



**Figure 1:** Environmental electron micrograph of the petal epidermis of *Rosa x hybrida* (Gr. X 600). The essential oil droplets seem to gather together due to environmental conditions in the microscope chamber [11].



**Figure 2:** Diversity of secretory trichomes among the *Lamiaceae* (A, B, C), *Solanaceae* (D, E) and *Rosaceae* (F): Environmental electron micrograph of peltate and capitate glandular trichome of *Mentha piperita* (A, Gr. x 400). Note the shadow (arrows) of the 8 head-cells before the secretory phase of peltate glands. Light micrographs of secretory trichomes of *Ajuga reptans* (B, Gr. x 400) with its thick stalk, *Lamium maculatum* (C, Gr. x 400) with the subcuticular oil droplet, *Lycopersicon esculentum* (D, Gr. x 400) with the 4 head-cells, *Nicotiana tabacum* (E, Gr. x 400) with the sticky secretion (thin arrow) and *Rosa x hybrida* (F, Gr. x 100) with the numerous cells [11].

Dai et al. presented an integrated “omics” database, TrichOME, to make possible the study of plant trichomes. A large volume of functional omics data is present in the database, and as a result is a precious and distinctive source for plant trichome research, because the genes and metabolites expressed in trichomes are often underrepresented in regular non-tissue-targeted cDNA libraries. TrichOME is freely available at <http://www.plantrichome.org> [34].

### Ducts and cavities

Secreting cells such as ducts and cavities often excrete gum, resin, paste or glue. For example, in conifers, diterpenoid resin acids are present in ducts and dissolved in volatile turpentine. Upon injury, the turpentine evaporates, and the resin forms a crystalline mass that may trap pathogens and insects [35]. Ducts and cavities are present in different plant families such as Apiaceae [36,37], Compositae [9], or Rutaceae [38], Heliantheae [39], and Rubiaceae [40]. Secretory ducts and non-GTs also exist in the leaves and stems of *Baccharis* species [41].

Surprisingly, numerous plants emit volatile organic compounds (VOCs) by non-specialized cells. For example, in the Brassicaceae, there are no specialized secretory tissues. Nevertheless, it was shown that volatile monoterpenoids and sesquiterpenoids are emitted from the green leaves of these plants directly or after injury. These VOCs may be emitted for the defense of the plant [42-44]. Only the liverworts (*Hepatics*), in the Bryophytes, contain oil bodies and biosynthesize large amounts of essential oils. Liverwort oil bodies are intracytoplasmic secretory structures bound by single membranes that originate from the dilatation of endoplasmic reticulum cisternae [10].

### Cellular Structure of Volatile Secreting Cells

Studies show that ducts, cavities, secreting trichomes, conical-papillate cells and other tissues that secrete essential oils and volatile organic compounds (VOCs) usually contain small vacuoles, dense cytoplasm and numerous mitochondria. Leucoplasts, plastoglobules and unusual figures of reticulum or dictyosomes, like periplastidial reticulum, smooth tubular reticulum, myelin-like lomasoma and osmiophilic vesicles or cisternae for example, are also sometimes observed [5-10].

The epidermal cells of osmophores have a visibly nonporous and mainly smooth cuticle, but cuticular blisters are observed. The secretory tissues consist of epidermal cells, numerous layers of small, sub-epidermal glandular parenchyma, large nuclei, and dense cytoplasm with numerous endoplasmic reticulum profiles. Many large idioblasts are observed with phenolic content and/or raphides in the secretory and ground parenchyma. In some parts of the osmophore tissue, lipids almost completely fill the cells. TEM observations [15] indicate the presence of large lipid droplets, mostly close to the outer wall of the epidermal cells. In the vacuoles, osmophilic precipitates and small vesicles are present. The presence of plasmodesmata is common in the walls of the epidermis and the adjoining parenchyma. In the cell walls of the osmophore cells, numerous pits with plasmodesmata occur to take part in symplastic transport of the aroma compounds [5,16,17].

Multiple of cells are present in the stalks of glandular trichomes. They often contain one or more sets of barrier cells that have noticeably impregnated lateral walls. It has been suggested that the impregnated cell walls permit the stalk cells to control directional transport of metabolites to the glandular cells above [28,45]. Stalk cells of peppermint peltate GTs have strongly impregnated lateral walls, but their ultrastructure suggests additional unknown functions. These peppermint cells are metabolically active at emission stage and

have several big, non-green, spherical plastids differing significantly in shape from the leucoplasts of the glandular cells. They also contain comparatively tiny vacuoles, several mitochondria and a comparatively large quantity of microbodies [33]. These specialized features were also reported for stalk cells of *Nepeta racimosa* L. and were found to be associated with the secretory phase of glandular trichome development [46].

### Role of leucoplasts and microbodies

Plant glands emitting hydrophobic essential oils and resins share a number of common features. They frequently enclose amoeboid leucoplasts that are occasionally bound by an enclosing layer of periplastic smooth endoplasmic reticulum. Several contacts of plastid and smooth endoplasmic reticulum membrane are observable [9,13,39,45,47].

Since the 1960s, leucoplasts have been linked to plant oil secreting glands and resin-secreting glands and their role in monoterpene biosynthesis has been well-recognized. Carde et al. comprehensively described the development, structure and function of plant oil glands leucoplasts from resin ducts, secretory cavities and GTs [24,48]. The leucoplasts of resin ducts and secretory cavities were found to be very analogous to those present in glandular trichomes, although only some of the leucoplasts they examined were from monoterpene-secreting trichomes [48].

In 1983, Gleizes et al. demonstrated that when provided with the terpenoid precursors IPP (isopentenyl pyrophosphate) and DMAPP (dimethylallyl pyrophosphate) *in vitro*, the isolated leucoplasts from secretory cavities of the exocarp of *Citrofortunella mitis* fruits were able to synthesize monoterpene hydrocarbons [49]. Then Chenicet and Carde carried out a large comprehensive study involving 45 species and showed that there is a strong correlation between the presence and volume of leucoplasts in gland cells and the quantity of monoterpenes in the secretion produced by the cells [48]. The study about the morphology of developing leucoplasts in *Pinus pinaster* resin ducts revealed that these leucoplasts enlarge continuously to form large complex structures with large central bodies having many deep pockets and small branches, which were all originated from a small number of proplastids present in newly-formed gland cells. The large leucoplast surface in a pine resin duct gland cell was in close contact with covering periplastic endoplasmic reticulum [50].

The microbodies in the cells of the peltate oil hairs of *Origanum dictamnus* (Lamiaceae), the oil cavities of *Citrus deliciosa* (Rutaceae) and the oil ducts of *Apium graveolens* (Apiaceae) have been studied to elucidate their unique structures. The microbodies appear in the cytoplasm of the head cells in the peltate hairs in a significant number during the stage of secretion and are globular to ovoid in shape with a 0.4  $\mu\text{m}$  average diameter. These have a single layer of membrane and their matrix consist of a loose specky substance with low electron density instead of being densely-arranged, fine granules as the typical leaf peroxisomes. These microbodies are dispersed and do not form close links with other cell organelles [51].

On the basis of ultrastructural, cytochemical and biochemical observations on origination of microbodies it has been concluded that the microbodies originated from the endoplasmic reticulum [52]. The particular enzymes involving in the biosynthesis of essential oil have been recognized cytochemically in the endoplasmic reticulum matrix [53]. So, this might provide a reasonable basis of the occurrence of the essential oil-biosynthetic enzymes within the microbodies. The association of the microbodies with the cytosol of the secretory cells in the oil glands studied might provide a ground of interpretation for a

possible presence of terpene biosynthetic enzymes in the microbodies. Supportive to these interpretations, the appearance of the microbodies during the stage of essential oil secretion demonstrated their relatively high numbers and divergences from the typical peroxisomes with respect to their structure and size [13,24,25,28,35].

### Role of the endoplasmic reticulum

The suggestion of the role for endoplasmic reticulum-leucoplast and endoplasmic reticulum-plasma membrane contact sites is persuasive for intracellular transport of terpenoids in the oil gland cells of plant. These membrane contact sites are common in plant essential oil-secreting cells. In many species, the extensive periplastic membrane contact sites found surrounding leucoplasts forms numerous clear associations between the smooth endoplasmic reticulum and the leucoplasts. Furthermore, previous studies suggest that membrane-contact sites are also common between the smooth endoplasmic reticulum and mitochondria as well as between the cortical smooth endoplasmic reticulum and the plasma membrane [15,18,27,28,47,53]. The physical properties of monoterpenes indicate that intracellular transfer of secreted monoterpenes could be facilitated by the numerous membrane-contact sites between the smooth endoplasmic reticulum and other organelles. Then these monoterpenes diffuse easily into outer leaflet lipid membranes [54,55]. Immunocytochemical localization experiments provided direct evidence that the enzymes catalysing the first committed steps of monoterpene biosynthesis reside within the stroma of gland cell leucoplasts [53,56].

### The role of plastids

The plastids of the glandular trichomes, osmophores, secretory cavities, and secretory ducts are often non-pigmented and amoeboid shaped, have few ribosomes, lack thylakoid membranes, appear to associate strongly with the presence of monoterpenes in the secretion product and hence are different from the chloroplast of neighbouring chlorenchyma tissue [48]. The plastids of glandular cells of some plants, including the GTs and apical glandular cells of some members such as the *Solanaceae*, *Asteraceae* [57] and *Nicotiana tabacum* L. [58] contain functional chloroplasts as well as copious amount of photosynthesis related enzymes. In contrast, the GTs of *Artemisia annua* L. contain five layers of cells, three of which contain well-developed chloroplasts and two of which contain leucoplast-like plastids, suggesting potentially different physiological specializations for the different cell layers [27,48]. Similar GTs with cells having chloroplasts and cells containing apparent leucoplasts come out to be widespread in the *Asteraceae* and have been illustrated from *Sigesbeckia* and *Helianthus* [59,60].

### The role of peroxisomes

The studies of different researchers have revealed that the enzymes of biosynthetic pathways of essential oils are present in peroxisomes. A study of Sapir-Mir et al. [61] revealed that the two isoforms of IPP (Isopentenyl pyrophosphate) isomerase, catalyzing the isomerization of IPP to DMAPP (Dimethylallyl pyrophosphate), are found in the peroxisomes. Further studies also showed the peroxisomal localization of the last two enzymes of the mevalonic acid pathway, including 5-phosphomevalonate kinase and mevalonate 5-diphosphate decarboxylase leading to IPP [62]. This result was emphasized by the later report, which revealed that using IPP and DMAPP as substrates, a short isoform of farnesyl diphosphate, is also localized to this organelle [63]. Hence, the classical compartmentation of isoprenoid biosynthesis between plastids and cytosol/endoplasmic reticulum within plant

cells can be reconsidered by including the peroxisome as an added isoprenoid biosynthetic compartment [64].

### Secretion Mechanism of Essential Oils

The transport of essential oils from one region to another within the plant cell or to outside the plant body occur by different mechanisms, but the two most significant mechanisms of secretion include granulocrine and eccrine mechanisms [65,66]. In granulocrine mechanisms, vesicles of reverse pinocytosis directly fuse with the plasma membrane or are surrounded and detached from the cytoplasm by invaginations of the plasma membranes [64,65].

On the contrary, the eccrine mechanism is the diffusion or active transport of oil droplets across membranes [65]. These mechanisms are studied by histochemical methods using Sudan stains, NaDi's reagent, Nile blue A and Fluoral Yellow 088 [65-67]. Other stains, such as nitrosophenol for monoterpenes phenols [68] and ferrous thiocyanate for sesquiterpenes [11] are also used occasionally. Such cytochemical studies are thus very helpful to locate oil droplets containing terpenes. Nevertheless, in numerous electron micrographs, vesicles of essential oils have been precisely located in specialized cells [66].

In secretory trichomes, mechanisms of oil secretion have led to a range of hypotheses. In *Origanum dictamnus* (*Lamiaceae*) [69], *Mentha x piperita* (*Lamiaceae*) [33] and *Lavandula pinnata* (*Lamiaceae*) [70] the operative secretion mechanism is eccrine. In *Nepeta racemosa* (*Lamiaceae*), *Artemisia annua* (*Apiaceae*) [46,71] and *Leonotis leonurus* (*Lamiaceae*) [72] plasmic membrane budding are involved in secretion whereas in *Prostanthera ovalifolia* (*Lamiaceae*) [68] clear granulocrine secretion mechanism is involved. In osmophores, ducts, and cavities, a granulocrine process is most often suspected with observations of oil droplets often originating not only from plastids, periplastidial reticulum and smooth reticulum but also sometimes from dictyosomes or other organelles [10,38-40,44,73].

The plant *Pterodon pubescens* Benth is a unique example of the involvement of holocrine as well as eccrine and granulocrine processes in the same secretory system, which is not common in other plant species. In secretory cavities, due to the presence of oil droplets adjacent to the plasma membrane of the epithelial cells and in the lumen, an eccrine secretion mechanism is suggested. In addition, a granulocrine secretion process is suggested due to the abundance of vesicles filled with dense contents in the peripheral cytoplasm and adjacent to the plasma membrane. The disruption of the dark epithelial cells followed by the release of their contents into the lumen cavity characterizes a holocrine secretion process [66].

In the family Pedaliaceae, two secretion mechanisms are reported in long and short trichomes of *Ceratotheca triloba*. A marked circular area in the upper part of each head cell of the long trichome is provided with micropores to secrete the secretory product directly onto the leaf surface by an eccrine pathway. The short trichomes secretion mode granulocrine and involves two morphologically and histochemically distinct vesicle types. One is the Ruthenium Red test positive small Golgi-derived vesicles for mucilaginous polysaccharides, and the subsequent type is similar to that of long trichomes and consists of dark large microbodies with low extent [66].

In the Leguminosae family, scent-secreting cells are distributed restrictedly on petals of *Caesalpinia pulcherrima*, *Anadenanthera peregrina*, *Inga edulis*, and *Parkia pendula*, comprising mesophilic osmophores and in a disperse way in *Bauhinia rufa*, *Hymenaea courbaril*, *Erythrostemon gilliesii*, *Poincianella pluviosa*, *Pterodon pubescens*, *Platygyamus regnellii*, *Mucuna urens*, and *Tipuana tipu*. The

mechanism of emission of essential oil from the petals of these plants is diffuse release [74]. In *Rosa x hybrida*, changes taking place during maturation of rose petal cells have been studied. Like in other secretory cells, plastoglobules are often observed. Furthermore, at the stage of maximal scent emission, tightly whorled structures, supposed to be lipophilic in nature, and other vesicular material of unknown function were observed. These vesicles could be associated with the cell wall and putatively concerned with the secretion of petal monoterpenes. It could well play a role in an eccrine process [75].

### Factors Effecting Essential Oil Biosynthesis

Although secondary metabolites in the medicinal and aromatic plants are controlled conventionally by their genotypes, their biosynthesis is strongly affected by factors of the environment, too. It means biotic and abiotic environmental factors affect the growth parameters, essential oil yield, and constituents of these oils [13]. Some of these factors are reviewed in comprehensive details in the following sections.

### Developmental stage of the plant

One of the most important factors that affect the essential oil biosynthesis is the dependence of the synthesis and accumulation of oil on the developmental stage of the related cells, tissues, organs and plants as well. The plants containing the leaves as a resource of commercially important essential oil differ in developmental stages of leaves, from the origin to growth to full maturity to finally loss through senescence, are principally vital. A close correlation between leaf development and oil biosynthesis and accumulation has been demonstrated in many aromatic plants of *Lamiaceae* family [76-78] and some of *Poaceae* [79] and *Asteraceae* [80] plants.

The stage of development also affects the composition of the essential oil. In *C. flexuosus*, citral, the chief component, reaches its maximum concentration at the twentieth day of development [79]. *Salvia officinalis* possesses camphor which is enhanced in concentration with leaf expansion [78]. In Japanese mint, the essential oil and main component of the oil, menthol, attain peak concentration at the stage of flower bud initiation. But individual leaves show a progressive decrease in oil percentage with the increase in dry matter and age of the leaf. The concentration of menthol in oil was increased up to the stage of leaf maturity and then started to decrease with further aging [81].

In *Artemisia annua*, the proportion of the major constituent, artemisia ketone, goes above 50% at the stage of peak flowering [82]. Studies conducted on *M. arvensis* [83,84] demonstrated that menthone is in peak concentration in young leaves and menthol in mature leaves. A similar gradual increase in monoterpene alcohols was also recorded in peppermint with advancement of leaf age [83,85,86]. The effects of the three maturity stages of lemongrass on chemical composition of essential oil and citral contents were studied by Tajidin et al. and it was found that maturity stage at harvest influenced essential oil and citral contents of lemongrass [87]. Thyme (*Origanum syriacum* L.) showed the effect of growth stages such as the pre-flowering stage and blooming stage on yield and composition of the essential oil. Total yield of oil was higher in the plant at blooming stage in comparison with that at pre-flowering stage. The higher levels of volatile compounds were noticed during the blooming stage. Exceptions were carvacrol, gamma-terpinene, alpha-terpinene, and para-cymene where higher levels were found in pre-flowering stage. Unexpectedly, carvacrol as structural isomer of thymol depicts higher concentration at pre-flowering stage [88].

In *Rosa damascene*, the essential oil is present in all parts of flower. Generally, when the petals develop into a cup shape and the stamens are bright yellow, the essential oil achieves the maximum yield generally. In the crown petals, the rate of biosynthesis of essential oil is highest. The composition of rose oil also fluctuates significantly with the developmental stages of flower. This is due to different rates of synthesis of different components. In the initial stages of development, flowers have more concentration of stearoptene, alpha pinene and myrcene as compared to the important alcohols, citronellol, geraniol, and nerol. The alcohol content quickly increases to about 60% in later stages of development [89]. In *Salvia sclarea*, the essential oil yield was less at flowering bud stage, highest at full bloom stage, and then decreased rapidly upon maturation. The oil consisted of linalool (main constituent), linalyl acetate,  $\beta$ -Humulene,  $\alpha$ -cadinene,  $\beta$ -caryophyllene, and sclareol, which showed a steady increase in percentage over the different stages of maturity, with no significant yield losses at maturation stage. The yield of essential oil and monoterpenes decreased at full maturity [90].

### The effect of ultraviolet radiations

Ultraviolet radiation is an important factor because, in many cases, it stimulates the production of secondary metabolite. The part of the ultraviolet daylight spectrum, which is particularly variable and of special attention, is the UV-B band with 280-315 nm. The research showed that in two different chemotypes of *Mentha spicata*, UV-B radiations caused a 50% increase in essential oil production on a dry-weight basis in one, while in the other chemotypes, the increase was not worth mentioning [91].

More detailed research was carried out on the biosynthesis of essential oil in sweet basil (*Ocimum basilicum* L.), in which the effect of ultraviolet radiation was of great significance. The effect of UV-B radiation increased with the plants' age and was positively different for 22 different essential oil components [92]. It was reported in a separate study that for normal development and filling of oil glands in sweet basil, UV-B radiation is needed [93]. Chang et al. studied that irradiating the *Ocimum basilicum* to supplementary UV-B light in the early morning in a restricted environment in room temperature produced shorter plants with higher dry mass, more axillary shoots, and thicker leaves [94]. In *Mentha piperita*, the oil contents were increased to some extent by UV-B radiation, but the menthol concentration was considerably decreased as the synthesis of menthone, menthofuran, and menthyl acetate was increased. The stem elongation of plant was significantly inhibited, along with changes in leaf area [95]. The application of UV-A radiation (360 nm) during the day considerably improved the total essential-oil content, especially menthofuran and menthol. The total leaf area and total phenols were increased as well. But during the night time when this plant was exposed to UV-A radiation, a typical shade-avoidance syndrome was produced with elongated stem, decreased leaf area, and less essential oil and menthol content [96]. The study of the effects of different UV-B radiation (280-315 nm) and photosynthetically active radiation (PAR, 400-700 nm) levels and ratios on yield and pattern of essential oil of peppermint showed that during flowering, the maximum essential oil yield was obtained at high PAR ( $1150 \mu\text{mol m}^{-2}\text{s}^{-1}$ ) and almost ambient UV-B radiation ( $0.6 \text{ Wm}^{-2}$ ). The menthol contents were reduced and menthone contents were increased in the absence of UV-B radiation and at low PAR ( $550 \mu\text{mol m}^{-2}\text{s}^{-1}$ ), and hence the oil quality was substantially decreased [97].

*Cymbopogon flexuosus* has also been analyzed to study the effect of supplemental Ultraviolet-B (sUV-B) radiation on yield and chemical composition of its essential oil. The exposure to sUV-B radiation

for different intervals of time (15 min, 30 min and 1 hr) showed an increased percentage of essential oil in aerial parts of the plant. There was no significant variation observed in essential oil percentage of sub-aerial parts. The analysis of essential oils of aerial parts showed an increase in citral percentage in UV treated plants as compared to the control. In sub-aerial parts of sUV-B-treated plants, neral and junipene were found in higher percentages [98].

### Effect of light quality

The light quality and light intensity can affect the chemical components of plants and percentage of accumulation of the secondary metabolites in plant tissues. In the aromatic plants, the essential oil yield and composition is affected by the quality of electromagnetic radiations [99]. In the leaves of geranium (*Pelargonium spp.*), the biosynthesis of the essential oil is affected by the quality of incident light of different wavelength regions of the spectrum. The irradiation to red light caused the biosynthesis of essential oil from  $^{14}\text{CO}_2$  as a primary precursor supplied externally [100]. The research conducted on dill (*Anethum graveolens* L.) to study the effect of light quality, concerning different wavelength regions like supplemental red, far-red and blue light treatments, and end of the day light treatments, showed no significant differences in the biomass yield of plant. The plants treated with far red light gave the highest essential oil yield. Consequently, the plants exposed to 4 hr of red and far red light produced oil containing more phellandrene and less myrsiticin. With an increase in the light level, the growth and essential oil yield increased in dill, and under full sunlight it was highest [101,102].

The influence of different wavelengths of lights on biosynthesis of essential oil has also been investigated in sage and thyme. The exposure to 45% of full sunlight resulted in the sage plants having peak yield of essential oil with (+)-thujonone as a major component and camphor in less concentration, as compared to the plants grown under other levels of sunlight. In thyme, the highest yield of oil and proportion of thymol and myrcene was obtained in full sunlight [103]. The nine-month-old seedlings of *Pothomorphe umbellata* (Piperaceae) were subjected to three shade levels (30%, 50%, 70%) and full sun to estimate the effect of shade on the yield and chemical composition of essential oil leaves. The highest essential oil concentration was observed in the plants grown under 30% shade and harvested in the second year of development. Twenty-six chemical substances were identified, with trans-nerolidol as the predominant substance [104].

Three important *Mentha* spp., including *M. arvensis*, *M. citrata* and *M. cardiac*, were grown under short-days, normal-days, or long-days conditions for 60 cycles and were analyzed for growth performance of plants, biosynthesis of essential oil, and yield and composition of essential oil. Under long-day conditions the species showed improved growth, which resulted in early flowering in *M. citrata*. The short-day plants revealed the highest rate of biosynthesis of the essential oil, and hence the highest yield of essential oil. The oil composition was also affected by this photoperiodic treatment [105]. Under different radiation levels of 23%, 46%, and 100%, *Mentha aquatica* and *Mentha x piperita* were also grown to analyze the effect of photoperiodic modulation in a separate study. In all plants subjected to the lowest level of radiation, leaf area, stem number, and total dry mass were found to be reduced. Although the essential oil yield and the percentage of menthol, menthone, linalool, and linalil acetate in this oil were decreased with reduction in radiation levels, there was no observable correlation between plant development and essential oil synthesis [106].

The growth and essential oil yield of African basil, *Ocimum*

*gratissimum* L., grown under watered and water-stressed field conditions in full sunlight and natural shade (26.7-44.2% full sunlight) conditions were investigated. Plant height and total leaf area of the African basil were decreased more by water stress than by the light or shade condition. Shade enhanced essential oil content and water stress boosted essential oil content under shade, but reduced oil content under full sunlight. The effects of water stress were only observed in plants in full sunlight. The results demonstrated that African basil will produce relatively high essential oil yields per plant when grown under natural shade, regardless of water stress and poor vegetative growth [107].

### Effect of salt stress

Salt stress is one of the major serious environmental factors restricting crop production in marginal agricultural soils in many arid and semi-arid parts of the world. Different aromatic plants show different effects of salinity stress on yield and composition of essential oils produced by them. The main harmful effects of salinity on growth of plant and yield are attributed to osmotic effect, ion toxicity and nutritional imbalance leading to photosynthetic efficiency and stomatal closure [108].

A field experiment conducted on three species and four varieties of basil to evaluate their behavior in saline soil in Egypt showed that *O. basilicum* Var. *Siam queen* and *O. tenuiflorum* were better in plant height and number of branches per plant, while *O. basilicum* Var. *genoveser* and *O. tenuiflorum* produced the highest values in fresh and dry weights in both season. *O. basilicum* var. *Siam queen* contained maximum essential oil yield, while the *O. basilicum* var. purple ruffles variety contained minimum yield of essential oil [109]. The effects of four levels of salinity on dill showed that mean essential oil yield was increased with increasing salinity. It was concluded that the dill plant is highly salt-tolerant, and it can perform well under NaCl salinities up to 12 dS/m [110].

The results of an experiment on peppermint (*Mentha piperita* L.) showed that an increase in the salinity led to reduced length of stem and root, fresh weight of stem and root, dry weight of stem and root, internodes length, total biomass and essential oil percent and yield. The highest values of growth parameters and essential oil percent and yield were observed under the non-salinity condition. The increase in menthone and the decrease in menthofuran with increasing salinity level improved the commercial quality of the distilled essential oil [111].

In *Mentha pulegium* L., the yield and composition and biosynthesis of shoot essential oil was shown to be affected by salt stress. The essential oil yield was increased by about 2.75 percent under salt stress, and the percentage of menthone (the major compound of oil) was also affected. Salt stress resulted in considerable changes affecting the size and distribution of trichomes on both sides [112]. The water stress enhanced the amount of antioxidant compounds in basil leaf tissues and the highest concentration was observed at an irrigation intensity of 25%. More decrease in irrigation level to 12% resulted in reduction of antioxidant compounds and antioxidant activities of basil extracts [113].

### Effect of arbuscular mycorrhizal fungi (AMF)

Arbuscular mycorrhizal fungi (AMF) are certainly a crucial element of the lower soil system and have a substantial documented effect on the sustainability and yield of agricultural systems. The first step of colonization of arbuscular mycorrhizal fungi is considered

to be the stimulation of synthesis of secondary plant metabolites such as flavonoids [114], pathogenesis-related proteins [115], and phenolics [116] in the roots of the host plant. But the accumulation of secondary compounds in the aerial parts of mycorrhizal plants has been considerable less. In *Origanum* species, the essential oil yields were found to be enhanced in the presence of arbuscular mycorrhizal fungi. The studies on *Coriandrum*, *Anethum* and *Foeniculum vulgare*, revealed that arbuscular mycorrhizal fungi root colonization altered the essential oil components, and as a result, the essential oil quality increased [117,118]. In mycorrhizal *Artemisia annua* the increased concentration of artemisinin was found to be correlated with a higher density of glandular trichome in leaves [119,120].

The experiments performed to compare the effectiveness of two arbuscular mycorrhizal fungi, *Glomus macrocarpum* (GM) and *Glomus fasciculatum* (GF), on three accessions of *Artemisia annua* showed that the synthesis of plant biomass, dry weight of shoot, nutrient status (P, Zn and Fe) of shoot, concentration of essential oil, and artemisinin in leaves was considerably enhanced in comparison with non-inoculated plants. The degree of growth, concentration of nutrients, and synthesis of secondary metabolites of plants changed with the fungus-plant accession combination. The efficacy of *Glomus fasciculatum* in increasing essential oil concentration in shoot was more than that of *Glomus macrocarpum*. While in two accessions, GM was more effective in enhancing artemisinin concentration than *Glomus fasciculatum* [121]. These two arbuscular mycorrhizal (AM) fungi also considerably enhanced the growth and concentration of essential oil of *Foeniculum vulgare*. The inoculation of arbuscular mycorrhizal fungi of plants along with phosphorus fertilizer application considerably increased growth, phosphorus uptake, and essential oil content of plants in contrast to either of the components used alone [118].

### Effect of fertilizers

The application of fertilizer usually affects the yield of essential oil by increasing the yield of biomass of plant in a unit area; however, fertilizer also shows the effect in a cultivar-specific manner. In oregano plants, two pot experiments were done in the seasons of 2006 and 2007 to study the effect of different levels of nitrogen fertilizer as ammonium sulphate on the fresh weight of plant and essential oil. The irrigation of plants every seven days and application of 1.2 g nitrogen in each pot was useful in increasing the yield of herb as well as yield of essential oil [122]. The plant *Java citronella* had been reported to show a positive response to nitrogen fertilizer with cultivars having herbage content difference by up to 42% and oil yields difference (per hectare) up to 36% at the same nitrogen levels [122]. Further, variation in herb yield with different types of urea used on the same cultivar has also been demonstrated [123]. In lemongrass, nitrogen application has been found to influence the citral content in oil, but the potassium requirement of lemongrass sometimes exceeds the nitrogen requirement required to produce best oil yield [124]. In *Rosa damascena*, at bud development stage better flower and oil yield has been found to be associated with levels of NPK in leaves, and in many countries rose flower yield has been enhanced with nitrogen application [125].

Manganese has been found to be the most valuable single micronutrient that increases the yield of essential oil. In the geranium, the application of magnesium often increases herb yield but oil concentration or composition has no significant influence [126]. In *C. winterianus*, chlorosis is produced in leaves due to iron deficiency, which reduces herb production and yield. However, their oil contents are not strongly affected [127]. In palmarosa, both herbage and oil have been found to significantly increase with the application of ferric

and manganese sulphates at the tillering time and before initiation of flowering [128].

Japanese mint has been found to require Zn for synthesis and accumulation of essential oil [129]. The Zn at 0.250 mg/L application to Khus-khus and at middle circumference position has given the maximum total oil % as well as the maximum khusimol and khusinol oil contents [130]. Zn applied as zinc sulphate (2.5 kg/ha) to palmarosa herb, in the presence of sufficient nitrogen and phosphate increases the yield of herb and oil [131]. Two field experiments carried out on chamomile showed that in calcareous soils the yield of flower and essential oil increased by foliar application of Fe and Zn. The application of Fe+Zn spray at stages of stem elongation and flowering had more beneficial effects on flower dry yield, essential oil percentage and essential oil yield as compared with spray at only one stage [132].

The experiment performed with peppermint grown under drip irrigation system to the west of Nile Delta of Egypt, revealed that a concentration of 15 ppm cobalt gave the best fresh and dry herb yield and the highest essential oil yield, as well as enhance the uptake of macro (N, P and K) and micro (Mn, Zn and Cu) nutrients. The principal components of oil, including menthone and isomenthone, increased with decreasing content of L-menthol relatively at the highest concentration of cobalt. The highest content of L-menthol was obtained at the low level of cobalt as compared to the control and other treatments. Thus, the comparatively high concentration of menthol in the peppermint oil implies that in newly reclaimed land in Egypt, peppermint essential oil of high commercial value could be successfully produced [133]. Inclusion of molybdenum and copper has led to an increased herb and oil yield in well-fertilized plants [89].

The effects of different amounts of complete fertilizer on the yield, fresh and dry weight, and essential oil composition of *Satureja hortensis* L. were studied by Alizadeh et al. The results showed that the use of fertilizer increased fresh and dry weight in *S. hortensis*. The effect of different amounts of fertilizer on the essential oil composition was very slight and was not significant. But the amounts of some components such as carvacrol,  $\gamma$ -terpinene and  $\alpha$ -terpinene were varied with fertilizer application [134].

The experiment was conducted by Ahmadian et al. on *Cuminum cyminum* to study the effects of water stress and application of manure on percentage of oil and its main components and yield of these components. Results showed that a relationship exists between the main components of cumin essential oil under water and manure application. The effect of water stress and manure were significant on essential oil and its constituents. The highest amount of cumin aldehyde and  $\rho$ -cymene and the lowest of  $\beta$ -pinene,  $\gamma$ -terpinene, and  $\alpha$ -pinene were obtained with manure treatment and three times irrigation [135].

### Concluding Remarks and Perspectives

The aromatic plant cells are important natural biosynthetic factories of essential oils with different morphologies, which are present on different parts of aromatic plants where these oils are biosynthesized, accumulate and hence reach the atmosphere by different reported secretion mechanisms. The research reviewed in this paper showed that different cell organelles within the plant cells play an important role in the entire process from the biosynthesis of essential oil to their secretion into the atmosphere by granulocrine or eccrine mechanisms or both, but their exact mechanism of action during all these processes still needs further exploration. There are many environmental factors that influence these natural processes of essential oil biosynthesis, their accumulation and secretion into the atmosphere. We have summarized

some of these factors in this paper, but there may be some other factors that have a role in all these processes. The generation of a cDNA library of oil secretory cell by using an isolated secretory cell can provide a tremendous resource of expressed sequence tag associated exclusively to biosynthesis and accumulation of essential oil, which can play a significant role in engineering terpenoid metabolism in plants. Some of the aromatic plants have been studied by this method, but many remain to be studied morphologically as well as genetically. Actually, recent years have witnessed a substantially increasing interest in the application of essential oils for the treatment of various diseases. In view of the diverse roles of essential oils in human physiology, future studies will be focused on investigating the potential therapeutic application of these essential oils.

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