



Effect of plant growth regulators on essential oil yield in aromatic plants

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ABSTRACT

The aroma and fragrance industry is billion dollar world market which grows at the rate of 18 billion per year and the essential oil in International trade increases 10% annually. In both developing and developed countries the requirement of aromatic plants containing essential oil has risen exponentially and it is expected that in future the demand of essential oil could be raised. Plant growth and their primary and secondary metabolite production is control by the plant growth regulators Plant growth regulators result the effects on plant like plant growth, number of essential oil storage structures and biosynthesis of essential oil which can alter the yield of essential oil. Phytohormones play significant role in enhancing the essential oil production. In most of the aromatic plants such as rose scented geranium, lemongrass, peppermint the quality and content of essential oil can be enhanced by the application of plant growth regulator.

Keywords: Plant growth regulators, Essential Oil, Terpenoid biosynthesis

INTRODUCTION

The aroma and fragrance industry is billion dollar world market which grows at the rate of 18 billion per year and the essential oil in International trade increases 10% annually. The large number of compounds used as aroma and flavor are chemically derived from petroleum derivatives. The required source for them is the use of hard metals in the catalysis process[1]. In Middle East since 5000BC aromatic plants also termed as spices and herbs are used in enhancing medicinal properties as a preservative and also used in increasing flavor and fragrance of foods[2,3]. In both developing and developed countries the requirement of medicinal plants containing essential oil has risen exponentially and it is expected that in future the demand of essential oil could be raised[4]. Essential oil also known as volatile or ethereal oil are obtained from different parts of the aromatic plant such as leaves, flowers, root, bark, wood, seeds, bud, twig and these contain odorous, volatile hydrophobic and highly concentrated compounds[5,6]. The essential oil depends on developmental stages of plants, environmental factors and genetic factors. The environmental changes may influence the physiological processes and biochemical pathway which alter plant metabolism and helps in essential oil biosynthesis [7]. Chemical composition and essential oil production can be affected by endogenous as well as exogenous application[8].

Due to therapeutic, antimicrobial and antioxidant activities of essential oils they are used in food and pharmaceutical industries. Beside this essential oil can be used as herbicides, pesticides and anticancer compounds due to their biological activities[9,10]. Plant growth and their primary and secondary metabolite production is control by the plant growth regulators. It has been demonstrated that the herb yield in basil is enhanced by plant growth regulator [11], similarly in coriander [12,13] and fenugreek [14] herb yield is enhanced [15].

Plant growth regulators results the effects on plant like plant growth, number of essential oil storage structures and biosynthesis of essential oil which can alter the yield of essential oil[16]. Several aromatic grasses of the genus

Cymbopogon like Palmarosa, Citronella and lemongrass are cultivated for their essential oil. Aromatic grasses have soil binding properties, have great significance in agro and social forestry plants and for wasteland recovery [17]. Farooqi *et al* studied the responses of plant growth regulators in aromatic grasses [7,17]. In aromatic plants such as rose scented geranium, lemongrass, peppermint the quality and content of essential oil can be enhanced by the application of plant growth regulators [18–20]. Since 1940, in agriculture areas to control the developmental processes like reproduction, maturation, vegetative growth, post harvest senescence and germination growth regulators have been used [21]. The effects of growth regulator on secondary metabolite production in agriculture have been little known although it is used from many years in agriculture [22].

Essential Oils

Essential oil is a complex mixture of chemical compounds mainly monoterpenes, diterpenes and sesquiterpenes. From the majority of secondary metabolite classes isoprenoids is the one whose name is related to five carbon structure isopentenyl diphosphate (IPP). In plants, isoprenoids occur as primary metabolites (ubiquinone, gibberellins, brassinosteroid, crotonoid, plastoquinones and others) [23]. Isoprenoids are also considered as secondary metabolites as they play an essential role in ecological functions such as attraction of pollinator and seed dispersion, protection against herbivores and alleopathy [24]. Some of the volatile terpenoids like hemiterpenoids (C₅), monoterpenoids (C₁₀), sesquiterpenoids (C₁₅) and diterpenoids (C₂₀) play role in interaction between plants and insect herbivores or pollinators and are also implicated in general defense or stress responses [25–27].

Terpenoid pathway: Terpenes are biosynthesized by two pathways: mevalonate and methylerythritol phosphate. Terpenoids are obtained from the basic structural units isopentenyl diphosphate (IDP) and its isomer dimethylallyl diphosphate (DMADP) [28–30]. In plants the mevalonate (MVA) pathway, which functions in the cytosol, and the methylerythritol 4-phosphate (MEP) pathway, which functions in the plastids play role in the synthesis of isopentenyl diphosphate and dimethylallyl diphosphate [23,31–33]. By the function of three prenyl-transferases, precursors of terpenoid such as Geranyl-di-phosphate (GDP, C₁₀), farnesyl-di-phosphate (FDP, C₁₅) and geranyl-geranyl-di-phosphate (GGDP, C₂₀) are formed. The formation of hemiterpenes (C₈), monoterpenes (C₁₀), sesquiterpenes (C₁₅) or diterpenes (C₂₀) from the substrates DMADP, GDP, FDP or GGDP are catalyzed by the primary enzymes known as terpene synthases. The addition of IPP units to prenyl-diphosphates with allylic double bonds to the diphosphate are catalyzed by prenyl-transferases. Depending on the individual prenyl-transferase, most of it bind to GDP or FDP [34–37]. In plants, formation of monoterpenes and sesquiterpenes are based on the GDP and FDP. In metabolic engineering experiments, this problem is reduced by the co-expression of GDP and FDP synthases with appropriate monoterpene and sesquiterpene synthases over-expressed in tobacco. The sesquiterpenes amorpho-4,11-diene and patchoulol and the monoterpene S-limonene production was increased by this technique and with targeting of the overexpression to the plastid compartment [38].

The third stage of terpene volatile biosynthesis includes conversion of the different prenyl diphosphates by the large family of terpene synthases DMADP, GDP, FDP and GGDP to hemiterpenes, monoterpenes, sesquiterpenes and diterpenes, correspondingly. By the condensation of two molecules of FDP or GGDP, triterpenes such as sterols and tetraterpenes such as carotenoids are formed. There is an evolutionary relationship between plant hemiterpene, monoterpene, sesquiterpene and diterpene synthases but they are structurally different from triterpene or tetraterpene synthases [39]. From different plant species many terpene synthases have been derived [40,41].

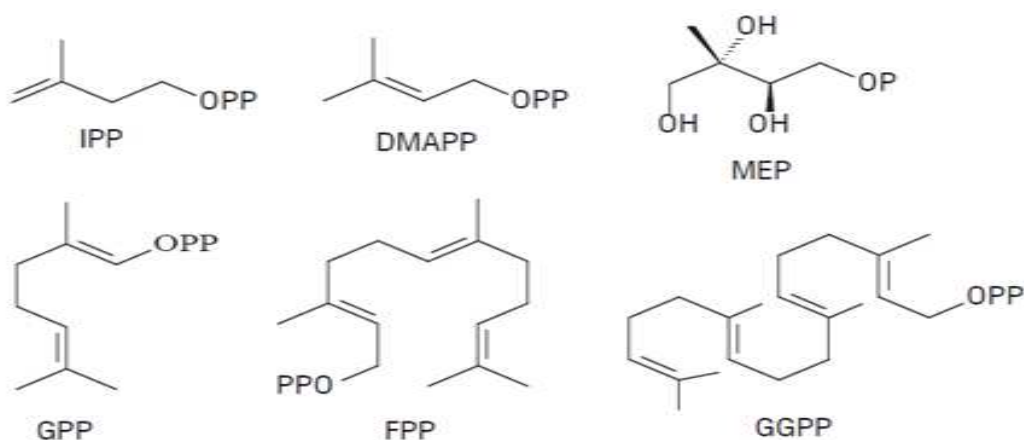


Fig.1: Terpenoid precursors (IPP and DMAPP) .MEP from chloroplast pathway and main precursors of terpenoid groups GPP, FPP and GGPP

The essential oil production can be changed by exogenous factors such as biotic, abiotic and endogenous factors such as development stage of whole plants and specific organs [7,42,43].

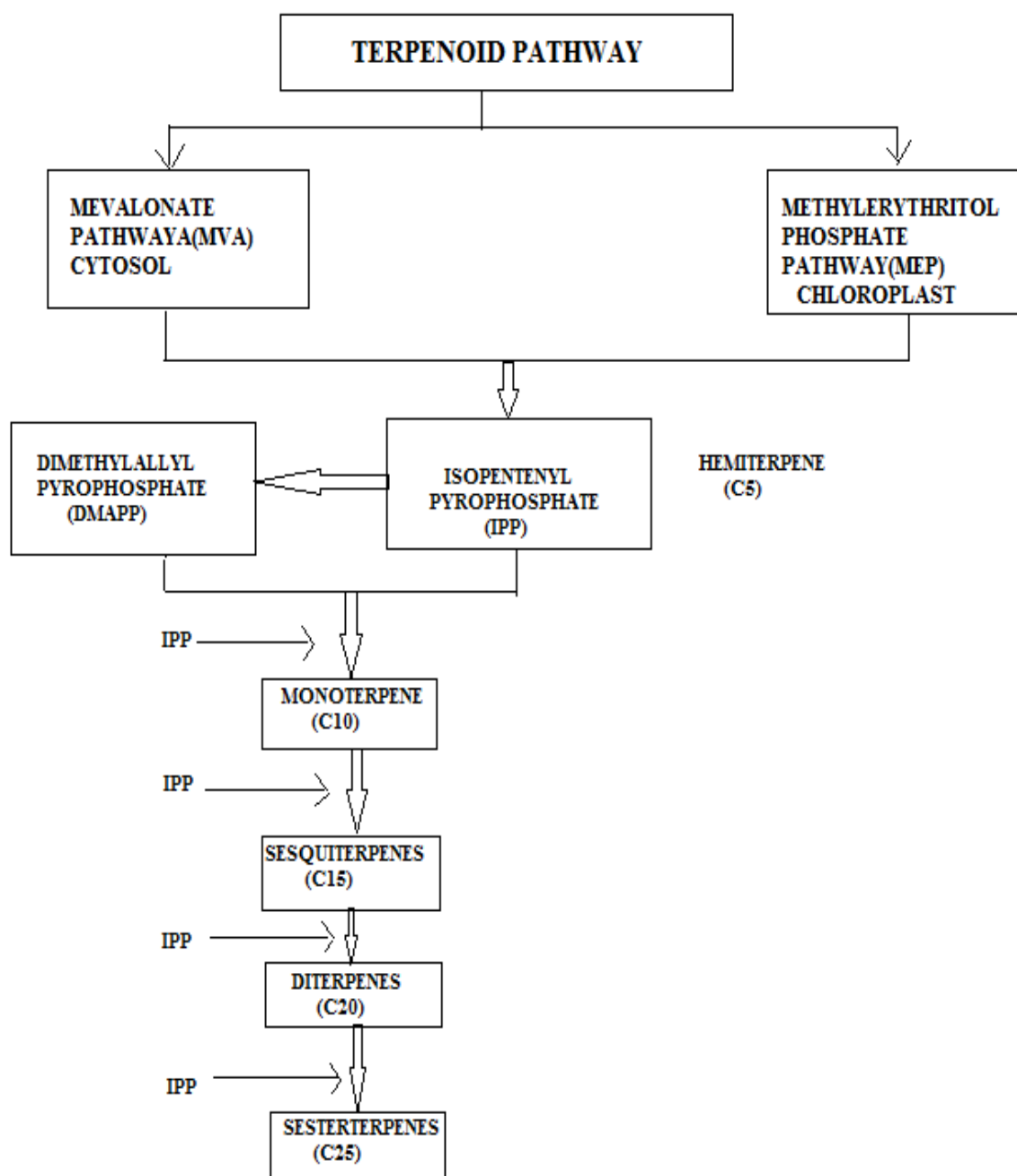


Fig.2: Terpenoid Pathway (Mevalonate and Methylethylol phosphate pathway)

The essential oil production and regulation is changed quantitatively and qualitatively by the factors such as photosynthetic rate, photoperiod, light quality, climatic and seasonal changes, ontogeny, nutrition, salinity, temperature, humidity, growth regulators and storage structure. Due to the positive effect on product quality in agricultural production the growth regulator use has been increased. This technology is used for the formation of better products with high yields and it is commonly used in small countries [22].

Plant Growth Regulators

In different aromatic plants, the plant growth and terpenoid biosynthesis is regulated by plant growth regulators which gives significant effect in both properties and content of terpenoids [44].

Terpenoid biosynthesis is based on primary metabolism such as photosynthesis and oxidative pathways for carbon and energy supply [45]. Triacantanol, a natural plant growth regulator plays significant role in enhancing biomass

production which results in increased biosynthesis of secondary products. Physiological changes like growth, photosynthesis, flowering and cell expansion in plant was observed by the application of phytohormone Gibberellic acid (GA3) [46,47]. The metabolic activity within pathways increased by the application of GA3 which results to stress and anthocyanin biosynthesis [48]. Plant growth regulator or phytohormone are active at low concentration and have particular effect on plant growth [49,50]. Plant growth regulators are classified as auxin, cytokinin, gibberellins, abscisic acid and ethylene [50], whereas jasmonate and brassinosteroids are also recognized as plant growth regulator [51].

Auxins (AUX) - Auxin play a role in cell elongation that's why it is classified as growth hormone and its function from embryo formation until tropic stimulus process. A wide number of genes are regulated by the auxin response. Indole acetic acid (IAA) is a predominant auxin in plants. In the young tissues, on the shoot and root meristematic apices biosynthesis of auxin is large. One of the factors which enhances the activity of auxin is the active transport and specificity and this property also differentiates it from other hormones [50,52,53].

Cytokinin (CYT) - Cytokinin functions in whole plant ontogeny from fertilized ovule to senescence and death. It plays a role in processes like cell division, shoot initiation and growth, senescence delay and photomorphogenic development, control of chloroplast division and growth, modulation of metabolism and morphogenesis in response to environmental stimulus [52,54-56].

Gibberellins (GA) - It is a phytohormone that is responsible for the regulation of plant height. Seed germination, flowering and stem elongation are regulated by gibberellins which are diterpenes. Gibberellins play an essential role in seed germination by activation of embryo vegetative growth and mobilization of energetic reserves from endosperm and they are also linked with juvenile to adult transition processes and promote fructification [51,57].

Abscisic acid (ABA) - It plays an essential role in seed germination and development processes because of induction of seed dormancy, tolerance to desiccation and inhibition of the embryonic to vegetative development, protein and lipid synthesis. ABA functions on the response generated by drought through stomata aperture in mature plants. Besides this it also functions in adaptation to stress conditions such as salinity, hypoxia, low temperature and in response to pathogen attacks. ABA is recognized as a hormone with inhibitory activity on growth in a simple way [7,52].

Ethylene (ET) - It is a phytohormone which functions on seed germination, shoot and root growth, flower development, flower and leaf senescence and abscission and fruit maturation. Emission by diffusion as a gas is the property of ethylene which differentiates it from other hormones. Ethylene is also linked with plant defense, acting on the induction of xylem inclusions and phytoalexins synthesis. The factors like ozone exposure, or mechanical injury, drought, inundation which linked it with stress response are responsible for effecting the ethylene production [51].

Brassinosteroid (BR) - Cell elongation and division is regulated by this phytohormone. The processes like plant curvature, reproductive and vascular development, membrane polarization and proton pumping, the source-sink relationship, and stress modulation through interaction with environmental signs are affected by BR. The essential components of BR are grains of pollen and immature seeds but in tissue the level of BR differs. BR concentration is greater in immature tissues and by this the effect of BR on young tissues can be explained. The dwarf plants are obtained when the mutant plants are treated with brassinosteroids and the growth pattern of plant is similar to the plant exposed to light even in the absence of BR. The altered leaf morphology can be seen in these mutant plants when they are exposed to light [57].

Role of PGR on Essential Oil in Aromatic Plants

Abbas *et al* (2012) carried out the study on lemongrass (*Cymbopogon citrates*) and they studied the effects on plant growth, hormonal content and essential oil content by the application of plant growth regulators such as indole butyric acid (IBA) and mepiquat chloride (MC). The application of plant growth regulators IAA and MC had no significant effect on essential oil content and growth in lemongrass [58]. The most important essential oil of species *Cymbopogon citrates* Stapf (lemongrass) belongs to family gramineae has high content of citral up to 75% [59]. In *rose-scented geranium* the content of essential oil was enhanced by the foliar application of tricentanol [60]. In *Mentha piperita*, plant growth regulators IAA and GA has no significant effect on herb and content of essential oil [61]. Stuart *et al.* reported that by the foliar application of mepiquat chloride (MC) plant growth was reduced such as the height of the plant and area of leaf [62,63].

In aromatic plant Basil (*O. gratissimum*), Hazzoumi *et al* (2014) studied the effect of plant growth regulator gibberellic acid (GA), indole 3-acetic acid (IAA) and benzyl-amino-purine (BAP) on the yield and composition of

essential oil and also on the main compound (methyl chavicol) and its isomer (the trans-anethole). The content of essential oil was enhanced by the application of IAA and IBA. The effect of GA, IAA and kinetin was studied in *O. basilicum* on the content and composition of essential oil. They concluded that the content of essential oil was reduced by the application of GA whereas by the application of IAA and kinetin the content of essential oil was enhanced. Due to this the main compound methyl chavicol was reduced from 75.16% in the control to 74.1% kinetin 73.2% IAA and 70.7% GA [64]. In *O. gratissimum* L. the content of essential oil is about 0.22% which is comparable to *O. basilicum* L. (0.213%) found in Egyptian [65], but the basil found in Turkish and Iran has higher concentration about 0.5% [66,67]. Salah el deen *et al* (1996) demonstrated that the composition and content of methyl chavicol was reduced and an increment in Eucalyptol. There is a small variation in the content of methyl chavicol by the application of IAA but the content of essential was enhanced [65]. In the species of *Lavandul adentata* L. Oudin *et al* (2007) demonstrated that by the application of BAP which functions like IAA results an increment in content of essential oil without any change in methyl chavicol composition [68,69]. Similar result was observed in *Salvia officinalis*, *M. spicata*, *Lavandul avera*, *M. suaveolens* [19] and *Cymbopogon citrates* L. [70]. In *Mentha piperita* the dry weight of plant was increased by the application of BAP (50 mg/L) [71].

In lemongrass, it was observed that the content, recovery and properties of essential oil was changed by the application of plant growth regulator [20]. Similarly it was observed in peppermint, sage, spearmint [19] and in rose scented geranium [18]. In rose scented geranium, the content of essential oil was enhanced by the foliar application of tricentanol and mixtalol which is a long chain aliphatic alcohol. Number of branches, height of the plant, composition and yield of essential oil was influenced [60].

In *Mentha piperita*, the essential oil yield and menthol was enhanced by the application of sodium salt of NAA and IAA [72]. It was noticed that by the application of N-phenyl pyridyl-thiourea the yield of essential oil was enhanced [73]. Similarly, increment in essential oil yield in isomenthone and neoisomenthol was observed by the application of cycocel, diaminozide and phosphon D [19]. In *M. citrata*, increment in herbage and essential oil content was noticed by the application of gibberellic acid (50ppm) and the linalool content in the essential oil was reduced. Kinetin (2 mg l⁻¹), sprayed on mint suckers shows significant effect on essential oil yield and green herb harvest [74]. It was demonstrated that biomass and essential oil content of peppermint was improved by the application of B9 (Alar) [75]. In *M. piperita* and *M. citrata* essential oil content was enhanced by the foliar application of cytokinin, kinetin and diphenylurea (1–10 ppm) [76]. Farooqi and Sharma 1988 observed increment in essential oil yield by the application of triacontanol [4]. In *Ocimum sanctum* and *Ocimum basilicum*, yield and properties of essential oil was enhanced by the application of GA₃ and chlomequat [77]. IAA and kinetin application reduced the basil growth [78]. In *Cymbopogon citrates* it was observed that essential oil yield and citral content was increased by the application of chlomequat [78] whereas in *C. jwarancusa*, GA and IAA showed significant effect in enhancement of essential oil yield [79]. In *Artemisia annua*, (Washington strain) Farooqi *et al* (1996) observed up to 42% increment in essential oil yield by the application of GA₃ and kinetin. By the application of tricentanol or GA₃ the quality parameter artemisia ketone of essential oil was enhanced up to 27% [80].

According to Farooqi *et al* (2003) the content of essential oil was enhanced by the use of 200 ppm of cinetine [7] which resulted in increasing biomass production in mint (*Mentha arvensis*) [81]. While in another experiment with *M. arvensis* L. var. *piperascens* Mal. Farooqi and Sharma (1988) identified the increase in content of essential oil treated with cytokinins and naphthalene acetic acid (NAA) but resulted in the reduction of plant size which was linked with a higher leaf production [4].

Essential oil are mostly obtained in flowers and leaves though they are present in whole plant. As compare to control plants the higher yield of essential oil was seen in sage (*Salvia officinalis*) treated with 100 mg L⁻¹ of gibberellic acid (GA). Due to this the number of leaves was increased according to author [22].

Plant height was decreased by the use of ethrel [(2-chloroethyl) phosphonic acid] [9], in concentrations of 50 and 100 mg L⁻¹ and when it is in contact with plant cell it is degraded and produces ethylene though in comparison to control plants there was an 38-42% increment in fresh and dry mass of flowers. While the plant height and flower production was decreased by the use of high concentrations 250 and 500 mg L⁻¹. Even the mass of single flower was not influenced [82]. In *Mentha arvensis* L. fresh matter of leaves and menthol production was increased by the use of various forms of brassinosteroids such as ketonic and lactonic spirostane [83].

Naeem *et al* (2012) observed that the triacontanol which is a natural plant growth regulator play significant role in enhancing growth, content, photosynthesis, protein synthesis, uptake of water and nutrients, nitrogen-fixation, enzymes activities and yield of free amino acids, reducing sugars, soluble protein, and active constituents of essential oil in different crops [84].

In an aromatic-antibacterial herb *Artemisia annua* L. Aftab et al (2010) studied stimulation of crop productivity, photosynthesis and artemisinin production in *Artemisia annua* L. by triacontanol and gibberellic acid application. The treatment of triacontanol with gibberellic acid significantly increased the artemisinin yield and essential oil content up to 29% and 61% higher values[85].

CONCLUSION

Thus, it can be concluded that the application of all the plant growth regulators (phytohormones) viz. Auxin, Gibberellin, Cytokinin, Abscissic acid, Ethylene and Brassinosteroids enhanced the physiological and biochemical parameters which directly influenced the terpenoid pathway. This in turn, improved the quality and quantity attributes of essential oil.

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