

Intimidating Effects of Heavy Metals on *Mentha* Species and Their Mitigation Using Scientific Approaches



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1 Introduction

As plants are sessile, they cannot escape the unfavorable environmental alterations to which they remain exposed during their life cycle that include biotic, climatic, and mechanical stresses (Tuteja 2007), but the plants have developed complex mechanisms for perceiving the stress-related signals. These mechanisms ultimately permit them to decelerate their growth and metabolism, thus escalating their ability to survive under stress conditions. They have developed the antioxidant system to tackle with the toxic effects of reactive oxygen species (ROS), which are generated in response to the stresses (Kanazawa et al. 2000; Parihar et al. 2015). However, the response of a plant to stress is a highly dynamic process which is dependent on the

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duration, severity of the stress, and the developmental stage and preparedness of the plant (Claeys and Inzé 2013). The various active oxygen species (AOS) like oxygen-free radicals cause membrane peroxidation and ultimately lead to tissue damage. The ramification includes inhibition of enzymes, peroxidation of lipids, protein oxidation, activation of the pathway leading to programmed cell death, and finally cell death (Mittler 2002).

The pollution of soils caused by heavy metals (HMs) is a major concern nowadays, and the HM pollution has affected a large part of agricultural land worldwide, making it barren and unproductive for the crop plants. The term HMs refers to a group of metals and metalloids having an atomic density greater than 5 g/cm^3 (Singh and Kalamdhad 2011; Alloway 2011; Tchounwou et al. 2012; Edelstein and Ben-Hur 2018). Some HMs like nickel (Ni), chromium (Cr), zinc (Zn), copper (Cu), molybdenum (Mo), and iron (Fe) are required by plants in minute quantities as they are essential trace elements and the plant cannot complete their life cycle if the deficiency persists. These trace elements are required by plants during many structural and biochemical functions including electron transport reactions, oxidation–reduction reactions, growth, and metabolic processes and are also the components of various enzymes (Hänsch and Mendel 2009, López and Magnitski 2011; Tripathi et al. 2015). Nonessential HMs such as cadmium (Cd), arsenic (As), silver (Ag), mercury (Hg), and lead (Pb) have unknown biological functions, and they are toxic to plants even at lower concentrations (Gaur and Adholeya 2004). Heavy metals originate from natural sources like volcanism and weathering of rocks that are always present in the soil at a background concentration (Lasat et al. 2000; Ghiyasi et al. 2010). However, the concentration of HMs has increased dramatically from past few decades due to the exasperation of anthropogenic intrusion, and these HMs are not biodegraded though they get accumulated in living systems and hamper the basic fundamental processes of living organisms (Pehlivan et al. 2009). These toxic HMs enter into the environment through anthropogenic disturbances like mining, smelting, use of pesticides, herbicides, and other industrial activities. The impact of abiotic stress on the environment ultimately affects plant life by altering their morphological, physiological, and developmental processes finally diminishing their productivity.

Contamination of soil and water over the years by toxic HMs has become a great concern (Ikenaka et al. 2010; Sayyed and Sayadi 2011; Raju et al. 2011; Prajapati 2014; Zojaji 2014). Heavy metals cause severe damage to plants due to oxidative stress. The excessive concentration of these HMs induces the formation of ROS such as superoxide radical (O_2^-), hydroxyl radical (OH \cdot), singlet oxygen ($^1\text{O}_2$), and hydrogen peroxide (H_2O_2) by creating the oxidative stress. These are produced during various metabolic pathways as well as membrane-linked activities as by-products (Lajayer et al. 2017; Berni et al. 2018; Soares et al. 2019), and these ROS are responsible for peroxidase damages to DNA, RNA, proteins, nucleic acids, and fatty acids, disrupt DNA synthesis, and alter mitotic activity and transcriptional processes and chlorophyll content in plants (Burzyński 1985; Gallego et al. 2002). However, plant cells use different antioxidants like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and peroxidase (POX) by employing

their antioxidant machinery which helps in scavenging the ROS by redox homeostasis (Noctor and Foyer 1998).

The pollution due to HMs across the globe has affected not only the crop plants but also the medicinal and aromatic plants (MAPs). The MAPs constitute a large segment of flora providing raw materials for use in pharmaceutical industries for therapeutic and aromatic purposes and culinary purposes. The WHO has estimated that 80% of people worldwide rely on herbal medicines, and 1500 species are known for their aroma and flavor. The genus *Mentha* is one among them and is an important member of the Lamiaceae family, and it has high medicinal and aromatic value (Šarić-Kundalić et al. 2009). The genus includes 18 species and 11 hybrids (some reports show the occurrence of more than 27 species). It is mostly grown around the temperate areas of the world, but nowadays it is cultivated throughout the world (Singh et al. 2015; Bhattacharya 2016). Due to its antioxidant properties, production of essential oil, and various other biological activities, it is used for the treatment of sinusitis and bronchitis and has antibacterial, antifungal, and antiviral activities besides their use in confectionery, perfumery, and pharmaceuticals (Liu and Lawrence 2007; Mahboubi and Haghi 2008; Nickavar et al. 2010; Chibane 2012; Benabdallah et al. 2016). The *Mentha* species are also known for their free radical scavenger properties, and most of these activities of *Mentha* species are due to the presence of essential oil obtained from different plant parts (leaves, aerial parts) (Ahmad et al. 2012; Singh et al. 2015). The EO consists of terpenoids and phenylpropanoids, and other components may also be present. The concentration of each component in EO varies and depends on the plant part used for the oil extraction (Figueredo et al. 2015).

2 The Genus *Mentha*

The genus *Mentha* grows well in the tropical and subtropical climate of Australia, Asia, Europe, China, Africa, Brazil, and North America. It consists of about 25 species and fewer hybrids. Various taxonomic names ascribed by the taxonomists to mint plants during the past 200 years reflect their great morphological variation (Kokkini 1992, Gupta et al. 2017). Different uses of genus *Mentha* are reported in the literature and found that its herbal products can cure bronchitis, nausea, anorexia, liver problems, and flatulence, owing to its antispasmodic, inflammatory, analgesic, antiemetic, sudorific, and stimulating effects (Cowan 1999; İşcan et al. 2002; Moreno et al. 2002). *Mentha arvensis* is an aromatic herb which belongs to the family Lamiaceae, and out of stupendous essential oil-bearing plants, its oil constitutes an important source for pharmaceutical, flavoring, and agrochemical industries worldwide (Misra et al. 2000; Tassou et al. 2004). There are about eight species of *Mentha* grown in India, namely, *M. arvensis*, *M. piperita*, *M. spicata*, *M. aquatica*, *M. sylvestris*, and *M. citrata*. Among the different species, *M. arvensis*, *M. piperita*, and *M. spicata* are widely grown in Uttar Pradesh, India.

3 Effects of HMs on *Mentha* Species

3.1 Effect of Cadmium (Cd)

Heavy metals are hazardous to the agricultural lands worldwide that impede the plants to outreach their full genetic potential and cause a greater loss by reducing their productivity (Yadav 2010). Among the various HMs, Cd is a nonessential and most pernicious heavy metal pollutant commonly released into the cultivable soil from various anthropogenic activities like industrial, mining, and farming operations (Wagner 1993). It is an overwhelming metal of extensive natural and world-related concern. It is broadly disseminated in the world outside at a normal grouping of about 0.1 mg kg^{-1} . It poses unpropitious effects on plant physiological and developmental processes. If it gets accumulated above the threshold levels, it causes the induction of various toxic responses in plants. The profound effect of Cd treatment was observed in different *Mentha* species. Amirmoradi et al. (2012) experimented on peppermint (*M. piperita*) by applying different concentrations of Cd (10, 20, 40, 60, 80, 100 ppm) on the plant. A decrease in fresh and dry weights, number of leaf and area per plant, main stem height, and number of nodes per main stem and EO of the plant was found as compared to control. Cadmium at 100 ppm proved very toxic for the plants as a maximum reduction was noticed in the above-studied parameters. Peyvandi et al. (2016) also reported a decrease in the growth parameters of *M. piperita* owing to the application of different concentrations of Cd, and there was a slight difference in the EO concentration of control and highest treated plants.

Furthermore, some findings regarding the effect of Cd were reported by Ahmad et al. (2018) and Zaid and Mohammad (2018) in the case of *M. piperita* and *M. arvensis*, respectively. A significant reduction occurred in various physiological and morphological parameters except for enzymatic and nonenzymatic antioxidants with the increasing concentration of Cd. The highest applied concentration of Cd decreased the yield of EO also. However, the content of EO in *M. piperita* was increased at the lowest concentration and decreased at the highest concentration.

3.2 Effect of Lead (Pb)

Lead is a naturally occurring (pale blue, dark) metal occurring in little sums in the world's outside layer. As Pb is already present in nature but due to the various anthropogenic activities like the burning of fossil fuels, mining and manufacturing of phosphate based fertilizers leads to the accumulation of these heavy metals in the environment (Casas and Sordo 2011). Contamination of soils with this HM affects the EO composition and production (Zheljazkov et al. 2006) and crop productivity

also (Sharma and Dubey 2005). Various experiments were carried out to evaluate the toxic effects of Pb on crop productivity and EO composition of both MAPs and cash crops. Toxic effects of Pb and Zn on *M. spicata* in nutrient solution were also reported (Bekiaroglou and Karataglis 2002). They reported that chlorophyll content and root growth of plants decreased with the increasing concentrations of Zn and Pb. Another experiment was carried out by Prasad et al. (2010) on mint species to evaluate the toxic effects of Pb and Cr (30 and 60 mg kg⁻¹ soil) on the chemical composition and yield of EO as well as to find out the phytoaccumulation of these HMs by using three mint species (*M. arvensis*, *M. piperita*, and *M. citrata*). The growth and yield of *M. citrata* were significantly reduced by the application of Pb and Cr. However, the above parameters were significantly enhanced in the case of *M. piperita*, and there was no significant effect of these HMs on *M. arvensis*. Furthermore, the effect of these HMs caused a decline in the EO content of all the tested mint species. The effects of absorption and localization of Pb and Cd on *M. arvensis* was reported by Jezler et al. (2015). *Mentha* plants were treated with 0, 8, 16, 32, 64, and 128 mg Pb kg⁻¹ of soil. However, little changes were brought about by applied Pb and Cd and were insufficient to influence the oil yield and composition of *M. arvensis*.

3.3 Effect of Mercury (Hg)

Mercury is a widespread environmental pollutant and strong phytotoxic HM ion that causes plant growth inhibition and has long-term effects on the fertility of the soil. The toxic HM enters into the food chain and causes body tissue alterations besides having a wide range of adverse health effects (Suszcynsky and Shann 1995; Bhan and Sarkar 2005). Sources of mercury pollution are numerous industrial processes including coal industry, dentistry (dental amalgams), untreated batteries, nuclear reactors, industrial and waste disposal, solvent for metal, mining of silver and gold, and the electrical industry (switches, thermostats, batteries) (Pilon-Smits and Pilon 2000; Tchounwou et al. 2003). As Hg is widely present in the environment, plants, animals, and humans are unable to avoid exposure to its different forms (Holmes et al. 2009). Manikandan et al. (2015) tested the effect of different concentrations of Hg (5, 10, 15, 20, and 25 mg L⁻¹) on *M. arvensis* plants. They noted that at the highest applied dose after 12 h, Hg declined the seedling growth and biomass. A similar decline in growth parameters was also observed by Mitchell and Barre (1995), Suszcynsky and Shann (1995), and Zhou et al. (2007). Another similar experiment was also carried out by Manikandan and Venkatachalam (2011) on *M. arvensis* by applying different concentrations of Hg (10, 20, and 40 mg L⁻¹) to the plant. Treatments of Hg reduced the root and shoot growth and activities of ascorbate peroxidase (APX) and catalase (CAT) beyond 40 mg L⁻¹. They also observed that different polymorphic bands were formed owing to the highest applied Hg (40 mg L⁻¹).

3.4 Effect of Chromium (Cr)

Among the various HMs, Cr is abundantly present on the Earth's surface and is the seventh abundant element of the Earth's crust, and it exists in different valence states from -2 to $+6$. The stable and common forms in the environment are its trivalent [Cr(III)] and hexavalent forms [Cr(VI)] (Katz and Salem 1994; Kimbrough et al. 1999; Jacobs and Testa 2005). The concentration of Cr in soils is $10\text{--}150\text{ mg kg}^{-1}$ (McGrath 1995). Chromium enters into the environment from several anthropogenic and natural sources with the colossal release coming from industries like stainless steel welding and metallurgical industries (Tchounwou et al. 2012). Elemental Cr enters into the environment at a rate of 2000–3000 tons in India alone through tanning industries annually, and the effluent concentration ranges between 200 and 500 mg L^{-1} (Chandra et al. 1997). Chromium is also an essential component of diet (Anderson 1997) and has ecological significance in soils, but the toxic form of Cr is hexavalent, a toxic carcinogen, and if ingested in large doses may cause death of animals, and this form of Cr has been estimated 10–1000 times more toxic than trivalent form (Ajmal et al. 1984; Bishnoi et al. 1993; Syracuse Research Corporation 1993). The Cr is accumulated by plants, and at different trophic levels, it gets biomagnified through the food chain (Rai et al. 2002). Chromium interferes with several metabolic processes like photosynthesis, water relations, nutrient status and enzymatic activities, induces chlorosis, generates toxicity in plants, and ultimately causes a reduction in growth, biomass, and finally plant death (Sharma 1995). Furthermore, Barouchas et al. (2014) studied the effect of different concentrations of trivalent and hexavalent Cr (0, 1, 5, 10, 15 mg kg^{-1}) on *M. piperita*, *M. Spicata*, and *Lippia citriodora*. They concluded that Cr affected the uptake of mineral elements in the studied plants and the total Cr concentration in vegetative parts was more in *M. piperita* than the other two plants.

3.5 Effect of Copper (Cu)

Copper (Cu) is an essential micronutrient required by the plants in minute quantities (Ghorbanpour et al. 2016; Lafmejani et al. 2018), but the excess amount of Cu persistent nowadays in the environment mainly in the soil has created loss of productivity in both agricultural and MAPs (Panou-Filotheou et al. 2001; Mostofa et al. 2015; Ibrahim et al. 2017). A study was conducted to find out the effect of copper sulfate (CuSO_4) and Cu nanoparticles with the concentrations of 0, 0.5, 1.0, and 1.5 g L^{-1} (Lafmejani et al. 2018) on *M. piperita*. The foliar application of 0.5 g L^{-1} of CuSO_4 and 1.0 g L^{-1} of Cu nanoparticles enhanced the dry matter and EO content of the crop as observed by them.

A field experiment was conducted to study the response of *M. arvensis* plant toward six micronutrients, namely, copper, boron, molybdenum, zinc, iron, and manganese (Rajput et al. 2002). These micronutrients are required by plants in

minute quantities and are important for the normal growth and maintenance of plants and enhance the essential oil production in *M. arvensis* L. f. *piperascens* Malinv. ex Holmes. The application of the micronutrients increased the plant height, leaf/stem ratio values, biomass, and essential oil yield (Rajput et al. 2002). Similarly, a correlation was studied between the activities of antioxidant enzymes and the level of lipid peroxidation in case of in *M. pulegium* under Cu^{2+} , Zn^{2+} , Mg^{2+} , Mn^{2+} , and Ca^{2+} (Candan and Tarhan 2003). According to their findings, the lipid peroxidation levels in *M. pulegium* organs, except roots, were higher (Ca^{2+} stress), and roots showed maximum increase under Cu^{2+} , Zn^{2+} , and Mn^{2+} stress. In the absence of Ca^{2+} and Mg^{2+} , the maximum lipid peroxidation levels were observed in leaves.

3.6 Effect of Vanadium (V)

Vanadium is a transition metal whose concentration in soil depends on the industrial pollution as well as on parent material, and its fate in soil depends on hydroxides, iron, and aluminum oxides that determine the mobility of this metal in soil and waters (Peterson and Girling 1981; WHO 1987; Naeem et al. 2007). Barouchas et al. (2019) studied the effect of V and Ni on *M. villosa* and *Lavandula angustifolia* and found that there were no visible symptoms of toxicity on the plants. However, the shoot and root dry matter of both plants decrease with increasing V concentration.

3.7 Effect of Arsenic (As)

Arsenic is ubiquitously present in the natural environment and is a nonessential toxic metalloid, and its presence in the soil above permissible limit adversely affects the plant growth, development, and productivity of crops (Sharma 2012; Srivastava et al. 2014; Chandrakar et al. 2018). Among various *Mentha* species, some are tolerant to HMs like *M. aquatica* and considered as an As-tolerant species. It was observed that *M. aquatica* had grown without any phytotoxicity symptoms in the contaminated soils (Száková et al. 2011) and a comparison in the mobility of arsenic species with *Phaseolus vulgaris*, *M. aquatica*, and *Pteris cretica* was also studied (Száková et al. 2009). *Mentha aquatica* was able to accumulate less As in comparison to *Pteris cretica*.

3.8 Effect of Nickel (Ni)

Nickel nowadays has become a toxic pollutant because its concentration is alarmingly increasing in the environment, notably in the soil and water across the world. It is a trace element which is required by the plants in minute quantities for their

normal growth and development as it is an important constituent of the enzyme urease. Hence, with the level of increasing Ni pollution in the environment, it is essential to understand both the functional roles and toxic effects of Ni in plants. Two *Mentha* species (*M. aquatica* and *M. sylvestris*) were evaluated for their phyto-accumulation potential under Ni exposure (1, 2, 4, and 8 mg Ni L⁻¹). Both the species accumulated higher levels of Ni in their roots and therefore can be used in case of phytoremediation (Zurayk et al. 2002). Similarly, *M. spicata* grown on soil is treated with sewage sludge (SS) and municipal solid waste (MSW), and it was observed that with the increase in concentration of the said treatments and if applied long term, there was found an increase in metal content in various parts of spearmint (Sorboni et al. 2013).

3.9 Effect of Cobalt (Co)

Cobalt is a nonessential transition metal, and in nature it occurs in various transition states. It is beneficial for plants at lower concentrations; however, if applied at higher concentrations, it proved deleterious for the growth of plants and its toxicity is very rare (Zaborowska et al. 2016; Lange et al. 2017; WaLwalaba et al. 2017), although the literature is very scanty in this regard. A study on the effect of Co and As was conducted on different herbs in Poland (*Achillea millefolium* L., *Comarum palustre* L., *Lysimachia vulgaris* L., *Lycopus europaeus* L., *Potentilla anserina* L., and *M. arvensis*). Workers observed that As was highly accumulated in the herbs as compared to Co and the maximum Co content was present in *M. arvensis* (Malinowska et al. 2018). Another study was carried out by Aziz et al. (2011) on *M. piperita* to find out the effect of Co on plant's growth and chemical composition. The lower doses of Co increased both the fresh and dry herbage yields besides increasing the essential oil yield and micro- and macronutrients. However, the higher doses of Co decreased the menthol content, while it increased the menthone and isomenthone content.

4 Mitigation of HMs Using Scientific Approaches

Several MAPs belonging to the family Lamiaceae that bear chemical compounds are used as medicinal herbs and spices and also play a significant role in plant defense (Gautam et al. 2012). Plants synthesize the primary metabolites (amino acids, carbohydrates, and lipids) which play an important role in the fundamental processes of plants, besides synthesizing the secondary metabolites like terpenoids, alkaloids, flavonoids, and steroids that are vital in the mechanism of plant defense under various environmental stresses (Yazaki 2006; Mazid et al. 2011; Lajayer et al. 2017). Therefore, in such stressful conditions, a process of elicitation is induced in plants owing to the application of elicitors. The elicitors are those substances which, when applied in small amounts, induces the biosynthesis of secondary metabolites

(Naik and Al-Khayri 2016; Thakur et al. 2018). Elicitors can be biotic and abiotic, depending on their nature, and can get utilized during the stressful conditions in plants to elevate the toxic response. The menthol or menthone and limonenes are the terpenoids that are involved in the secondary metabolism of plants besides their role in respiration and photosynthesis. As there is an increasing demand for menthol worldwide, therefore, new scientific approaches are employed by researchers all over the world to enhance its production. However, synthetic biology is an approach which leads to the increased production of menthol by developing and optimizing the complex metabolic pathways (Ribeiro and Shapira 2019). But due to its limitations, there is an urgent need to find out the constructive approach which can be used to obtain the menthol in large production directly from the plants. The consumption of menthol is more than 7000 tons per year (Heydari et al. 2018). Hence, there is a need to find out the possible link between the HMs and their effects on proteins and genes involved in the biosynthetic pathway of secondary metabolites.

As various abiotic stresses cause widespread loss of crop productivity worldwide, there is a necessity to investigate how abiotic stress affects plant growth and developmental processes at the biochemical, physiological, and molecular level so that the productivity of crops may be enhanced (Kazan 2015). The major environmental factors imposing stress on plants are HMs, salinity, drought, chilling, pathogens, and heat stress. The stress response on plants is dependent on the stages of plants, period and development of stress, and biotic and abiotic factors. Therefore, plants need to respond to various internal and external stimuli by regulating their growth and development (Wolters and Jürgens 2009, Feller and Vaseva 2014). It has already been reported that the exogenous application of plant hormones enhances stress tolerance in HM-affected plants (Rubio-Wilhelmi et al. 2011; Peleg and Blumwald 2011; Elobeid et al. 2011; Srivastava et al. 2012; Zhu et al. 2012; Krishnamurthy and Rathinasabapathi 2013). Additionally, the application of different kinds of elicitors improves the secondary metabolite production in MAPs under stress conditions. Some species of *Mentha* acts as hyperaccumulators and can be used for phytoremediation in the HM-contaminated soils.

Plants have developed the capability to perceive the stress signals among them and recruit various signaling molecules for the transduction of these signals systemically or locally. According to the definition of the Environmental Protection Agency (EPA), a plant growth regulator is “any substance or mixtures of substances intended, through physiological action, to accelerate or retard the rate of growth or maturation or otherwise alter the behavior of plants”. Plants naturally produce these hormones and are indispensable for regulating their growth, and they perform by modifying or controlling plant growth processes like the formation of flowers and leaves, elongation of stems, development, and ripening of fruits (Wani et al. 2016; Jagodzic et al. 2018; Jamwal et al. 2018).

The “classical” phytohormones that were identified during the first half of the twentieth century are abscisic acid (ABA), auxin, cytokinin, ethylene, and gibberellin (GA) (Baharycz and Konopińska 2007; Wheeler and Irving 2010). Recently, additional compounds are being added to the category of plant hormones and include brassinosteroids, salicylic acid, jasmonic acid, nitric oxide, and strigolactones (Grün et al. 2006; Browse 2005; Gomez-Roldan et al. 2008). Furthermore,

phytosulfokines (PSKs), S-locus cysteine-rich proteins (SCPs), ENOD40, CLAVATA3 (CLV3), polaris, plant natriuretic peptides (PNPs), and systemin are biologically active peptides which have been found to be key signaling players in different aspects of plant life (Bahyrycz and Konopińska 2007; Wheeler and Irving 2010).

The PGRs have a positive effect on the overall growth and development of a plant as they escalate the various physiological and biochemical responses. Various phytohormones, biomolecules, and chemicals are assigned diverse roles to cope with different kinds of stresses. They are the most important endogenous molecules for modifying various molecular and physiological reactions and are critically required for the survival of plants under HM stress (Fahad et al. 2015). Besides having a direct role, it is now well accepted that these molecules work through crosstalk among them and culminate the stress (Gaosheng and Jingming 2012; Dar et al. 2015; Rahimi et al. 2017). They can control and signal the response, growth, and development like regulation of secondary metabolites, enzyme activity, and cell membrane permeability (Wani et al. 2016) via circulating through the whole plant or part of it. The pivotal role of plant hormones in promoting plant acclimatization to ever-changing environments has been well established by mediating fundamental processes like growth, development, source–sink transitions, and nutrient allocation (Fahad et al. 2015; Jamwal et al. 2018; Wasternack and Strnad 2019).

Here, we have summarized the effects of some phytohormones, plant growth regulators, irradiated polysaccharides, and micro- and macronutrients on *Mentha* species toward mitigation of the HM stress. It has been observed that these PGRs have also been proven beneficial for the MAPs in various studies. Kavina et al. (2011) studied the effect of traditional PGRs, namely, GA₃ and ABA, and nontraditional PGRs (Difenoconazole (DIZ) on *M. Piperita*. Most of the parameters (fresh and dry weights, growth of roots, and photosynthetic pigments) are enhanced by the application of DIZ and ABA; however, the length of the stem was decreased. Different concentrations (1, 10, and 100 mM) of GA₃ and calliterpenone (CA, a phyllocladane diterpenoid) were applied on *M. arvensis* (Bose et al. 2013). They found that the exogenously applied CA was found better over GA₃ in improving plant biomass, leaf area, branching, leaf/stem ratio, and stolon yield. Higher number and density of glandular trichome were observed in CA-treated plants. Additionally, they noticed that the transcript level of menthol dehydrogenase/menthone reductase was found highly upregulated in CA-treated plants as compared to GA₃ treated, with increased content of both menthol and menthone in oil. Finally, they postulated that both PGRs positively regulated the yield by enhancing the density of trichomes and branching, resulting in a higher accumulation of essential oil.

Parić et al. (2017) tested different PGRs (auxin, indole-3-butyric acid (IBA), and the cytokinin, N6-benzyladenine (BAP), both individually and in combination with *M. piperita* plants. Authors reported that an increase in several shoots and roots occurred by applying the treatment with BAP and IBA, but the highest concentration of BAP affected the production of phenolic compounds. However, concentrations of BAP and IBA affected the antioxidants and antimicrobial activities of the plant.

Few reports are available about the use of rhizospheric microorganisms on *Mentha* species. In this regard, Kumar et al. (2015) experimented on *M. piperita* and *M. arvensis* under two HMs (Cd and Ni), to find out their interaction with arbuscular mycorrhizae (AM). The AM fungal inoculation had no significant effect on the oil yield of *M. piperita* under control or soils amended with Cd as they reported. However, the oil yield significantly enhanced under the soils amended with Ni. More toxicity of these HMs was observed in case of *M. arvensis* as the yield of this species decreased in addition to application of the AM fungi inoculation. Another experiment was carried out by Kunwar et al. (2015) on *Ocimum basilicum* and *M. spicata* under Cu, Cd, and Pb. A significant variation in the EO of *O. basilicum* was observed, while there was no change in the EO composition of *M. spicata* recorded in the HM-amended soils.

Moreover, Cd-induced reduction on various growth, physiological and biochemical parameters, and EO production was studied by Ahmad et al. (2018) and Zaid and Mohammad (2018) in case of *M. piperita* and *M. arvensis*, respectively. *M. piperita* plants were treated with salicylic acid, and the *M. arvensis* plants were treated with methyl jasmonate (with and without nitrogen). Application of both PGRs significantly alleviated the Cd stress in case of *Mentha* species.

5 Radiation-Processed Polysaccharides Act as Plant Growth Promoters

Natural bioactive polymers have transmogrified the agricultural field because of the meagreness of toxicity and propensity to act as effective plant growth promoters. Ionizing radiation-mediated depolymerization of polymers has emerged as a recent and promising technology for boosting the productivity of crops (Hien et al. 2000; Nagasawa et al. 2000; Kume et al. 2002; Naeem et al. 2012a, b). Application of these radiation-processed polysaccharides (oligomers of sodium alginate, carrageenan, and chitosan) through foliar sprays on plants promotes various biological activities like plant growth (shoot and root growth, seed germination), production of flowers, enhanced content and yield of oil in aromatic plants, induction of phytoalexins, and amelioration of HM stress (Aftab et al. 2011; Dela Rosa et al. 2002; Hegazy et al. 2009; Khan et al. 2011; Sarfaraz et al. 2011; Idrees et al. 2013; Naeem et al. 2012a, b; Sadiq et al. 2017; Ahmad et al. 2019). Furthermore, application of these oligomers can abbreviate the harvesting period, and the use of chemical fertilizers and insecticides can be minimized by the application of oligomers (Hafiz et al. 2003; Luan et al. 2003). A considerable work has been done till now regarding the beneficial effects of the irradiated polysaccharides on a number of MAPs including *M. arvensis*, *Catharanthus roseus*, *Trigonella foenum-graecum*, *Cymbopogon flexuosus* Steud, *M. piperita*, *Foeniculum vulgare*, and *M. spicata* by various workers (Sarfaraz et al. 2011, Naeem et al. 2011, 2012a, b, 2015a, b, 2019, Dar et al. 2015, Singh et al. 2017, Sadiq et al. 2017; Ahmad et al. 2019).

6 Crosstalk Mechanism of the Biosynthetic Pathway in Plants

The volatile organic compounds are mainly derivatives of fatty acids, benzenoids, amino acids, and terpenoids and synthesized from different pathways. These organic compounds can be monoterpenes, diterpenes, tetraterpenes, sesquiterpenes, triterpenes, and sterols. The different pathways involved in their synthesis are 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway, mevalonate (MVA) pathway, lipoxygenase (LOX) pathway, and shikimate–phenylalanine pathway. Through crosstalk mechanism, these pathways result in the formation of precursors of various secondary metabolites. Among these pathways, the two pathways involved in the formation of secondary metabolites in case of MAPs are MEP and MVA pathways. These two pathways through crosstalk and in combination are responsible for the synthesis of precursors of different kinds of terpenes. MEP and MVA are the two compartmentally separated pathways which are responsible for the formation of five-carbon isoprenoid precursors, isopentenyl diphosphate (IPP), and dimethylallyl diphosphate (DMAPP), and these provide the precursors for geranylgeranyl diphosphate (GGPP) and geranyl diphosphate (GPP). IPP, the precursor for farnesyl diphosphate (FPP), is obtained from the MVA pathway (Sharma et al. 2003; Orlova et al. 2009; Mishra et al. 2017; Heydari et al. 2018). These precursors ultimately result in the formation of various kinds of terpenes. By overexpressing the genes responsible for the activity of the enzymes which are accountable for the production of secondary metabolites in plants, the overall production of essential oil and their active constituents could be enhanced. The hypothetical model shows the synthesis of various kinds of terpenes given in Fig. 1.

7 Conclusion

The HMs strongly affect the primary and secondary metabolite production in the plants either by increasing or decreasing their production depending on the period, concentration, and the plant type. Heavy metals alter the biosynthetic pathways in case of essential oil-bearing plants by generating the harmful reactive oxygen species (ROS), and by the application of biotic or abiotic elicitors using different scientific strategies, the deleterious effects on plants might be declined. The application of various plant growth hormones that combat the deleterious effects of HMs is comprehensively discussed in this chapter. A few studies carried out on *Mentha* species depict that the growth, herbage, and productivity of essential oils including the content or yield are negatively affected by the HMs. Only a few species of *Mentha* could be used as hyperaccumulators but up to a certain limit of HMs, and by achieving the threshold limit and beyond, these species also show decline in growth and photosynthetic, enzymatic, and quality of essential oils. The studies on nanotechnology, radiation biology, proteomics, metabolomics, and genomics could promote

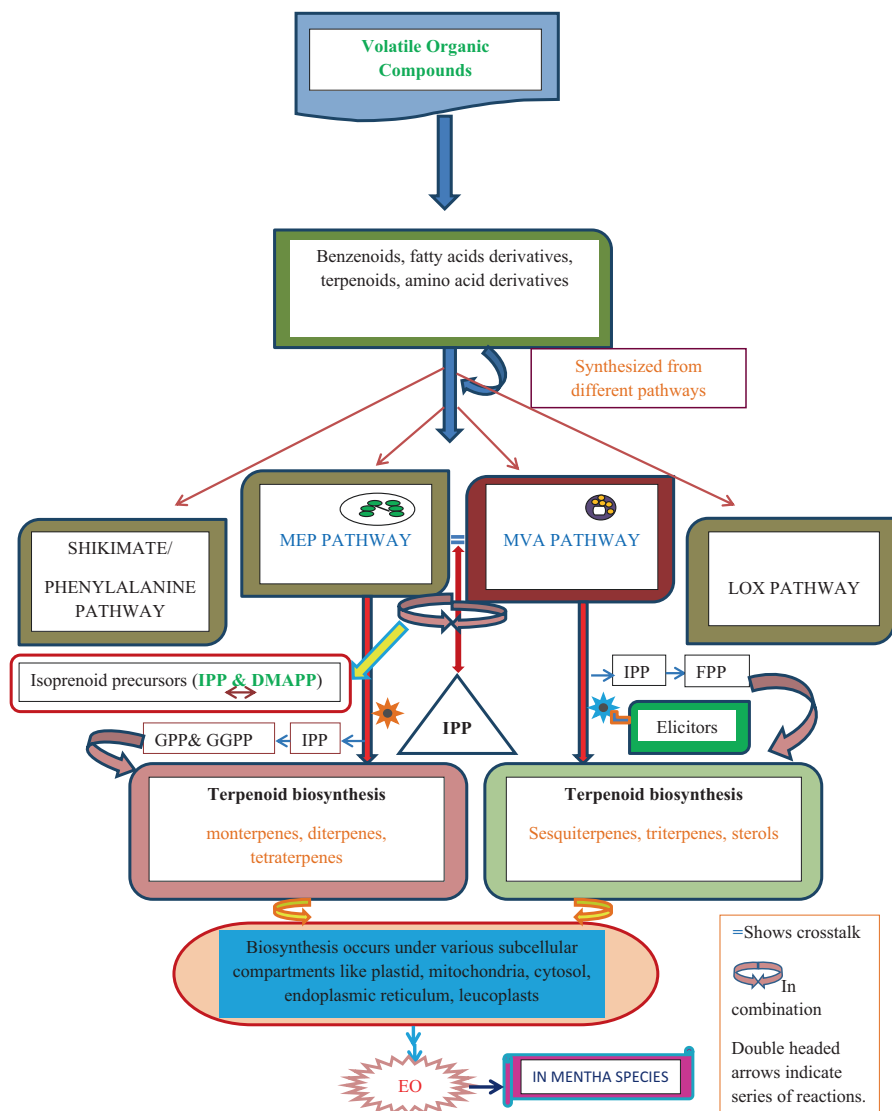


Fig. 1 Showing the synthesis of terpenoids (EO) from the two pathways methylerythritol (MEP) and mevalonic acid (MVA) occurring in different cellular compartments. In *Mentha* species, the synthesis occurs from precursors isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). IPP derived from the MVA acts as precursor for farnesyl diphosphate (FPP), and the one from MEP pathway is a precursor for geranyl diphosphate (GPP) and geranylgeranyl diphosphate (GGPP) and finally results in the formation of terpenes. Lipoxxygenase (LOX) and shikimate pathway leads to the formation of other organic compounds

new insights in combating the HM stress both in crop and non-crop plants. Also, more studies need to be carried out on various *Mentha* species to find out the specific hyperaccumulator species, and those could be used in the process of phytoremediation in the heavy metal-contaminated soils. Furthermore, the studies on crosstalk mechanism of newly discovered hormones interacting with each other in the biosynthetic pathway need to be explored in the modification of stress conditions.

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