



REVIEW ARTICLE

Integrated biotechnological and agronomic approaches for enhancing essential oil yield in mint (*Mentha* spp.)

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Abstract

Essential oils (EO) derived from mint (*Mentha* spp.) have high commercial value and are experiencing increasing global demand across the food, pharmaceutical and cosmetic industries. This review synthesises the recent advances in biotechnological and agronomic strategies for enhancing the EO yield and quality of mint and evaluates the potential of integrating these approaches for sustainable, resilient and high-value production systems. Engineering the methylerythritol phosphate (MEP) pathway, especially overexpression of 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), together with menthofuran synthase silencing has been shown to increase the oil yield by 60–78 % in multi-year field experiments while at the same time, minimising the production of unnecessary substances, such as menthofuran and pulegone. Complementary agronomic practices including integrated nutrient management, deficit irrigation (e.g., partial root-zone drying), biochar, mycorrhizae, biostimulants, nano-elicitors and intercropping effectively boost biomass, glandular trichome development and menthol-rich oil composition. Emerging tools such as CRISPR/Cas9 and trichome-specific promoters offer precise regulation of biosynthetic genes. Major challenges remain in understanding regulatory networks of EO biosynthesis, developing real-time precision tools for irrigation and nutrition and assessing environmental safety of nanomaterials. Integration of biotechnological advances with optimised agronomic management provides synergistic, field-validated improvements in oil yield and quality of mint, paving the way for sustainable, resilient and high-value production systems.

Keywords: essential oil; metabolic engineering; mint (*Mentha* spp.); precision agronomy; sustainable production; trichome development

Introduction

Essential oils (EO) derived from mint plants are valuable natural products and key drivers of the global market for EO. The market is expected to grow from USD 10.8 billion in 2020 to exceed USD 24.7 billion in 2030 (1). The market share for the mint oil sector is expected to increase from USD 177.8 million in 2018 to exceed USD 330 million in 2025 with various applications in food flavouring, pharmaceuticals, cosmetics and bio-based pesticides/biocontrol agents (1).

Mint oil crops are based on several important *Mentha* species such as *Mentha arvensis* L., *M. piperita* L., *M. spicata* L., *M. suaveolens* Ehrh. and *M. longifolia* (L.) Huds. which include various chemotypes. Peppermint (*M. piperita*) oil is rich in menthol (20–54 %) and menthone (5–43 %), while oil from spearmint (*M. spicata*) is mainly composed of carvone (35–68 %). Other *Mentha* spp. produce oil rich in linalool (e.g., *M. citrata*) or piperitenone oxide (e.g., *M. suaveolens*) (2). Novel fruit-scented oils with various chemotypes (such as grapefruit, ginger and pineapple) are produced by naturally occurring mint chemotypes (3).

The biosynthesis of important monoterpenes occurs through the methylerythritol phosphate (MEP) pathway in the

chloroplasts, where 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) is the major limiting factor for the production rate. Overexpression of DXR increases the flux to precursors without affecting composition, while combination with downstream gene modifications has been shown to achieve 60–78 % higher oil yields in controlled field trials. Suppression of (+)-menthofuran synthase reduces undesirable menthofuran and pulegone by over 50 %, improving oil quality by redirecting carbon flux toward menthol (4).

Oil yield is also determined by the density and size of glandular peltate trichomes, the anatomical sites of oil synthesis and storage. Agronomic and nano-elicitor approaches notably titanium dioxide (TiO₂) nanoparticles increase trichome density and diameter, raising oil content by approximately 39 % and nearly doubling yield while increasing menthol concentrations (5). However, the process of production is highly susceptible to stress from the environment. Irrigation with water deficit in combination with biostimulants increases the efficiency of water utilisation as well as oil production due to increased photosynthesis and antioxidant activity (6). Verticillium wilt, caused by the soil-borne fungus *Verticillium dahliae*, is one of the key diseases limiting mint production. The pathogen colonises the vascular system, leading to

stunted growth, leaf chlorosis and premature defoliation, which can significantly reduce biomass yield and EO content while also altering oil composition (7).

Genetic manipulation becomes difficult due to polyploidy and reproductive sterility. The sequenced *M. longifolia* reference genome enriched for disease resistance and EO biosynthesis genes now enables molecular breeding and metabolic engineering (8). Field-validated pathway engineering has delivered stable yield gains of up to 78 % with concurrent reductions in undesirable components (9). However, engineering alone may impose growth limits and agronomic improvement without elite genetics cannot overcome inherent limitations. The integrated approach involving both elite genetics and improved agronomics is thus, crucial for achieving consistent high production and quality. The integration of genetic improvement with improved agronomic approaches is illustrated in Fig. 1.

Biotechnological strategies to enhance mint essential oil yield

Metabolic engineering of monoterpene pathways

Enhancing precursor supply through methylerythritol phosphate pathway engineering

Monoterpene precursors (isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are primarily produced in mint glandular trichomes by the plastidial MEP pathway, which means that their supply can serve as a bottleneck during EO biosynthesis. Overexpressing DXR greatly increases enzyme activity and oil production in peppermint, all without altering the monoterpene composition, confirming a major influx of precursor flux as one of the primary rate-limiting factors (4). Conversely, some transgenic lines showing co-suppression of the DXR transgene exhibited strongly reduced DXR enzyme activity as a direct consequence of co-suppression, which in turn led to secondary effects including decreased chlorophyll content, impaired growth and lower oil yield. This provides evidence that balanced pathway regulation is required for optimal results (4). Subsequent studies have used metabolic flux analysis to show that synchronised enhancement of precursor supply and metabolic sink capacity

through coordinated expression of other MEP-pathway genes such as DXS (1-deoxy-D-xylulose-5-phosphate synthase), CMK (4-diphosphocytidyl-2-C-methyl-D-erythritol kinase) and HDR (4-hydroxy-3-methylbut-2-enyl diphosphate reductase) that maximises overall flux utilisation in the MEP pathway (10, 11). Elevated CO₂ concentration has been shown to upregulate MEP pathway gene expression and increase EO accumulation in related *Lamiaceae* species, indicating that environmental factors can directly influence terpenoid biosynthetic flux (12). A simplified monoterpene biosynthesis pathway in *Mentha* spp. is illustrated in Fig. 2.

Manipulating downstream monoterpene pathway branches

Suppression of menthofuran synthase to improve oil quality

The concentration of (+)-menthofuran and (+)-pulegone, both compounds linked to negative flavour notes and hepatotoxicity greatly influences peppermint oil quality. Using menthofuran synthase antisense suppression, the accumulation of menthofuran was reduced by greater than 50 % (non-stressing and stressing environments) without affecting plant morphology, demonstrating the potential for significant increases in oil safety and quality through single-enzyme suppression (4). Simultaneous suppression of menthofuran synthase, in combination with overexpression of MEP-pathway genes, resulted in lower levels of both menthofuran and pulegone (down to < 1 % relative concentration per oil) without affecting the accumulation of menthol-type monoterpenes (13). Such branch-point engineering using antisense or RNA interference (RNAi) is now recognised as a robust strategy to enhance menthol and carvone rich chemotypes across the genus *Mentha* (2).

Engineering terpene synthases for novel oil profiles

Downstream pathway engineering has enabled the generation of novel and biochemically traceable oil profiles. Introduction of a (+)-limonene synthase gene (generated by site-directed mutagenesis of spearmint (–)-limonene synthase) into peppermint resulted in high limonene accumulation, providing a biochemical marker capable of distinguishing engineered oils during commercial processing (13). Targeted redesign of menthol and

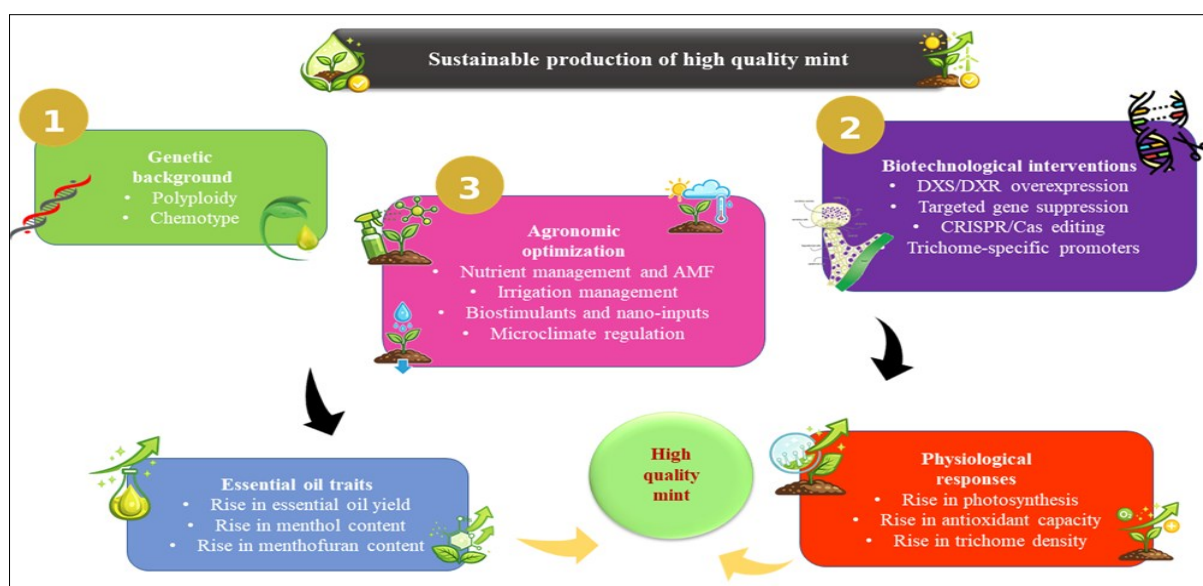


Fig. 1. Integrated biotechnological-agronomic framework for mint essential oil enhancement.

DXS: 1-deoxy-D-xylulose-5-phosphate synthase, DXR: 1-deoxy-D-xylulose-5-phosphate reductoisomerase, AMF: arbuscular mycorrhizal fungi.

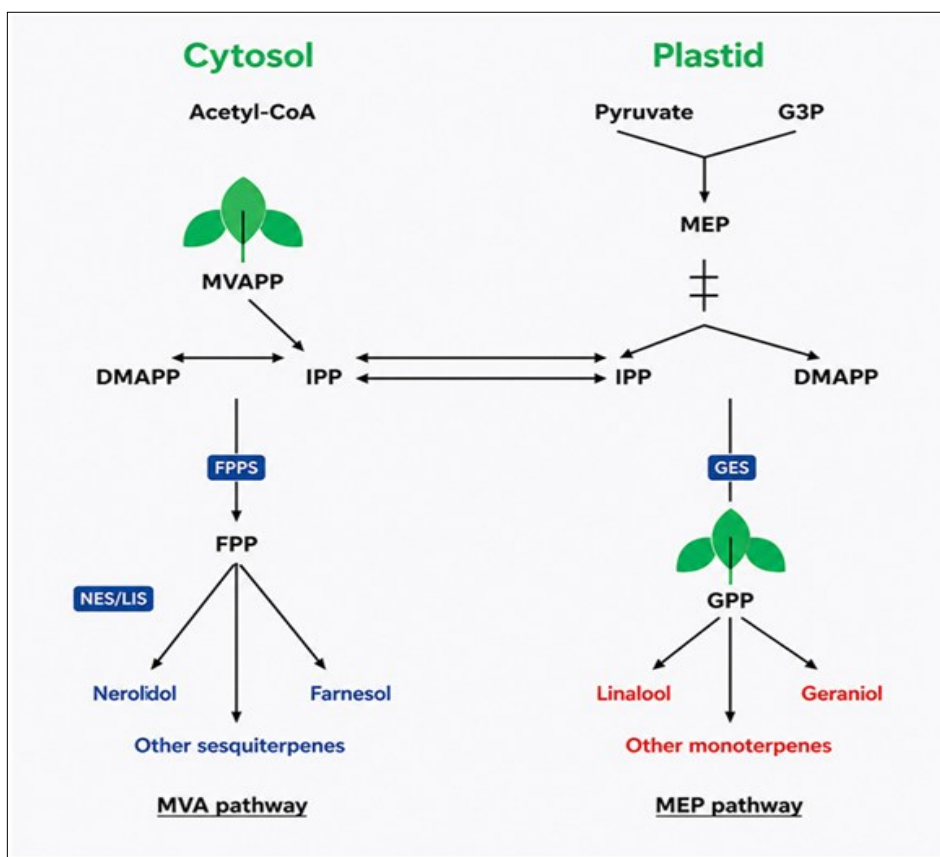


Fig. 2. Simplified monoterpene biosynthesis pathway in *Mentha* spp. (12).

Acetyl-CoA: acetyl coenzyme A, MVAPP: mevalonate-5-pyrophosphate, DMAPP: dimethylallyl diphosphate, IPP: isopentenyl diphosphate, FPPS: farnesyl diphosphate synthase, FPP: farnesyl diphosphate, NES/LIS: nerolidol/linalool synthase, MVA: mevalonate/mevalonic acid, G3P: glyceraldehyde-3-phosphate, GES: geraniol synthase, GPP: geranyl diphosphate, MEP: 2-C-methyl-D-erythritol 4-phosphate.

carvone pathways through manipulation of terpene synthases and heterologous enzymes is emerging as a powerful approach for customising mint oils to meet specific industrial application requirements (14).

Stacked-gene strategies for simultaneous yield and quality improvement

A major advance in mint metabolic engineering has been the development of stacked-gene strategies that simultaneously enhance precursor supply and suppress competing branches. Peppermint lines overexpressing DXR while suppressing menthofuran synthase produced up to 61 % higher oil yield with substantially reduced menthofuran and pulegone under greenhouse conditions (13). Future advances are anticipated from integrating pathway engineering with transcription factor (TF) manipulation, transporter modulation and growth-regulator interactions to further optimise carbon allocation and improve regulatory precision (15).

Genetic manipulation and emerging genomic tools

Transgenic mint: Achievements and constraints

Essential oil biosynthesis in *Mentha* is well characterised at the molecular level, enabling targeted redirection of carbon flux toward desirable monoterpenes through overexpression and RNAi-mediated suppression of endogenous and heterologous enzymes (14). Key enzymatic control points include DXS, DXR, monoterpene synthases and the enzymes regulating the menthol and carvone branches. Transgenic strategies have also introduced biochemical markers such as elevated limonene accumulation to facilitate product traceability during processing (13). Foundational work established that advances in pathway knowledge and plant

transformation protocols enable systematic engineering of EO traits toward commercially relevant phenotypes (16).

Resistance to verticillium wilt remains a major breeding target, yet current genetic modifications have mainly focused on metabolism rather than resistance enhancement, which is dependent on the utilisation of new genomics (8).

Bottlenecks in transgenic improvement

Cultivated mints are typically polyploid and reproductively sterile, which significantly complicates conventional breeding, backcrossing and trait segregation analysis (17). These constraints also limit the precision of regulatory refinement and multigene stacking strategies. However, multi-year performance of genetically engineered peppermint in the field demonstrates the viability of this strategy for producing sustainable EO (18). The presence of biochemical markers in genetically engineered lines, as well as the retention of the traditional taste, indicates compliance with traceability standards and consumer preferences.

Prospects for CRISPR/Cas9 and precision genome editing

Genomic foundations and candidate targets

CRISPR/Cas9 allows genome editing of genes responsible for disease resistance, abiotic stress tolerance and synthesis of specific metabolites in order to increase plant productivity and medicinal content in *Mentha* spp. (19). The reference genome of *M. longifolia* (353Mb) contains 35597 predicted protein-coding genes, including 292 disease-resistance gene homologs and 9 genes encoding enzymes of the p-menthane monoterpene biosynthesis pathway (e.g., (-)-limonene synthase,

(+)-menthofuran synthase and (+)-pulegone reductase) that determine EO characteristics, based on a high-density linkage map and over two million simple sequence repeats (SSRs) (8).

Cisgenic, intragenic and promoter-level editing

Native trichome-specific promoters from *M. longifolia* have been successfully used to modulate oil composition in peppermint without non-native regulatory elements. Promoter-level engineering in mint focuses on glandular trichome-specific promoters controlling key genes in the menthol biosynthesis pathway, such as (-)-limonene synthase (LS), (-)-limonene-3-hydroxylase (L3OH) and menthol dehydrogenase (MD). These promoters contain regulatory sequences known as *cis*-regulatory elements, including binding sites for TF such as MYB (15). The use of such promoters allows tight regulation of transgene expression in a precise manner, which minimises pleiotropy while maximising oil synthesis (20).

A summary of the principal biotechnological strategies employed to enhance EO yield and quality in mint is presented in Table 1.

Agronomic strategies to enhance essential oil yield and quality

Soil fertility and nutrient management

Integrated nitrogen management

The availability of nitrogen (N) plays a major role in influencing vegetative growth and EO production in mint. However, optimum yields are generally achieved through moderation of mineral N coupled with organic or nanofertilisers rather than through excessive amounts of inorganic N. Plant height and leaves as well as EO percentage and yield are increased when urea is mixed with vermicompost for both peppermint (*M. piperita*) and japanese mint (*M. arvensis*) (21). The optimal percentage of EO and its yield were observed at a mixture containing 25 % urea and 75 % vermicompost, thus indicating the ability of organic N to promote vegetative growth and EO synthesis (22). Likewise, by substituting 50 % mineral fertiliser with nanochelated fertiliser, optimal biomass, EO concentration (2.7 %) and EO yield (6.6 g m⁻²) are achieved (23).

Phosphorus, potassium and biological interactions

Nutritional balance of both P and K is crucial not only to enhance biomass yield but also to maintain control of oil quality parameters. When grown under water deficit, peppermint treated with 50 % P fertiliser along with arbuscular mycorrhizal fungi (AMF) inoculation had relatively higher concentration of leaf P and EO

yield by about 11 % compared to unfertilised plants (24). Use of both mineral and nanofertilisers led to the further increase in menthol and menthone contents (23).

Impact on soil health and essential oil quality

Nutrient integration especially when it involves the use of both organic and inorganic nutrients like vermicompost or farmyard manure increases soil quality through increased organic carbon content, cation exchange capacity and water retention capacity (25). Improved soil properties result in improved nutrient availability and absorption, hence resulting in increased vegetative growth and biomass (26). The enhanced qualities of the soil will increase the amount of EO produced because of the relationship between the amount of leaves and shoot biomass with EO yield (21), the optimal combination being 25 % urea with 75 % vermicompost (22).

Mycorrhizae and biofertilisers

Arbuscular mycorrhizal fungi considerably improve nutrient absorption, abiotic stress tolerance and EO accumulation in mint plants. In peppermint, inoculation with AMF increased the leaf dry weight by 5.3 % and EO production by 10.7 % and the highest effect was recorded in the case of 50 % P fertilisers + AMF treatment, leading to peak levels of P concentration (0.41 %) in leaves and gas exchange in the absence of adequate water (24). Under agrivoltaic conditions, AMF improved the fresh herb biomass production by 43.4 % in *M. arvensis* and menthol content by 30.38 % in shaded peppermint (27).

The effect of biofertiliser consortia is greater than that of individual biofertilisers. In peppermint, effective microorganism (EM) formulations improved growth, EO yield, hormonal balance and antibacterial activity of the extracted oils (28). In *M. arvensis*, combining biofertilisers, bioformulations and recommended NPK fertiliser produced 3.77g EO plant⁻¹ and 0.99 % oil content without reducing menthol concentrations (29).

Special soil amendments: Biochar and treated wastewater

The application of biochar had a positive influence on the development, mineral content and quality of EO in peppermint and spearmint, due to enhanced shoot biomass, chlorophyll, leaf N, magnesium (Mg), manganese (Mn), iron (Fe) and zinc (Zn) concentrations (30). Under treated wastewater irrigation, biochar application decreased the cadmium (Cd) and lead (Pb) content in the above-ground plant biomass, yet at the same time, contributed to an increase in EO content, phenolic compounds concentration and antioxidant activity. Notably, biochar resulted in the improvement of EO profile due to increased menthol and

Table 1. Summary of biotechnological strategies to enhance essential oil yield and quality in mint

Strategy	Target pathway/component	Major outcome	Field relevance	Key references
DXR overexpression	MEP pathway (precursor supply)	↑ Essential oil yield (50–78 %) with stable composition	Proven in multi-year field trials	(4)
MEP flux balance	MEP pathway	↑ Precursor availability	Improves metabolic flux	(13)
Menthofuran synthase suppression	Competing monoterpene branch	↓ Menthofuran and pulegone (> 50 %)	Improves oil safety and quality	(4)
Stacked gene approach	Yield and quality pathways	↑ EO yield (61–78 %) with stable menthol profile	Commercially robust	(13)
Terpene synthase engineering	Oil composition profile	Novel and traceable chemotypes	Enables industry-specific customisation	(13)
Trichome-specific promoters	Oil biosynthesis and storage sites	↑ Trichome-localised EO synthesis	Precision metabolic regulation	(20)

DXR: 1-deoxy-D-xylulose-5-phosphate reductoisomerase, EO: essential oil, MEP: methylerythritol phosphate.

decreased pulegone contents (31).

The irrigation of the soil with treated wastewater alone also increased biomass and EO yields for peppermint and spearmint, as well as changed the oil composition in favour of menthol and carvone, but not menthone and pulegone contents (31, 32). The combined biochar + treated waste water strategy maximised EO yield while minimising heavy-metal contamination risks through mechanisms such as metal immobilisation and improved microbial activity, providing a productive and environmentally more sustainable production system.

Irrigation, microclimate and water management

Moderate water stress frequently enhanced EO concentration and menthol content while simultaneously reducing total water consumption. In peppermint, mild deficit irrigation produced the highest EO content (0.97 %) and increased menthol proportion despite some reduction in total shoot biomass (25). In *M. arvensis*, irrigation at 70 % field capacity (FC) with biostimulants ensured continuous biomass production, increasing EO yields and improving water use efficiency (WUE) (33).

Further improvement in water productivity was obtained with partial root-zone drying (PRD); PRD at 70 % FC (PRD70) resulted in the maximum EO yield (23.7 kg ha⁻¹), whereas PRD at 55 % FC (PRD55) attained maximum water productivity based on oil (0.0191 kg m⁻³) (34). Soil mulching improved moisture retention and EO yield; synthetic polyethylene films showed superior biomass and oil production compared to other mulches (35, 36).

Moderate shading intensity increased EO concentration and antioxidant activity in several Lamiaceae species including mint (37, 38). Shade and agrivoltaic systems modified the crop microclimate and water relations illustrated in Fig. 3. Under dynamic agrivoltaic configurations, evapotranspiration declined by 81 % beneath the solar panels, EO percentage increased above unshaded controls and between-panel zones maintained competitive biomass levels with higher oil concentration, demonstrating the potential of integrated light-water co-management for commercial EO production systems (39).

Plant growth regulators (PGR), biostimulants and elicitors

Combined application of gibberellic acid (GA₃) with irradiated chitosan increased EO content by 27.9 %, EO yield by 61.3 % and menthol yield by 15.4 % in peppermint (40). Exogenous 24-epibrassinolide application increased EO yield per plant by 67–78 % and menthol yield by 100 %, primarily through enhanced glandular trichome density and secretory cell size (1).

The use of elicitors obtained from smoke, such as plant smoke water and karrikinolide KAR1, increased the EO concentration by 32 to 37 % and the EO production by 67 to 72 % under drought conditions and enhanced menthol, menthone and menthyl acetate proportions in the soil (41). The formulations of biostimulants, comprising seaweed extracts, humic acid, silicon and nanobiostimulants, positively affected EO yield, antioxidant capacity and WUE under deficit irrigation regimes (6).

Nanotechnology-based inputs

Silicon-based nanomaterials, including nano-silicon and bulk silicon, have also been reported to enhance EO yield and quality (42). Application of nano-kinetin boosted the EO content and EO yield by 46.6 and 50.8 % respectively, in peppermint mainly through improving the efficiency of photosynthesis and the characteristics of trichome development (43). The application of TiO₂ nanoparticles increased the EO yield by 105 % and menthol yield by 124 % due to an increase in trichome density and diameter (5). Silicon dioxide (SiO₂) nanoparticles resulted in a 62.5 % increase in EO content and improved the menthol and menthyl acetate fractions in peppermint while reducing oxidative stress (44). Nanoparticles of selenium and cerium oxide (CeO₂) increased tolerance to salt stress and EO composition towards commercial value (45, 46).

Cropping system design and cultural practices

Ridge planting in *M. arvensis* maximised EO yield (209.8 kg ha⁻¹), shortened crop duration and reduced irrigation water requirements (47). Intercropping with legumes particularly peppermint-faba bean and peppermint-soybean systems increased EO yield and improved oil quality by raising menthol fractions and reducing menthofuran and pulegone, with

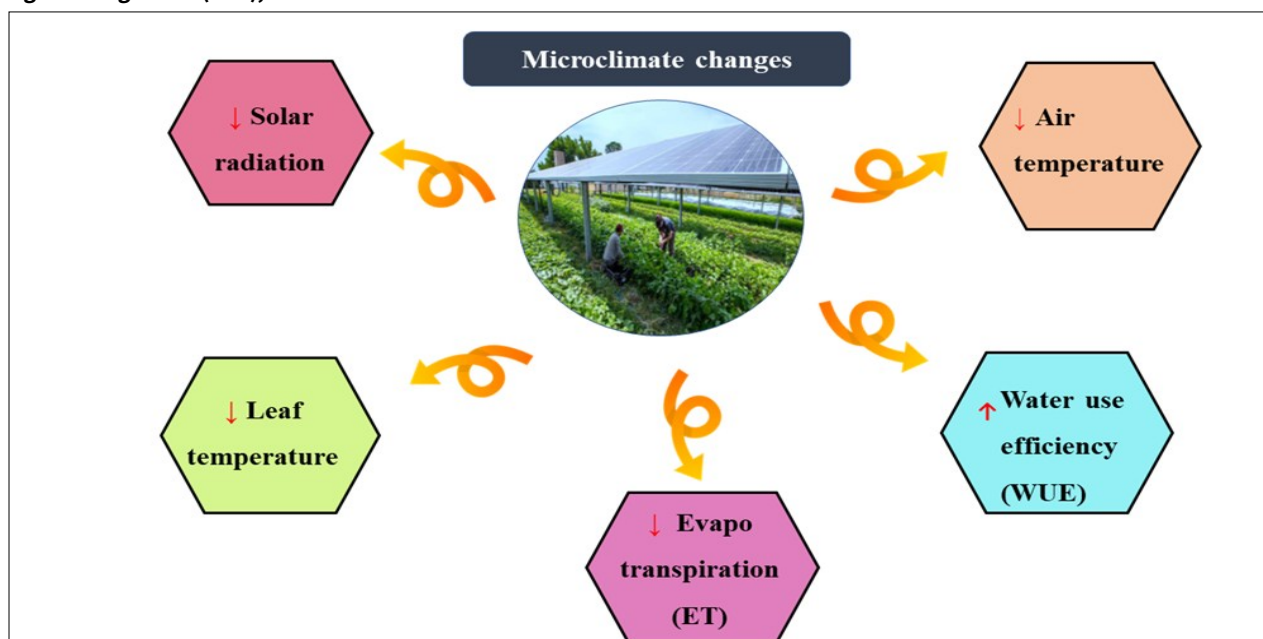


Fig. 3. Impact of agrivoltaic systems on microclimate (39).

optimum performance achieved at plant density ratios of 40 : 60 to 60 : 40 (48, 49). Peppermint-soybean strip intercropping further demonstrated compatibility with mechanised harvesting while maintaining oil quality advantages (50).

Cultivar selection strongly influenced both EO yield and composition. Fruit-scented chemotypes exhibited high linalool or piperitone oxide fractions and superior antioxidant activity relative to standard peppermint cultivars (3). Pollination mode affected chemotype stability and plant vigour, with wide variation observed in pulegone, piperitenone oxide, carvone and limonene fractions across pollination treatments (51).

Pest and disease management in reduced-chemical systems

Biological protection products consistently increased shoot biomass and EO yield while reducing disease incidence in mint production systems. Integrated use of biofertilisers, bioformulations and moderate inorganic NPK fertilisation doubled herbage and EO yield compared with exclusive chemical fertiliser inputs, while simultaneously improving soil fertility indicators (29). These integrated systems minimise pesticide residue risks in distilled oils and support sustainable, high-quality EO production aligned with market requirements (1).

Integrated biotechnological-agronomic strategies

Bio-optimised mint production

Integrated biotechnological-agronomic strategies form the conceptual basis of bio-optimised mint production, in which genetic improvement and field management are jointly designed to maximise shoot biomass, EO yield and oil quality with efficient resource utilisation. Metabolic engineering in peppermint has generated elite high-oil genotypes through overexpression of MEP-pathway enzymes and suppression of competing biosynthetic branches, achieving 61–78 % higher EO yield in replicated field trials alongside reduced menthofuran and pulegone concentrations (13). Comprehensive reviews confirm that targeted manipulation of the menthol and carvone pathways together with associated TF can redirect carbon flux toward desirable monoterpenes and improve compositional profiles (2). These studies collectively establish genetic control of terpene metabolism as a powerful platform for developing chemotype-specific, high-performance mint cultivars.

Field optimisation is essential to express the full genetic potential of engineered genotypes. In peppermint, 50 % conventional fertiliser combined with nano-chelated nutrients maximised biomass, leaf nutrient status and EO product ion (6.6 g m^{-2} ; 2.7 % oil content) while halving total fertiliser inputs, providing an environmentally efficient agronomic strategy (23). Integrated nutrient management combining mineral fertilisers, biofertilisers and plant growth regulators (PGR) consistently enhances EO yield, composition and climate resilience across mint species (1). Thus, coupling metabolically engineered genotypes with optimised nutrient nano-PGR management regimes constitutes the core of a bio-optimised mint system capable of simultaneously improving yield, oil quality and production sustainability.

Organic and sustainable production systems

Increasingly, sustainable mint cultivation systems are adopting vermicompost, biofertiliser, mycorrhizal inoculum, intercrops and nonchemical treatments for sustaining the economic yield of EO and improving the oil quality through decreasing chemical

reliance. The use of vermicompost promotes soil fertility, dry matter production and menthol production (21, 22, 26, 52). The use of biofertilisers and AM fungi inoculant promotes biomass and EO yield and enables the reduction in fertiliser dosages (29); mycorrhizal inoculation modifies EO composition by increasing the menthone fraction and lowering the pulegone fraction and total antioxidant capacity (53). Intercropping between peppermint and faba beans increases EO yield and menthol content but lowers menthofuran and pulegone (48). Biochar treatment promotes shoot biomass and decreases heavy metal uptake; it also promotes EO yield and menthol production (31).

Knowledge gaps and future research directions

Systems-level understanding of essential oil regulation

The regulatory networks governing EO biosynthesis including TFs, intracellular signalling cascades, epigenetic modifications and metabolism-trichome feedback loops remain largely unmapped and experimentally unvalidated in mint (54). No trichome resolved multi-omics datasets currently exist that would enable the identification of master regulatory hubs controlling oil biosynthesis flux (2). The mechanistic insights into the regulation of biosynthesis of these elicitors of stress responses like exogenous melatonin, which has been reported to increase EO levels up to 143 % - remain unknown and no predictive systems-level modelling approaches exist yet to establish the link between changes in gene expression and EO metabolism (55). Future research needs to focus on establishing specific genomic, proteomic and metabolomic data from trichomes to perform network analysis and pinpoint the regulatory targets.

Precision agronomy and digital decision-support tools

Although sensor-based irrigation scheduling (e.g., IoT sensors, remote sensing) and compositional optimisation are technically feasible, no real-time sensor-driven irrigation systems have been validated specifically for EO optimisation in commercial mint production. Optimal irrigation regimes identified experimentally such as PRD at 55–70 % FC have not been translated into farmer-accessible digital decision-support tools (33, 34). Fertigation and microclimate management systems are not yet dynamically integrated with EO quality optimisation algorithms (39). Furthermore, EO compositional shifts under deficit irrigation are not currently used to define site-specific irrigation thresholds and multi-objective optimisation frameworks balancing yield, quality and water productivity lack commercial validation (34). Development of precision digital agriculture platforms integrating sensor networks (IoT sensors, remote sensing), machine learning models and real-time EO prediction represents a priority for the field.

Environmental safety of nanotechnology-based inputs

Despite promising agronomic results, critical data are lacking regarding the environmental fate, chemical transformation and soil-profile leaching behaviour of applied nanoparticles including SiO_2 , TiO_2 and CeO_2 beyond single growing seasons (5, 42). Ecotoxicological effects of accumulated nanoparticles on soil microbial communities and beneficial insect populations remain uncharacterised. Phytotoxicity thresholds - for example, CeO_2 at concentrations exceeding 100 mg L^{-1} and the impacts of chronic, multi-year exposure are undefined (44, 46). Most published studies are based on pot or greenhouse experiments; multi-year field trials and integrated environmental risk assessment frameworks are

urgently needed before large-scale commercial deployment of nanotechnology-based inputs can be responsibly recommended (46).

Breeding for stress resilience and chemotype stability

No genomic associations between EO chemotypes and specific stress-tolerance traits have been established in *Mentha* and trade-offs between terpene production and defence metabolite synthesis remain unquantified (56). The complex polyploid genome structure and unusual reproductive biology of cultivated mints have not yet been fully integrated into modern marker-assisted or genomic selection breeding strategies (17). Causal genes responsible for chemotype differentiation especially pulegone, carvone and menthone biosynthesis genes have not yet been identified (3, 51). The stability of elite chemotypes under various growth conditions has also not been tested yet (3, 51). In future breeding programs, the newly developed reference genome sequence (8), as well as additional SSRs and single nucleotide polymorphisms (SNPs) markers coupled with genomic selection strategies should be considered for developing stable, high-oil and environment-tolerant lines.

Economic, social and regulatory challenges for commercial translation

Despite technical advances, transgenic mint oils face major regulatory barriers (4, 13). In the EU, Directive 2001/18/EC restricts the deliberate release of genetically modified organisms (57), while Regulation (EC) No 1829/2003 mandates the authorisation and labelling of all GM-derived food and feed (58). Essential oils derived from genetically modified crops fall under this regulation. Consumer acceptance of GM-derived EO remains unstudied. Although refined EO contain no detectable transgenic DNA or protein, which could theoretically improve acceptance, studies on consumer perception are absent (59). Scale-up from greenhouse to field is hindered by the polyploid, sterile nature of commercial mint cultivars and by transgene stability across environments (4). Multi-year field trials have confirmed transgene stability, but multi-location data are scarce and no published cost-benefit analysis exists for transgenic mint oil production (4, 13). The intellectual property landscape for *Mentha* innovations is complex. Key enabling technologies for DXR overexpression and menthofuran synthase suppression are protected by intellectual property rights, which may restrict freedom-to-operate for small enterprises. Overlapping patents on transformation methods and CRISPR-Cas9 applications may further complicate development (60). Future efforts must integrate techno-economic assessments, multi-location field validation, consumer perception studies and intellectual property landscaping to enable sustainable, commercial mint oil production.

Conclusion

The integration of advanced biotechnological tools with precision agronomy has led to significant breakthroughs with the potential to revolutionise mint essential oils (EO) production. By employing metabolic engineering, mainly via overexpression of 1-deoxy-D-xylulose-5-phosphate reductoisomerase coupled with inhibition of menthofuran synthase, scientists have succeeded in generating significant and consistent yields of 60–78 % through field experiments over several years while making remarkable advances in the quality of oils produced. When applied to genetically elite cultivars grown under the most optimal

agronomic practices such as precise nutrition, controlled deficit irrigation and use of biostimulants and nano-elicitors, these genetic gains translate into enhanced harvestable biomass and superior oil composition. This synergy confirms that genetic potential in mint is most fully expressed under carefully optimised production conditions. CRISPR-enabled gene editing approaches coupled with improved genomic resources of *Mentha* provide a promising way forward toward addressing polyploid and reproductively sterile bottlenecks through pathway engineering strategies. However, further progress in optimising these combined technologies is contingent upon answering several outstanding questions regarding EO regulation, developing reliable decision-making support systems based on plant phenotyping sensor technologies for irrigation and fertilisation control, establishing safe environmental profiles of nanomaterials involved in the process and defining genomic connections between abiotic stress tolerance and chemotype production. Further advancements in the study of trichomes through multi-omics approaches, digital agriculture and environmental risk assessment methodologies will play key roles in the development of sustainable production systems. These research endeavors are pivotal to sustainably meet the growing demands for mint EO in the food, pharmaceutical and cosmetic industries.

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Authors' contributions

JJ carried out the writing of review article, collected the literatures and drafted the manuscript. MS conceived the study, participated in its flow and direction and critically revised the manuscript for important intellectual content. PT assisted in manuscript preparation. LRD contributed to manuscript editing and technical guidance. AT participated in literature review and manuscript refinement. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

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