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BIOPROSPECTING THE POTENTIAL OF PLANT GROWTH PROMOTING RHIZOBACTERIA-ASSISTED CULTIVATION PRACTICES TO REDEFINE THE PRODUCTIVITY OF PEPPERMINT (*MENTHA PIPERITA* L.)

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ABSTRACT

Aim To evaluate the potential of the plant growth-promoting rhizobacterium (PGPR) *Agrobacterium radiobacter*, isolated from the rhizosphere of peppermint genotype MPS-3637, in enhancing biomass, essential oil yield, and menthol content of peppermint (*Mentha piperita* L.) variety CIM-Suras under field conditions.

Methods and results *Agrobacterium radiobacter* was aseptically isolated from the rhizospheric soil of peppermint genotype MPS-3637 and applied to suckers of peppermint variety CIM-Suras under field conditions, either alone or in combination with vermicompost (@ 5 kg ha^{-1}). Compared with untreated control plants, inoculation with *A. radiobacter* alone increased biomass by 1.5-fold, resulting in an 80.70% increase in essential oil content and a 13.78% increase in menthol content. The combined application of *A. radiobacter* and vermicompost further enhanced biomass by 2.5-fold, increased essential oil content by 54.39%, and improved menthol content by 11.08% over the control.

Conclusion The application of *Agrobacterium radiobacter*, either alone or in combination with vermicompost, significantly enhanced biomass production, essential oil yield, and menthol content in peppermint variety CIM-Suras. These findings demonstrate the potential of this PGPR as a sustainable and environmentally friendly approach for improving peppermint productivity and profitability while reducing reliance on conventional agricultural inputs.

Impact statement Plant growth-promoting rhizobacteria offer a sustainable alternative to conventional inputs, but their effectiveness depends on the selection of compatible host–microbe combinations. This study demonstrates that *Agrobacterium radiobacter* can substantially enhance peppermint biomass, essential oil yield, and menthol content under field conditions. These findings support the development of targeted microbial bioinoculants for medicinal and aromatic crops, contributing to sustainable peppermint cultivation, improved farmer profitability, and reduced reliance on chemical inputs.

KEYWORDS: *Agrobacterium* spp., biopriming, essential oil, *Mentha* spp., menthol, PGPR.

INTRODUCTION

Peppermint (*Mentha piperita* L.), a member of the family Lamiaceae is a medicinally important aromatic herb, widely grown in the temperate to sub-temperate areas of the world (Prasad et al. 2021). It is mainly cultivated in Europe, China, Brazil, America, and India as one of the most important essential oil-bearing crops (del Rosario Cappellari et al. 2015, Hayes et al. 2007). The essential oil (EO) of the crop, rich in terpenoids, flavonoids, polyphenols, carotene, alpha-tocopherol, betaine, and choline, is extracted by steam distilling its fresh herbs (Farnad et al. 2014). Other secondary metabolites of peppermint, extracted from this crop are hesperidin, didymin, rosmarinic acid, and diosmin which are reported by Rizvi et al. (2022). Its essential oil along with dried and fresh herbs are used in the sectors of pharmaceuticals as well as Fast-moving Consumer Goods (FMCG). EOs of peppermint are extensively used in the food and confectionary industries for adding flavours to chewing gums, candies, toothpaste, mouthwashes, etc. (del Rosario Cappellari et al. 2015). Along with contributing to aromatherapy, peppermint is also used as an antipyretic, antispasmodic, analgesic, carminative, diaphoretic, anti-diarrhea, and antimicrobial agent (Chiappero et al. 2019, Zheljazkov et al. 2010). The extracts of M. piperita are well known to cure various diseases in humans like the inflammation of the pharynx and liver, gastrointestinal tract disorders, nausea, vomiting, cramps, and dyspepsia (Shalayel et al. 2017). The production of EO from peppermint is chiefly associated with the abundance of peltate and capitate types of glandular trichomes present over the upper as well as lower surface of the plant (Werker 2000). The accumulation of monoterpenes in peppermint is reported to occur only in the peltate type of glandular trichomes and EO gets secreted into the cavity formed by the separation of layers of cuticular material (del Rosario Cappellari et al. 2015). The globally accepted major constituents of its essential oil include menthol (36–46%), menthone (15–25%), iso-menthone (2.0–4.5%), 1,8-cineole (4.0–6%), menthofuran (1.5–6%), neo-menthol (2.5–4.5%), menthyl acetate (3.0–6.5%), and pulegone (0.5–2.5%) (Prasad et al. 2020). Even after being highly demanded in the food, pharma, and aroma industries the cultivation of peppermint is not that much popular among its growers as compared to menthol mint (*Mentha* arvensis L.). This comparably lower popularity of the crop is

due to its lower oil yield, generating lower profit for its growers. As it is a vegetatively propagated crop its genetic improvement is a major area of concern among breeders (Prasad et al. 2021). Although, there are numerous proficient varieties with improved morpho-chemical characteristics are being released by the breeding experts but genetic improvement of peppermint through the application of plant growth-promoting rhizobacteria (PGPR) is anticipated as a sustainable solution to the problem.

Bacteria inhabiting the rhizospheric region of root system of plants, assisting via directly or indirectly to improve the yield or quality of the crop is termed as plant growth promoting rhizobacteria (PGPR) (Singh and Kumar 2023). Solubilization of minerals like N, P, K, and increasing their availability for plant uptake is mediated through direct mechanisms while regulation of phytohormones and release of plant defensive chemical molecules/ biocontrol molecules to improve plant health, and growth is facilitated via indirect mechanisms of PGPRs (Singh et al. 2020). Inoculating aromatic plants with helpful microorganisms frequently improves plant growth indices and secondary metabolic responses. There are numerous reports available depicting the positive influence of PGPR inoculation with medicinal and aromatic crops (Banchio et al. 2009, Santoro et al. 2011, del Rosario Cappellari et al. 2013; Naik et al. 2020, Arora and Fatima, 2022, Singh and Kumar, 2023). Therefore, the present study is designed to harness the role of PGPR in improving growth and quality attributing traits of peppermint.

MATERIALS AND METHODS

Soil sample collection

The sampling of rhizospheric soil with intact root system was performed during October 2022 from the rhizospheric region of a morphologically distinct, menthofuran-rich line/genotype MPS36-37 of peppermint, growing at the experimental farm of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India (25.89ºN latitude, 80.98ºE longitude). The topsoil containing dry matter was removed with the help of sterile equipment and plants with their upper and underground stems were collected by digging up to 5-10cm of depth. Sterile plastic bags were used to carry the sample from the experimental farm to the Seed Quality Lab, Plant Breeding & Genetic Resource Conservation Division of CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India.

Isolation and morphological characterization of rhizobacteria

The isolation of bacteria inhabiting the rhizospheric region of peppermint line/genotype SP36-37 was performed over Nutrient Agar medium (NA), and Kings B medium (KB) (HiMedia Laboratories Pvt. Ltd) at 28±2°C by following the technique of serial dilution up to six dilutions (10-6). All the dilution plates, spread with 1ml aliquotes of each dilution over 2 selected growth mediums were examined for typical bacterial colonies. The well-defined single colonies were selected for streaking over fresh plates of their respective media for 3-5 days (Ashrafuzzaman et al. 2009, Saharan and Shuchita. 2015). Loopful cultures of purified single colonies from these freshly grown plates were streaked again over KB medium and incubated for 3 days at 28±2°C and examined under UV light of 365nm to observe the fluorescence production ability of the isolates (Nepali et al. 2018). Further, the morphology of the isolates was observed based on the shape, size, color, margin, elevation, and pigmentation of the colonies. Gram staining of the isolates was performed, using Gram Stains Kit (HiMedia Laboratories Pvt. Ltd.) following the manufacturer's instructions. After examining and recording the morphological description of isolates they were stored in glycerol stock at − 80 °C until further analysis.

Screening of isolates for their plant growth promotion activities

All the bacterial isolates from 2 different media (NA and KB) were screened for Indole acetic acid production, NH3 production, phosphate solubilization, and siderophore production to access the plant growth-promoting potentials of isolates.

IAA production assay

IAA production of isolates was estimated by following the calorimetric method (Okon et al. 1977). Bacterial cultures were inoculated with Luria Bertini broth (7.0 ± 2 pH) amended with 2% L-tryptophan and incubated under the orbital shaking incubator with the speed of 150 rpm and at the temperature of 28 ± 2°C for 5 days. Salkowski reagent was prepared by using the steps described by (Bric et al. 1991). The five days old cultures were centrifuged at 10000rpm for 10 minutes under a cold centrifuge and cell-free supernatant was mixed with Salkowski reagent in a 1:2 ratio. This mix was immediately placed in dark for 30 minutes and the absorbance was recorded at 530nm under spectrophotometer (Lebrazi et al. 2020a). The standard curve was prepared by using varying concentrations of IAA and results were recorded in µg/ml over control (Gordon and Weber, 1951).

NH_3 production test

The assessment of isolates for NH_3 production was performed by inoculating the bacterial isolates into 10ml peptone water (7.2 ± 2 pH) and incubated for 5 to 7 days at 28 ± 2°C under orbital shaking incubator of speed 150rpm (Iwata et al. 2010). The analysis of NH3 production was done by adding 1.0ml of Nessler's reagent into each tube, producing yellow to brown colour (Cappuccino and Sherman 2007). Afterwards, spectrophotometric estimation was done at 450nm for the quantitative analysis of ammonia production by each isolate. The standard was prepared by using different concentrations of ammonium sulphate ranging from 2µM to 10µM and the ammonia production was estimated in ppm (mg/L).

Inorganic phosphate solubilization

Invitro evaluation of all the isolates for phosphate solubilization was performed by point inoculating the bacterial cultures over Pikovaskaya's agar medium (HiMedia Laboratories Pvt. Ltd), enriched with 0.4% tricalcium phosphate, and the plates were incubated at 32 ± 2°C (Singh, 2015) for 5 days. The formation of a clear halo zone around the point inoculated culture represents the phosphate solubilization activity index (PSI) of the isolates (Premono et al., 1996). The formula calculated the efficiency of isolates to solubilize phosphate:

$$PSI = \frac{\text{Colony diameter (cm)} + \text{Halo zone diameter (cm)}}{\text{Colony diameter (cm)}}$$

Siderophore production

The siderophore production assay of isolates was performed by the protocol described by (Louden et al. 2011). Firstly, Chrome Azurol S (CAS) reagent was prepared and autoclaved by following the steps elaborated by (Schwyn and Neilands 1987). Afterward, 100ml of CAS reagent (pH 6.8) was mixed with sterilized 900ml of King's B agar medium and plates were incubated at 28 ± 2°C for 3-5 days and were analyzed after incubation (Alexander and Zuberer 1991). The formation of yellow to orange zone around the culture over blue-coloured CAS media, confirms the siderophore production ability of isolates (Saharan and Shuchita 2015).

Identification of potential PGPR

On the basis of plant growth promotion efficiency tests single isolate, giving positive results for NH3 production, K solubilization, IAA production and siderophore production along with giving fluorescence under UV light when inoculated over King's B Agar medium (Nepali et al. 2018) was selected for identification. The genomic DNA of the PGPR was isolated with the help of XploregenTM Bacterial DNA extraction Kit by following the manufacturer's instructions. Thereafter, the isolated DNA was amplified at V3-V4 regions of the 16S rRNA gene by using the universal primers 27F (5′-AGA GTT TGA TCC TGG CTC AG- 3′) and 1492R (5′- CGGT TAC CTT GTT ACG ACT T- 3′) by making 20µl of reaction mixture. The reaction mixture was prepared by adding, 2µl of PCR assay buffer (10X), 2µl of (2mM) dNTPs, 0.6µl of forward and reverse primers (10pmol), 2µl of Taq DNA polymerase (3U/µl) (Takara Bio India Pvt Ltd.) along with 1.5µl of DNA template (50ng/µl) and rest of the volume is made up with Millipore distilled water. The amplification of the reaction mixture was carried out under the Proflex PCR system from Applied Biosystems with initial denaturation at 95°C for 5 minutes followed by 35 cycles of denaturation at 95°C for 20 seconds, annealing at 55°C for 1 minute, extension at 65°C for 2 minutes, followed by a final extension at 72°C for 5 minutes. Further, this amplified PCR product was sent to a sequencing service (Anuvanshiki (OPC) Private Ltd., Kishangarh, India). The 16S rDNA gene of approximately 1400bp consensus sequence was resolved on an Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA). The identification of received sequences was analyzed by comparing their nucleotide homology with the genomic sequences available in the NCBI database (Jamalzadeh et al. 2021, Kumar et al. 2017). Afterward, the gene sequence was also submitted to GeneBank and the accession number was assigned.

In vivo analysis of plant growth promotion efficiency of isolate

The field experiment was carried out in Randomized Block Design (RBD) in a triplicate manner at the experimental farm of CSIR-CIMAP, Lucknow. A menthol-rich, high-yielding, newly released variety of peppermint, CIM-Suras was selected to access the plant growth promotion efficiencies of potential PGPR strain. A bacterial culture SPK-8, growing in Luria Bertini broth (adjust the pH to 7 ± 2) with a cell density of about 106cfu/ml was used to inoculate/bioatize the 20-day-old vegetative propagules of peppermint variety CIM-Suras. The suckers of CIM-Suras of size 5-10cm were surface sterilized with 70% ethanol and 0.5% sodium hypochlorite and were used to treat with different treatment conditions (Table 1). All the seven treatments, T1: surface sterilized suckers of CIM-Suras, biotized with isolate SPK-8; T2: surface sterilized suckers amended with vermicompost of 5 tons/ha on dry weight basis; T3: surface sterilized suckers amended with Farm Yard Manure (FYM) of 25 tons/ha on dry weight basis; T4: surface sterilized suckers amended with required dose of fertilizer (RDF) (120:60:60); T5: Suckers of CIM-Suras biotized with SPK-8 and amended with sterilized vermicompost (5 tons/ha); T6: Suckers of CIM-Suras biotized with SPK-8 and amended with sterilized Farm Yard Manure (FYM); T7: Suckers of CIM-Suras biotized with SPK-8 and amended with recommended dose of fertilizer (RDF). The surface sterilized suckers of peppermint variety CIM-Suras without any biotization and amendment were used as control. All seven sets of treatment along with control were transplanted end to end in 4-5cm deep furrows into eight rows of equal spacing (50cm) into 8 different beds with bed size 3×4 m2 and with 20cm plant to plant distance, during the mid-January month of 2023 (Table 1). The irrigation was provided once a week up to 20 days of transplanting after that the fields were irrigated as and when required to maintain the humidity of soil up to 60-70% throughout the experiment. A booster dose of the bacterial culture of the same strength (10^6cfu/ml) was diluted with distilled water (1:100) and sprayed again, into the soil near the root zone of plants, before the flowering initiation stage of the crop i.e., 45 days after transplantation. The metrological data of daily temperature fluctuation, precipitation, and average relative humidity were recorded during the experimentation period from January to May 2023. Also, the physiochemical properties of the field soil were analyzed and recorded during the onset of the experiment. The morphological data including plant height (cm), number of branches, number of leaves/branch and canopy diameter (cm) were recorded at the maturity stage of plants (90 DAP) and the plants were harvested during the first week of May 2023, after 110 days of transplanting to record the herb yield per bed (kg/bed). The collected herb was steam distilled to evaluate the oil content (%) as well as chemical constituents of the extracted essential oil by performing GC analysis.

Data collection and Statistical analysis

A one-way Analysis of Variance (ANOVA) was analyzed with the help of recorded data of treatments (T1, T2, T3, T4, T5, T6, T7), and control to assess the effect of plant growth promoting bacterial (PGPB) application over peppermint variety CIM-Suras. In the mean table CD at a 5% level ($p \leq 0.05$) was used for comparing treatment means according to Katsenios et al. (2022). The estimation of genotypic and phenotypic correlations (Dewey and Lu, 1959) and D^2 analysis (Mahalanobis, 1936) was performed using the software OriginPro 9.1 and Microsoft Excel program.

RESULTS AND DISCUSSION

Morphological characterization of isolates

A total of 14 bacterial isolates growing over both Nutrient Agar medium (NA), and Kings B medium (KB) were isolated from the line/genotype MPS-36-37 of peppermint and named according to the medium used for their isolation (Table 2). Fluorescence production by the isolates was also examined and recorded with other morphological characters of the isolates under Table 2. Isolates from SPN-1 to SPN-6 were pure cultured from the dilution plates of Nutrient Agar medium

(NA) and from SPK-1 to SPK-8 were pure cultured from Kings B medium (KB) plates. Out of 14 isolates, 8 bacterial isolates were gram-positive and 6 were gram-negative. However, only 4 out of 14 were fluorescence-producing, SPN-4 and SPK-8 were producing green pigment whereas SPK-1 and SPK-5 were producing yellow pigment when analyzed under 365nm of UV illumination (Table 2).

Plant probiotic activities of isolates

IAA production

All the morphologically characterized isolates were examined for their IAA production efficiency as the ability of microbes to produce IAA, an auxin-derived crucial phytohormone, is suggested as an effective measure for the screening of plant probiotics (Maharana 2019). Matsuda et al. (2018) describe IAA as a phytohormone directly influencing plant growth by interacting with plants and microbes. Cell division initiation, elongation, and enlargement in root cells are the properties of IAA described by Lebrazi et al. (2020a), increasing the surface area of the root thereby improving the absorption of soil nutrients and ultimately influencing plant health. In general, various organic compounds including L-Trp have been reported to be utilized by soil bacteria for the biosynthesis of IAA by involving processes like decarboxylation, deamination and/or hydrolysis reactions (Lebrazi et al. 2020b). The most likely pathway involved for the biosynthesis of IAA through rhizobacteria is the Indole-3-pyruvic acid pathway whereas, several other pathways such as Indole-3- acetonitrile pathway, tryptamine pathway, and Indole-3-acetamide pathway are also reported to occur in some species (Lee et al. 2004). The qualitative evaluation of collected rhizospheric samples for IAA production ability of isolates depict that out of 14 isolates only 6 (42.8%) were positive for IAA production test (Table 2). Those 6 isolates were quantitatively accessed further for their IAA production efficiency and results revealed that SPK-8 (24.8µg/ml) was highest IAA producer followed by SPK-2 (16.5µg/ml), SPN-2 (13.5µg/ml), SPK-4 (12.4µg/ml), SPK-7 (11.4µg/ml) and SPN-4 (10.8µg/ml) (Table 2). A significantly higher production of IAA, than our findings were recorded by Vasant et al. (2023), ranging from 20.7-133µg/mL. Whereas, during the study conducted by Singh et al. (2020), IAA production by the isolates ranged between 7.01-35.56µg/mL which was almost similar to the results we have obtained. The maximum IAA production in the study conducted by Bessai et al. (2022) was 67.14µg/mL and was about 37% higher than the concentration of IAA recorded in our study.

Ammonia production

The cellular synthesis of plant's enzymes is chiefly dependent on biologically fixed nitrogen which also promotes the synthesis of chlorophyll, proteins, DNA and RNA in plants (Hayat et al. 2010). The puissant plant growth-promoting microflora assists in fixing atmospheric nitrogen for plants either by associating symbiotically with them or by being present in free-living state. In the current study morphologically characterized rhizobacterial population was accessed for their ammonia production efficiency and results revealed that only 5 (35.7%) bacterial isolates out of 14 were able to produce ammonia in a range from 19.5mg/L to 49.4mg/L (Table 2). Similar findings of ammonia production through isolates were suggested by the study conducted by Vasant et al. (2023). A comparatively lower range of ammonia (5.0µM/ml-12.5µM/ml) produced through plant growth promoting isolates was recorded by Alotaibi et al. (2022).

Phosphate solubilization

Phosphorous is suggested as an essential macronutrient involved in proliferation and development of new tissues, production of nucleic acids, and regulation of protein synthesis as well as energy regulation in plants (Elhaissoufi et al. 2022). It is reported to increase the yield of crop by inducing the development of roots and root hairs formation, cell signalling and redox homeostasis (Elhaissoufi et al. 2020, Siedliska et al. 2021). According to Wang et al. (2022) most of the phosphate solubilizers induces IAA production along with plying their role in P cycling. Under the current study, the phosphate solubilization efficiency by isolates was estimated and only 3 out of 14 isolates, SPN-2, SPK-1 and SPK-8 were giving positive results. The phosphate solubilization index (PSI) recorded for potential phosphate solubilizers was in the range from 2.05 to 3.63 (Table 2) which was in accordance with the findings of Manzoor et al. (2017) and Rfaki et al. (2015). A slightly higher range of PSI, between 2.9 to 4.1 was recorded during the study conducted by Janati et al. (2022). However, the phosphate solubilization index by plant growth-promoting isolates ranged from 1.2 to 2.3, as reported by Mohamed et al. (2019).

Siderophore production

Siderophores are the low molecular weight (200-2000 Da) secondary metabolites produced from the rhizo-microbial niche of the plants during iron stress conditions, composed of small peptides with low molecular weight and high iron chelating potentials (Arora and Verma 2017). The iron-binding ligands, known as siderophores, released from the rhizobacteria facilitate the binding of ferric ions and transport them through the membrane receptor molecules into the cell thereby increasing the availability of iron to host plant (Niehus et al. 2017). It is reported to improve the health and growth of plants either by improving the iron availability to crop or by reducing its accessibility to phytopathogens (Santoro et al. 2015). After 7 days of incubation all of the isolates were accessed for siderophore production efficiency and results revealed that only one (SPK-8) out of 14 was giving positive results for the siderophore production test (Table 2).

Identification of potential PGPR

Out of 14 bacterial isolates cultured from the rhizospheric soil of line/genotype MPS36-37 of peppermint; single isolate SPK-8 was selected based on its strong ability to depict plant growth promotion activities. The 16S rRNA sequence identification of strain SPK-8 showed that the selected isolate belonged to the species *Agrobacterium radiobacter* DSM 30147 with 99.7% similarity and was assigned with accession no. PV682772. The selected isolate was applied further to assess its plant growth-promoting efficiency with the runners of variety CIM-Suras of peppermint in field conditions.

Field Experiment

A field experiment was established at CSIR-Central Institute of Medicinal and Aromatic Plants, Lucknow, India with the temperature fluctuation from a low range of 4˚C in January to a high temperature of 45˚C in June during the year 2023. A significant variation in relative humidity was recorded to range from 26% to 95% from January to May 2023. The mean

average precipitation during these five months was recorded 20.69mm. The soil type used for the field trial was sandy loam with pH 7.5 (soil to water ratio 1:2.5), electrical conductivity of 0.344mS/cm with medium range of Organic C (5.4 mg/L), medium concentration of available N2 (398.27 Kg/ha), high amount of available phosphorous (41.9 Kg/ha) and moderate K+ (193.5 Kg/ha).

The surface sterilized suckers of CIM-Suras of size 5-10cm without any treatment and any amendment (control) were transplanted into the first bed of size 3×4 m2 and with 20cm plant-to-plant distance. Further, the surface-sterilized suckers administered with selected PGPB (SPK-8) without any amendment (T1) was transplanted into the second bed. For treatment, T2, vermicompost with the rate of 5 tons/ha was used to amend the surface sterilized suckers of CIM-Suras, transplanted in the third bed. The surface-sterilized suckers of variety CIM-Suras of the same size i.e., 5-10cm were transplanted into fourth bed with the amendment of Farm Yard manure (FYM) of rate 25 tons/ha (T3). Similarly, for T4 surface sterilized suckers were amended with N:P:K taken in ratio the 120:60:60, T5 (PGPR+vermicompost@5tons/ha), T6 (PGPR+FYM@25tons/ha) and T7 (PGPR+RDF (N:P:K)@120:60:60) were transplanted in the fifth, sixth, seventh, and eighth beds respectively. Regrowth/leaf initiation into the transplanted suckers was initiated after 15 days of transplanting. The morphological data of the plants was collected after 90 days of transplanting and their mean was recorded under Table 4. The analysis of the variance of the morpho-chemical results shows that the mean square of all treatments along with the control was found to be highly significant ($p<0.01$) for all the studied qualitative and quantitative parameters (Table 3). A similar trend of highly significant variability within morpho-chemical traits with PGPR treatments in peppermint (Chiappero et al., 2019) has been reported previously. The estimations of genotypic and phenotypic variance, genotypic and phenotypic coefficient of variation, along with heritability and genetic advance for all the morphometric characters were calculated and recorded under Table 5. The phenotypic coefficient of variation for the studied characters like number of branches/plant, number of leaves/branch, canopy diameter, biomass (kg/bed) and oil content (%) were found to be slightly higher than the genotypic coefficient of variation depicting that the environmental effects have minorly influenced the studied traits up to some extent. the difference between GCV and PCV was found to be maximum for the trait: number of leaves/branch, showing the maximum influence of the environmental factors over this specific trait. The values of PCV in comparison to GCV recorded to be slightly higher in peppermint was reported earlier by Prasad et al. (2020) and similar results were depicted for different spp of *Mentha* by Mishra et al. (2016) and Venkatesha et al. (2019). However, the values of genotypic coefficient of variation and phenotypic coefficient of variation for the traits: the content of menthol (%), menthofuran (%), pulegone (%), and menthone (%), were recorded to be almost similar, suggesting that these traits were nearly uneffected by the influence of the environmental factors. The maximum value for the genotypic coefficient of variation was recorded for menthofuran (%) (36.81%), followed by menthone (%) (25.44%) and biomass (kg/bed) (21.87%) indicating the scope of further improvement of these traits through treatments (Prasad et al. 2021). A high heritability (>75%) was recorded for all the studied characters and high genetic advance as a percentage of the mean (>20%) was recorded for all the morphometric characters except for plant height, number of branches/plant, number of leaves/branch, canopy diameter and menthol content (Table 5). The highest genetic advance as a percentage of the mean was recorded for the menthofuran content (75.78%). The high heritability for all characters and high genetic advance for characters like biomass (kg/bed), oil content (%), menthofuran (%), pulegone (%) and menthone (%), suggests that these traits are highly influenced by the treatments used for the study.

The study of correlation determines the magnitude and nature of the association between different studied characters and reveals about the character responsible for selecting the most favourable treatment (Aftab et al. 2024). However, the relationship between phenotypic and genotypic correlation becomes important when the results of studied traits are contemplated to choose the best-applied treatment of the study (Toth et al. 2020). Table 6 shows that for almost all the characters except for canopy diameter for oil content, menthofuran, and biomass, the genotypic correlation was found higher than the phenotypic correlation, showing prominent inherent interconnection among the characters (Table 6, Fig.1). An Array of correlations among morpho-chemical parameters shows that plant height (cm) is positively and significantly correlated with biomass (kg/bed), oil content (%), menthol, and menthofuran (%). Oil content (%) was positively and significantly correlated with menthol (%), menthofuran (%), menthone (%), and biomass (kg/bed). The content of menthol showed a positive correlation between menthofuran (%) along with biomass (kg/bed) and menthofuran was correlated with biomass (kg/bed) (Table 6). The segregation of correlations into direct and indirect effects depicts the complex inter-relationship among different studied traits. Path coefficient analysis is simply standardized partial regression coefficient which split the correlation coefficient into direct and indirect effects on predicted variable (Neelam et al. 2016). A highly positive direct effect on biomass (kg/bed) was imparted by the content of pulegone (1.309), followed by menthofuran (1.135), menthol (0.352), and plant height (0.104) (Table 7). The residual effect of 0.077 indicates that the experimental model was reasonably fit (MacCallum et al. 1994). The study, therefore determines that including traits like pulegone, menthofuran, menthol, and plant height are important parameters for selecting treatments to improve the biomass (kg/bed) of the peppermint variety CIM-Suras.

The clustering of applied treatments using Mahalonobis D^2 analysis and Tocher's method was performed and the results of the study grouped all eight treatments including control into five different clusters (Fig.1). The findings indicate that cluster I, cluster II, cluster III had the highest number of genotypes i.e., two followed by cluster IV, and cluster V with one genotype in each cluster. Cluster I, comprised of two treatments T5 and T7 with favourable improvement in plant height, biomass (kg/bed), oil content (%), and menthol content (%) were grouped together. Cluster II was comprised of T4 along with control, showing almost similar for the studied traits like number of leaves/plant, oil content (%), menthol (%), and menthofuran content (%) (Fig.2). The third cluster comprised treatments like T3 and T6 showing almost similar values for traits like plant height, number of branches/plant, and the content of menthofuran, pulegone, and menthone. The first treatment (T1) was solely lying under cluster IV, showing maximum oil content (%) and methol content (%). Whereas, cluster V contained a single treatment T2, having the highest values for the content of pulegone. As per Singh

(1981), the trait with the highest contribution to treatmental effect was number of branches/plant with contriution of 207.7381 % followed by number of leaves/branch with contribution of 132.4405 %. The inference regarding the means of the traits for clusters depicts that all clusters performed differently for various studied treatments in peppermint variety CIM-Suras (Table 8). Cluster I showed the highest mean performance for plant height, oil content, menthol content, and menthofuran (%). Cluster II showed the maximum mean performances for the number of leaves/branch, pulegone, and menthone contents. Cluster III had the highest intracluster distance (470.42) followed by Cluster II (327.52) and Cluster I (307.54) (Table 8, Fig.2). Among five clusters the largest (7385.55) inter-cluster distance was within Cluster IV and Cluster III, followed by Cluster III and Cluster II (4985.91), and the minimum inter-cluster distance (833.4) was within Cluster I and Cluster IV (Table 8, Fig.2). This inter-cluster distance signifies the proportion of divergence in treatments, clubbed between different clusters and eases down the process of selecting the most favorable treatment. The treatments grouped under the clusters showing minimum inter-cluster distance are considered to exhibit more similarity in traits. Out of a total of five clusters, during this study T1, lying under Cluster IV, and T5 and T7 lying under Cluster I showed maximum inter-cluster difference or divergence from other clusters signifies that selecting these treatments (T1, T5 & T7) would be more favorable for improving the studied traits in peppermint variety CIM-Suras.

The deliberate analysis of mean data of morpho-chemical traits for various treatments revealed that a highly significant improvement throughout the experiment, over the control was recorded for plant height, biomass (kg/bed), and content of menthol (%). The maximum improvement (42.72%) in plant height and biomass (kg/bed) was recorded for T5 (SPK-8+vermicompost@5tons/ha) which was in accordance with Singh et al. (2007) and Padmavathiamma et al. (2008) when crops like sugarcane and chili were implemented with vermicompost and growth promoting bacterial inoculum (Gopinath et al. 2010). Similar results have been obtained for *Mentha* arvensis L. also with the same treatment conditions (Lothe et al. 2021). The suckers of peppermint variety CIM-Suras, amended with vermicompost (T2) was recorded to show maximum improvement (27.50%) in number of leaves/branch along with increasing the oil content (%) by 28.75% and menthol content by 7.43%, over the control (Table 4). Ram et al. (2012) reported that the amendment of vermicompost with peppermint increases the morphometric parameters of the crop. The experiment conducted by Pourhosseini et al. (2024) suggests that implementing vermicompost with peppermint significantly increases the number of leaves/plant and doubles the essential oil content along with increasing its menthol content. Implementing the isolate SPK-8 (T1) with the suckers of CIM-Suras was recorded to maximumly improve the content of essential oil (80.70%) and menthol (13.81%), over the control which was in accordance with the results derived from the study conducted by Pourhosseini et al. (2024) where growth promoting bacteria, employed with peppermint have increased the content of essential oil and menthol. The treatment of vegetative propagules of CIM-Suas with isolate SPK-8 amended with FYM @ 25tons/ha was recorded to double the biomass (kg/bed) and improve the plant height by 28.49%, oil content by 35.09% and 6.92% improvement in menthol content, over the control (Table 4). Verma et al. (2016) have reported that the application of Farm Yard Manure (FYM) with *Mentha piperita* was found to increase the biomass and essential oil yield of the crop significantly. The overall analysis of the results determines that T1 was most suitable for improving oil content (%) along with T5 showing the most suitability in improving biomass (kg/bed) by keeping the essential oil quality of CIM-Suras up to the level of globally accepted essential oil quality of peppermint.

CONCLUSIONS

This study was designed with an interest of improving the growth and yield of peppermint variety CIM-Suras without deflecting its essential oil quality. To achieve this objective a plant growth-promoting bacterial inoculum of SPK-8 (*Agrobacterium radiobacter* DSM 30147), isolated from the rhizospheric region of a peppermint line/genotype MPS-36-37 was introduced with the suckers of newly released, menthol rich peppermint variety CIM-Suras. This study compared the performance of the implementation of this plant growth-promoting bacterial inoculum solely as well as in combination with various organic and inorganic additives like vermicompost @5tons/ha, farm yard manure @25tons/ha, and recommended dose of NPK (120:60:60) over the variety CIM-Suras. The results of the study revealed that the application of different treatments showed improvement in plant height ranging from (80.83cm to 89.77cm); biomass (kg/bed) ranged from (3.57-5.00); oil content (%) from (0.55-1.03); menthol (%) from (69.98-80.85); and menthofuran (%) from (1.43-3.41). The treatment of suckers solely with SPK-8 (T1) showed maximum improvement in oil content (80.70%) with the highest improvement in menthol content (13.78%) along with keeping the menthofuran content in the acceptable range of US-type peppermint. The T1 was also found to be the most divergent from other applied treatments, interpreted from the clustering analysis. However, the highest improvement in plant height (42.72%), biomass (kg/bed) (150.0%), and content of menthofuran (122.88%) in peppermint variety CIM-Suras was analyzed for the suckers treated with SPK-8, amended with vermicompost (T5). Whereas, the administration of the same bacterial isolate (SPK-8) with vermicompost amendment is most favorable to improve the biomass as well as plant height of the variety CIM-Suras. Therefore, on the basis of designed experiment, T1 i.e., implementation of bacterial isolate *Agrobacterium radiobacter* with the suckers of CIM-Suras is the best-suited treatment condition to improve oil content of CIM-Suras with essential oil quality lying in the range of globally acceptable oil quality of peppermint. Thus, it may be suggested to peppermint growers to cultivate variety CIM-Suras with the implementation of plant growth-promoting bacterial isolate *Agrobacterium radiobacter* to harness its maximum growth potential. However, the cultivation of variety CIM-Suras amended with SPK-8 (*Agrobacterium radiobacter*), at varying locations and climatic conditions is essential to analyse the accurate performance for its growth and yield attributes.

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Author contributions

Vagmi Singh: Writing - original draft, Writing - review & editing, Formal analysis. Rajesh Kumar Verma: Conceptualization, Visualization, Methodology, Investigation. Rakesh Kumar: Visualization, Methodology, Investigation. Akanksha Singh: Conceptualization, Visualization, Investigation. Neelam: Visualization, Data curation. Ram Kishor: Visualization, Data curation. Akancha Gupta: Visualization, Data curation. Nashra Aftab: Visualization, Data curation. Birendra Kumar: Conceptualization, Visualization, Supervision, Writing - review & editing, Validation.

Conflicts of interest

The authors have declare no competing financial interests or personal relationships.

Data availability

The 16S rRNA gene sequences of the bacterial isolates have been deposited in the NCBI GenBank database under accession number PV682772 (https://submit.ncbi.nlm.nih.gov/subs/genbank/).

Ethical approval statement

This study did not involve human participants, human tissues, vertebrate animals, or endangered species. All experimental procedures were conducted using plant materials and microbial isolates in accordance with the institutional guidelines and applicable national regulations. Therefore, formal ethical approval and informed consent were not required for this study.

REFERENCES

1. Aftab, N., Gupta, A., Prasad, P., Kushwaha, H. K., Kishor, R., Singh, V., Chandra, S., & Kumar, B.. Eploring genetic diversity for high CBD content in cannabis accessions in tropical and subtropical regions of India. Biochemical Genetics 2024; 1–31. doi:10.1007/s10528-024-10914-2
2. Alexander, D., & Zuberer, D. Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. Biology and Fertility of Soils 1991; 12, 39–45. doi:10.1007/BF00369386
3. Alotaibi, F., St-Arnaud, M., & Hijri, M. In-depth characterization of plant growth promotion potentials of selected alkanes-degrading plant growth-promoting bacterial isolates. Frontiers in Microbiology 2022; 13, 863702. doi:10.3389/fmicb.2022.863702
4. Arora, N. K., & Fatima, T. Role of plant growth promoting microbes in managing sustainability of stressed agroecosystems. Environmental Sustainability 2025; 5(1), 1–3. doi:10.1007/s42398-022-00222-z
5. Arora, N. K., & Verma, M. Modified microplate method for rapid and efficient estimation of siderophore produced by bacteria. 3 Biotech 2017; 7(6), 381. doi:10.1007/s13205-017-1008-y
6. Ashrafuzzaman, M., Hossen, F. A., Ismail, M. R., Hoque, A., Islam, M. Z., Shahidullah, S., & Meon, S. Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. African Journal of Biotechnology 2009; 8. DOI:10.5897/AJB2009.000-9198
7. Banchio, E., Xie, X., Zhang, H., & Pare, P. W. Soil bacteria elevate essential oil accumulation and emissions in sweet basil. Journal of Agricultural and Food Chemistry 2009; 57, 653–657. doi:10.1021/jf8020305
8. Bessai, S. A., Bensidhoum, L., & Nabti, E. H. Optimization of IAA production by telluric bacteria isolated from northern Algeria. Biocatalysis and Agricultural Biotechnology 2022; 41, 102319. doi:10.1016/j.bcab.2022.102319
9. Bric, J. M., Bostock, R. M., & Silverstone, S. E. Rapid in situ assay for indoleacetic acid production by bacteria immobilized on a nitrocellulose membrane. Applied and Environmental Microbiology 1991; 57, 535–538. doi:10.1128/AEM.57.2.535-538.1991
10. Cappuccino, J. G., & Sherman, N. 2007. Microbiology: A laboratory manual. Benjamin-Cummings Publishing Company. https://books.google.co.in/books/about/Microbiology.html?id=EM1UPgAACAAJ&redir_esc=y
11. Chiappero, J., del Rosario Cappellari, L., Alderete, L. G. S., Palermo, T. B., & Banchio, E.. Plant growth promoting rhizobacteria improve the antioxidant status in *Mentha piperita* grown under drought stress leading to an enhancement of plant growth and total phenolic content. Industrial Crops and Products 2019; 139, 111553. doi:10.1016/j.indcrop.2019.111553
12. del Rosario Cappellari, L., Santoro, M. V., Reinoso, H., Travaglia, C., Giordano, W., & Banchio, E. Anatomical, morphological, and phytochemical effects of inoculation with plant growth-promoting rhizobacteria on peppermint (*Mentha piperita*). Journal of Chemical Ecology 2015; 41(2), 149–158. doi:10.1007/s10886-015-0549-y
13. del Rosario Cappellari, L., Santoro, M. V., Nievas, F., Giordano, W., & Banchio, E. Increase of secondary metabolite content in marigold by inoculation with plant growth-promoting rhizobacteria. Applied Soil Ecology 2013; 70, 16–22. doi:10.1016/j.apsoil.2013.04.001
14. Dewey, D. R., & Lu, K. A correlation and path-coefficient analysis of components of crested wheatgrass seed production. Agronomy Journal 1959; 51(9), 515–518.
15. Elhaissoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y., & Bargaz, A. Phosphate bacterial solubilization: A key rhizosphere driving force enabling higher P use efficiency and crop productivity. Journal of Advanced Research 2022; 38, 13–28. doi:10.1016/j.jare.2021.08.014
16. Elhaissoufi, W., Khourchi, S., Ibnyasser, A., Ghoulam, C., Rchiad, Z., Zeroual, Y., Lyamlouli, K., & Bargaz, A. Phosphate-solubilizing rhizobacteria could have a stronger influence on wheat root traits and aboveground physiology than rhizosphere P solubilization. Frontiers in Plant Science 2020; 11, 979. doi:10.3389/fpls.2020.00979
17. Farnad, N., Heidari, R., & Aslanipour, B. Phenolic composition and comparison of antioxidant activity of alcoholic extracts of peppermint (*Mentha piperita*). Journal of Food Measurement and Characterization 2014; 8(2), 113–121. doi:10.1007/s11694-014-9171-x

18. Gopinath, K. A., Venkteswarlu, B., Mina, B. L., Natraja, K., & Devi, K. G. Utilization of vermicompost as a soil amendment in organic crop production. Dynamic Soil, Dynamic Plant 2010; 4 (Special Issue 1), 48–57. https://api.semanticscholar.org/CorpusID:73632095
19. Gordon, S. A., & Weber, R. P. Colorimetric estimation of indoleacetic acid. Plant Physiology 1951; 26(1), 192–195. doi:10.1104/pp.26.1.192
20. Hayat, R., Ali, S., Amara, U., Khalid, R., & Ahmed, I. Soil beneficial bacteria and their role in plant growth promotion: A review. Annals of Microbiology 2010; 60(4), 579–598. doi:10.1007/s13213-010-0117-1
21. Hayes, J., Stavanja, M., & Lawrence, B. M. Mint: The genus *Mentha*. Boca Raton 2007; FL: CRC Press/Taylor & Francis.
22. Iwata, K., Azlan, A., Yamakawa, H., & Omori, T. Ammonia accumulation in culture broth by the novel nitrogen-fixing bacterium, Lysobacter sp. E4. Journal of Bioscience and Bioengineering 2010; 110(4), 415–418. doi:10.1016/j.jbiosc.2010.05.006
23. Jamalzadeh, A., Darvishnia, M., Khodakaramian, G., Bazgir, E., & Zafari, D. Genetic diversity and plant growth-promoting activity of the dominant bacteria from canola plants in Western Iran. Egyptian Journal of Biological Pest Control 2021; 31, 1–9. doi:10.1186/s41938-021-00442-1
24. Janati, W., Mikou, K., El Ghadraoui, L., & Errachidi, F. Isolation and characterization of phosphate-solubilizing bacteria naturally colonizing legumes rhizosphere in Morocco. Frontiers in Microbiology 2022; 13, 958300. doi:10.3389/fmicb.2022.958300
25. Katsenios, N., Andreou, V., Sparangis, P., Djordjevic, N., Giannoglou, M., Chanioti, S., & Efthimiadou, A. Assessment of plant growth-promoting bacteria strains on growth, yield, and quality of sweet corn. Scientific Reports 2022; 12(1), 11598. doi:10.1038/s41598-022-16044-2
26. Kumar, V., Menon, S., Agarwal, H., & Gopalakrishnan, D. Characterization and optimization of bacterium isolated from soil samples for the production of siderophores. Resource-Efficient Technologies 2017; 3(4), 434–439. doi:10.1016/j.reffit.2017.04.004
27. Lebrazi, S., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. Screening and optimization of indole-3-acetic acid production by Rhizobium sp. strain using response surface methodology. Journal of Genetic Engineering and Biotechnology 2020a; 18, 1–10. doi:10.1186/s43141-020-00035-9
28. Lebrazi, S., Niehaus, K., Bednarz, H., Fadil, M., Chraibi, M., & Fikri-Benbrahim, K. Screening and optimization of indole-3-acetic acid production and phosphate solubilization by rhizobacterial strains isolated from Acacia cyanophylla root nodules and their effects on its plant growth. Journal of Genetic Engineering and Biotechnology 2020b; 18, 1–12. doi:10.1186/s43141-020-00090-2
29. Lee, S., Flores-Encarnación, M., Contreras-Zentella, M., Garcia-Flores, L., Escamilla, J. E., & Kennedy, C. Indole-3-acetic acid biosynthesis is deficient in Gluconacetobacter diazotrophicus strains with mutations in cytochrome c biogenesis genes. Journal of Bacteriology 2004; 186(16), 5384–5391. doi:10.1128/JB.186.16.5384-5391.2004
30. Lothe, N. B., Mazeed, A., Pandey, J., Patairiya, V., Verma, K., Semwal, M., & Verma, R. K. Maximizing yields and economics by supplementing additional nutrients for commercially grown menthol mint (*Mentha* arvensis L.) cultivars. Industrial Crops and Products 2021; 160, 113110. doi:10.1016/j.indcrop.2020.113110
31. Louden, B. C., Haarmann, D., & Lynne, A. M. Use of blue agar CAS assay for siderophore detection. Journal of Microbiology & Biology Education 2011; 12(1), 51–53. doi:10.1128/jmbe.v12i1.249
32. MacCallum, R. C., Roznowski, M., Mar, C. M., & Reith, J. V. Alternative strategies for cross-validation of covariance structure models. Multivariate Behavioral Research 1994; 29(1), 1–32. doi:10.1207/s15327906mbr2901_1
33. Mahalanobis, P. C. On the generalized distance in statistics. Sankhyā B: The Indian Journal of Statistics 2018; 80 (Suppl. 1), S1–S7. doi:10.1007/s13171-019-00164-5
34. Maharana, P. K. Isolation and characterization of IAA-producing plant growth-promoting rhizobacteria (PGPR) from rhizospheric soil of ornamental marigold plant. International Journal of Life Science 2019; 7(2), 333–336. https://www.researchgate.net/publication/334095640_Isolation_and_characterization_of_IAA_producing_plant_growth_promoting_rhizobacteria_PGPR_from_rhizospheric_soil_of_ornamental_Marigold_plant
35. Manzoor, M., Abbasi, M. K., & Sultan, T. Isolation of phosphate-solubilizing bacteria from maize rhizosphere and their potential for rock phosphate solubilization–mineralization and plant growth promotion. Geomicrobiology Journal 2017; 34(1), 81–95. doi:10.1080/01490451.2016.1146373
36. Matsuda, R., Handayani, M. L., Sasaki, H., Takechi, K., Takano, H., & Takio, S. Production of indoleacetic acid by strains of the epiphytic bacteria Neptunomonas spp. isolated from the red alga Pyropia yezoensis and the seagrass Zostera marina. Archives of Microbiology 2018; 200(2), 255–265. doi:10.1007/s00203-017-1439-1
37. Mishra, A., Lal, R. K., Chanotiya, C. S., & Dhawan, S. S. Genetic elaborations of glandular and non-glandular trichomes in *Mentha* arvensis genotypes: Assessing genotypic and phenotypic correlations along with gene expressions. Protoplasma 2017; 254(2), 1045–1061. doi:10.1007/s00709-016-1011-x
38. Mohamed, A. E., Nessim, M. G., Abou-El-Seoud, I. I., Darwish, K. M., & Shamseldin, A. Isolation and selection of highly effective phosphate-solubilizing bacterial strains to promote wheat growth in Egyptian calcareous soils. Bulletin of the National Research Centre 2019; 43(1), 1–13. doi:10.1186/s42269-019-0212-9
39. Naik, K., Mishra, S., Srichandan, H., Singh, P. K., & Choudhary, A. Microbial formulation and growth of cereals, pulses, oilseeds and vegetable crops. Sustainable Environment Research 2020; 30(1), 1–18. doi:10.1186/s42834-020-00051-x
40. Neelam Chouksey, N. C., Priyanka Agarwal, P. A., Satish, Y., & Rajesh Chouksey, R. C. Direct, indirect and residual effects through path analysis using Microsoft Office Excel. International Journal of Agriculture Statistical Sciences 2016;12(1), 75–80.

41. Nepali, B., Bhattarai, S., & Shrestha, J. Identification of Pseudomonas fluorescens using different biochemical tests. International Journal of Applied Biology 2018; 2, 27–32. DOI: 10.13140/RG.2.2.23860.40328
42. Niehus, R., Picot, A., Oliveira, N. M., Mitri, S., & Foster, K. R. The evolution of siderophore production as a competitive trait. Evolution 2017; 71(6), 1443–1455. doi:10.1111/evo.13230
43. Okon, Y., Albrecht, S. L., & Burris, R. H. Methods for growing Spirillum lipoferum and for counting it in pure culture and in association with plants. Applied and Environmental Microbiology 1977; 33(1), 85–88. doi:10.1128/AEM.33.1.85-88.1977
44. Padmavathiamma, P. K., Li, L. Y., & Kumari, U. R. An experimental study of vermi-biowaste composting for agricultural soil improvement. Bioresource Technology 2008; 99(6), 1672–1681. doi:10.1016/j.biortech.2007.04.028
45. Pourhosseini, S. H., Azizi, A., Seyedi, F. S., & Hadian, J. Bio-fertilizer as a pathway to minimize nitrate leaching from chemical fertilizer in high-yield peppermint production. Journal of Cleaner Production 2024; 468, 143100. doi:10.1016/j.jclepro.2024.143100
46. Prasad, P., Kishor, R., Gupta, A., Saxena, V., Zaidi, S., Kushwaha, H., Singh, V., Verma, R. S., & Kumar, B. Genetic variability and character association among morphometric traits and essential oil constituents in eight half-sib seed progenies of peppermint (*Mentha piperita* L.). Trends in Phytochemical Research 2020; 4(3), 143–152. https://www.researchgate.net/publication/350397829_Genetic_variability_and_character_association_among_morphometric_traits_and_essential_oil_constituents_in_eight_half-sib_seed_progenies_of_peppermint_*Mentha*_piperita_L
47. Prasad, P., Singh, V., Aftab, N., Gupta, A., Kishor, R., Kushwaha, H. K., Verma, R. S., & Kumar, B. Gamma irradiation-induced variability in morpho-agronomic and oil quality traits of *Mentha piperita* L. International Journal of Radiation Biology 2021; 97(6), 737–745. doi:10.1080/09553002.2021.1893855
48. Premono, M. E., Moawad, A., & Vlek, P. L. G. Effect of phosphate-solubilizing Pseudomonas putida on the growth of maize and its survival in the rhizosphere. International Journal of Crop Sciences 1996; 11(1), 13–23.
49. Ram, M., Singh, M., Prajapati, A. K., Ram, D., & Rawat, G. Towards sustainable production of peppermint (*Mentha piperita* L.) through integrated use of vermicompost and cowpea green manuring with synthetic nitrogen fertilization. Archives of Agronomy and Soil Science 2012; 58(3), 293–301. doi:10.1080/03650340.2010.514266
50. Rfaki, A., Nassiri, L., & Ibijbijen, J. Isolation and characterization of phosphate-solubilizing bacteria from the rhizosphere of faba bean (Vicia faba L.) in Meknes region, Morocco. British Microbiology Research Journal 2015; 6(5), 247–257. doi:10.9734/BMRJ/2015/14379
51. Rizvi, A., Ahmed, B., Khan, M. S., El-Beltagi, H. S., Umar, S., & Lee, J. Bioprospecting plant growth-promoting rhizobacteria for enhancing the biological properties and phytochemical composition of medicinally important crops. Molecules 2022; 27(4), 1407. doi:10.3390/molecules27041407
52. Saharan, B., & Verma, S. Evaluation of rhizospheric bacteria from Ocimum sp. as potential PGPR. Journal of Microbial and Biochemical Technology 2015; 7(2), 88–95. doi:10.4172/1948-5948.1000187
53. Santoro, M. V., Zygadlo, J., Giordano, W., & Banchio, E. Volatile organic compounds from rhizobacteria increase biosynthesis of essential oils and growth parameters in peppermint (*Mentha piperita*). Plant Physiology and Biochemistry 2011; 49(10), 1177–1182. doi:10.1016/j.plaphy.2011.07.016
54. Schwyn, B., & Neilands, J. B. Universal chemical assay for the detection and determination of siderophores. Analytical Biochemistry 1987; 160(1), 47–56. doi:10.1016/0003-2697(87)90612-9
55. Shalayel, M. H. F., Asaad, A. M., Qureshi, M. A., & Elhussein, A. B. Anti-bacterial activity of peppermint (*Mentha piperita*) extracts against some emerging multi-drug resistant human bacterial pathogens. Journal of Herbal Medicine 2017; 7, 27–30. doi:10.1016/j.hermed.2016.08.003
56. Siedliska, A., Baranowski, P., Pastuszka-Woźniak, J., Zubik, M., & Krzyszczak, J. Identification of plant leaf phosphorus content at different growth stages based on hyperspectral reflectance. BMC Plant Biology 2021; 21(1), 1–17. doi:10.1186/S12870-020-02807-4
57. Singh, B. P. Molecular and functional diversity of PGPR fluorescent pseudomonads based on 16S rDNA-RFLP and RAPD markers. Journal of Environmental Biology 2015; 36, 1169–1175.
58. Singh, K. P., Suman, A., Singh, P. N., & Srivastava, T. K. Improving quality of sugarcane-growing soils by organic amendments under subtropical climatic conditions of India. Biology and Fertility of Soils 2007; 44(2), 367–376. doi:10.1007/s00374-007-0216-8
59. Singh, T. B., Sahai, V., Ali, A., Prasad, M., Yadav, A., Shrivastav, P., & Dantu, P. K. Screening and evaluation of PGPR strains having multiple PGP traits from hilly terrain. Journal of Applied Biology & Biotechnology 2020; 8(4), 38–44. doi:10.7324/JABB.2020.80406
60. Singh, V., & Kumar, B. A review of agricultural microbial inoculants and their carriers in bioformulation. Rhizosphere 2023; 26, 100843. doi:10.1016/j.rhisph.2023.100843
61. Toth, J. A., Stack, G. M., Cala, A. R., Carlson, C. H., Wilk, R. L., Crawford, J. L., Viands, D. R., Philippe, G., Smart, C. D., Rose, J. K. C., & Smart, L. B. Development and validation of genetic markers for sex and cannabinoid chemotype in Cannabis sativa L. GCB Bioenergy 2020;12(3), 213–222. doi:10.1111/gcbb.12667
62. Vasant, G., Bhatt, S., & Raghav, R. Isolation and molecular characterization of plant growth-promoting rhizobacteria from groundnut (Arachis hypogaea L.) rhizosphere. Current Agriculture Research Journal 2023; 11(1). doi:10.12944/CARJ.11.1.30
63. Venkatesha, K. T., Singh, V. R., Padalia, R., Verma, R., Upadyay, R., Kumar, R., & Chauhan, A. Genetic variability, D^2 analysis and character association among quantitative and qualitative traits of spearmint (*Mentha* spicata L.). Trends in Phytochemical Research 2019; 2(2), 101–108.

64. Verma, R. K., Verma, R. S., Rahman, L. U., Kalra, A., & Patra, D. D. Integrated nutrient management on biomass, oil yields and essential oil composition of peppermint (*Mentha piperita* L.) and residual fertility in a hilly soil. Journal of Essential Oil Bearing Plants 2016; 19(3), 582–591. doi:10.1080/0972060X.2014.905758
65. Wang, Z., Zhang, H., Liu, L., Li, S., Xie, J., Xue, X., & Jiang, Y. Screening of phosphate-solubilizing bacteria and their abilities of phosphorus solubilization and wheat growth promotion. BMC Microbiology 2022; 22(1), 296. doi:10.1186/s12866-022-02715-7
66. Werker, E. Trichome diversity and development. Advances in Botanical Research 2000; 31, 1–35. doi:10.1016/S0065-2296(00)31005-9
67. Zheljazkov, V. D., Cantrell, C. L., Astatkie, T., & Ebelhar, M. W. Productivity, oil content, and composition of two spearmint species in Mississippi. Agronomy Journal 2010; 102(1), 129–133. doi:10.2134/agronj2010.0446

Table 1. Details of treatments applied over peppermint variety CIM-Suras to analyse their effect over its growth improvement

SL No.	Treatment names	Treatment details
1	Control	surface sterilized suckers of CIM-Suras without any treatment
2	T1	surface sterilized suckers administered with *Agrobacterium radiobacter* (SPK-8)
3	T2	surface sterilized suckers amended with vermicompost of 5 tons/ha on dry weight basis
4	T3	surface sterilized suckers amended with Farm Yard Manure (FYM) of 25 tons/ha on dry weight basis
5	T4	surface sterilized suckers amended with required dose of fertilizer (RDF) (120:60:60)
6	T5	surface sterilized suckers of CIM-Suras biotized with SPK-8 and amended with sterilized vermicompost (5 tons/ha)
7	T6	surface sterilized suckers of CIM-Suras biotized with SPK-8 and amended with sterilized Farm Yard Manure (FYM)
8	T7	surface sterilized suckers of CIM-Suras biotized with SPK-8 and amended with recommended dose of fertilizer (RDF)

Table 2. Details of rhizospheric bacteria isolated from rhizospheric soil of MPS-36-37 genotype of *Mentha piperita* L.

Isolates	Shape	Size	Colour	Surface	Margin	Florescence Praduction	Gram stain	Siderophore Production	IAA production (µg/ml)	Ammonia Production (mg/L)	Phosphate solubilization Index
SPN-1	Round	Small	Yellowish	Smooth	Entire	-	+	-	-	-	-
SPN-2	Irregular	Medium	Creamish	smooth	Irregular	-	+	-	13.510	-	2.771
SPN-3	Round	Small	Yellowish	Smooth	Irregular	-	+	-	-	-	-
SPN-4	Round	Small	White	Smooth	Entire	Green	-	-	10.833	23.217	-
SPN-5	Round	Small	Yellowish	Smooth	Entire	-	+	-	-	-	-
SPN-6	Irregular	Small	Creamish	Smooth	Irregular	-	-	-	-	-	-
SPK-1	Round	Small	White	Shiny	Entire	Yellow	+	-	-	38.922	2.053
SPK-2	Round	Small	Yellowish	Shiny	Entire	-	-	-	16.477	-	-
SPK-3	Round	Small	White	Smooth	Irregular	-	+	-	-	19.513	-
SPK-4	Round	Small	Creamish	Smooth	Entire	-	-	-	12.423	2.160	-
SPK-5	Round	Small	Creamish	Smooth	Entire	Yellow	-	-	-	-	-
SPK-6	Irregular	Medium	White	Rough	Irregular	-	+	-	-	-	-
SPK-7	Round	Small	Yellowish	Smooth	Entire	-	-	-	11.432	-	-

SPK-8	Round	Small	White	Smooth	Entire	Green	+	+	24.792	4.941	3.632

Table 3. Analysis of Variance of morphochemical traits of peppermint variety CIM-Suras undergone through various treatments.

Source of Variation	DF	Plant Height (cm)	No. of Branch/plant	No. of Leaf/branch	Canopy Diameter (cm)	Biomass (kg/bed)	Oil yield (%)	Menthol (%)	Menthofuran (%)	Pulegone (%)	Menthone (%)
Replication	2	2.57	0.04	0.13	0.53	0.016	0.010	0.306	0.004	0.005	0.0012
Treatments	7	189.78**	20.18**	38.55**	29.80**	2.272**	0.081**	43.18**	1.452**	0.018**	5.6078**
Error	14	3.37	2.47	6.17	1.60	0.019	0.003	0.146	0.0007	0.0004	0.0016
Total	23										

*p<.05 and **p<.01

Table 4. Effect of various treatments on morpho-chemical parameters of peppermint variety CIM-Suras

Treatments	Plant Height (cm)	No. of Branch/plant	No. of Leaf/branch	Canopy Diameter (cm)	Biomass (kg/bed)	Oil content (%)	Menthol (%)	Menthofuran (%)	Pulegone (%)	Menthone (%)
Control	62.90	28.67	30.33	48.00	2.00	0.57	71.06	1.53	0.61	5.73
T1	86.40**	24.67**	30.67	46.50	4.53**	1.03**	80.85**	2.34**	0.60	5.16**
T2	82.00**	22.67**	38.67**	47.80	4.17**	0.80*	76.34**	1.43**	1.97**	6.15**
T3	81.50**	16.00**	26.33	45.73*	4.27**	0.55	72.48**	1.49	0.55**	6.67**
T4	83.55**	16.33**	30.67	44.33**	4.00**	0.63	69.98**	1.58*	0.48**	5.30**
T5	89.77**	23.33**	35.67*	45.50*	5.00**	0.88**	78.93**	3.41**	0.37**	4.93**
T6	80.83**	22.00**	28.67	38.10**	4.00**	0.77**	75.98**	1.50	0.45**	5.78
T7	82.27**	17.00**	22.33**	43.73**	3.57**	0.75**	76.35**	1.44**	0.51**	6.27**
SEm	1.061	0.907	1.434	0.730	0.079	0.030	0.221	0.016	0.012	0.023
CD (5%)	3.22	2.75	4.351	2.214	0.239	0.0906	0.669	0.047	0.037	0.069
CD (1%)	4.46	3.82	6.039	3.073	0.332	0.1258	0.928	0.066	0.051	0.096

T1: surface sterilized suckers administered with SPK-8; T2: surface sterilized suckers amended with vermicompost of 5 tons/ha on dry weight basis; T3: surface sterilized suckers amended with Farm Yard Manure (FYM) of 25 tons/ha on dry weight basis; T4: surface sterilized suckers amended with required dose of fertilizer (RDF) (120:60:60); T5: Suckers of CIM-Suras biotized with SPK-8 and amended with sterilized vermicompost (5 tons/ha); T6: Suckers of CIM-Suras biotized with SPK-8 and amended with sterilized Farm Yard Manure (FYM); T7: Suckers of CIM-Suras biotized with SPK-8 and amended with recommended dose of fertilizer (RDF).

Table 5. Estimates of genetic parameters for various morpho-chemical traits of peppermint variety CIM-Suras undergone through different sets of treatments

Sl no.	Characters	σ2g	σ2p	GCV	PCV	h^2	GA as % of mean
1	Plant Height (cm)	62.134	65.507	9.713	9.974	94.85	19.487
2	No. of Branch/plant	5.905	8.375	10.322	12.293	79.259	17.854
3	No. of Leaf/branch	10.792	16.964	10.512	13.180	82.587	17.272

4	Canopy Diameter (cm)	9.400	10.997	6.819	7.376	85.469	12.986
5	Biomass (kg/bed)	0.751	0.770	21.872	22.141	96.858	44.507
6	Oil content (%)	0.026	0.029	21.600	22.651	90.649	42.277
7	Menthol (%)	14.343	14.489	5.033	5.059	98.992	10.316
8	Menthofuran (%)	0.484	0.485	36.814	36.841	97.565	75.784
9	Pulegone (%)	0.006	0.006	15.012	15.513	99.655	29.922
10	Menthone (%)	1.869	1.870	25.440	25.451	99.563	52.385

Table 6. The genotypic and phenotypic correlation coefficient among ten morpho-chemical traits on biomass (kg/bed) of peppermint variety CIM-Suras

Characters		No. of Branches	No. of Leaf/ plant	Canopy Diameter (cm)	Oil content (%)	Menthol (%)	Menthofuran (%)	Pulegone (%)	Menthone (%)	Biomass (kg/bed)
Plant Height (cm)	G	-0.485*	0.170	-0.235	0.641**	0.603**	0.529**	-0.042	-0.294	0.978**
	P	-0.428*	0.147	-0.227	0.585**	0.583**	0.508*	-0.047	-0.287	0.931**
No. of Branch/plant	G	1	0.494*	0.337	0.338	0.295	0.312	0.168	-0.447*	-0.385
	P		0.438*	0.296	0.255	0.252	0.282	0.146	-0.393	-0.324
No. of Leaf/branch	G		1	0.421*	0.365	0.235	0.426*	0.629**	-0.457*	0.297
	P			0.369	0.285	0.199	0.381	0.574**	-0.402	0.292
Canopy Diameter (cm)	G			1	-0.023	-0.051	0.142	0.442*	-0.011	-0.178
	P				-0.037	-0.050	0.144	0.407*	-0.007	-0.180
Oil content (%)	G				1	0.959**	0.618**	0.106	-0.571**	0.614**
	P					0.897**	0.565**	0.099	-0.543**	0.552**
Menthol (%)	G					1	0.599**	0.090	-0.340	0.597**
	P						0.587**	0.090	-0.339	0.585**
Menthofuran (%)	G						1	-0.306	-0.741**	0.571**
	P							-0.300	-0.728**	0.553**
Pulegone (%)	G							1	0.313	0.017
	P								0.310	0.021
Menthone (%)	G								1	-0.274
	P									-0.270

Table 7. The direct (in bold letters) and indirect effect of 10 morpho-chemical traits on biomass (kg/bed) of peppermint variety CIM-Suras.

Characters	Plant Height (cm)	No. of Branches/plant	No. of Leaf/branch	Canopy Diameter (cm)	Oil content (%)	Menthol (%)	Menthofuran (%)	Pulegone (%)	Menthone (%)
Plant Height (cm)	0.104	0.300	-0.125	0.089	-0.322	0.212	0.600	-0.055	0.175
No. of Branches/ plant	-0.050	-0.618	-0.363	-0.127	-0.170	0.104	0.354	0.220	0.266
No. of Leaf/branch	0.018	-0.306	-0.735	-0.159	-0.183	0.083	0.484	0.823	0.272
Canopy Diameter (cm)	-0.024	-0.208	-0.309	-0.377	0.012	-0.018	0.162	0.579	0.006
Biomass (kg/bed)	0.066	-0.209	-0.268	0.009	-0.502	0.338	0.701	0.139	0.341
Menthol (%)	0.062	-0.183	-0.172	0.019	-0.481	0.352	0.680	0.118	0.202
Menthofuran (%)	0.055	-0.193	-0.313	-0.054	-0.310	0.211	1.135	-0.401	0.442

Pulegone (%)	-0.004	-0.104	-0.462	-0.167	-0.053	0.032	-0.347	1.309	-0.187
Menthone (%)	-0.030	0.276	0.336	0.004	0.287	-0.120	-0.840	0.410	-0.596

Table 8. Cluster means of different clusters for the morpho-chemical traits in peppermint variety CIM-Suras

Trait	cluster I	cluster II	cluster III	cluster IV	cluster V
Plant Height (cm)	88.08333	82.13333	73.22333	81.5	80.83333
No. of Branches/plant	22.66667	23.5	25.66667	25.66667	21.66667
No. of Leaf/branch	31.66667	33.66667	30.5	26.33333	32
Canopy Diameter (cm)	46	45.76667	46.16667	45.73333	38.1
Biomass (kg/bed)	4.333333	4	3	4.533333	4
Menthol (%)	0.958333	0.775	0.6	0.55	0.766667
Menthofuran (%)	79.88833	76.345	70.52167	72.48333	75.98
Pulegone (%)	2.876667	1.433333	1.556667	1.49	1.503333
Menthone (%)	0.488333	1.236667	0.541667	0.546667	0.453333
Plant Height (cm)	5.045	6.208333	5.515	6.67	5.78

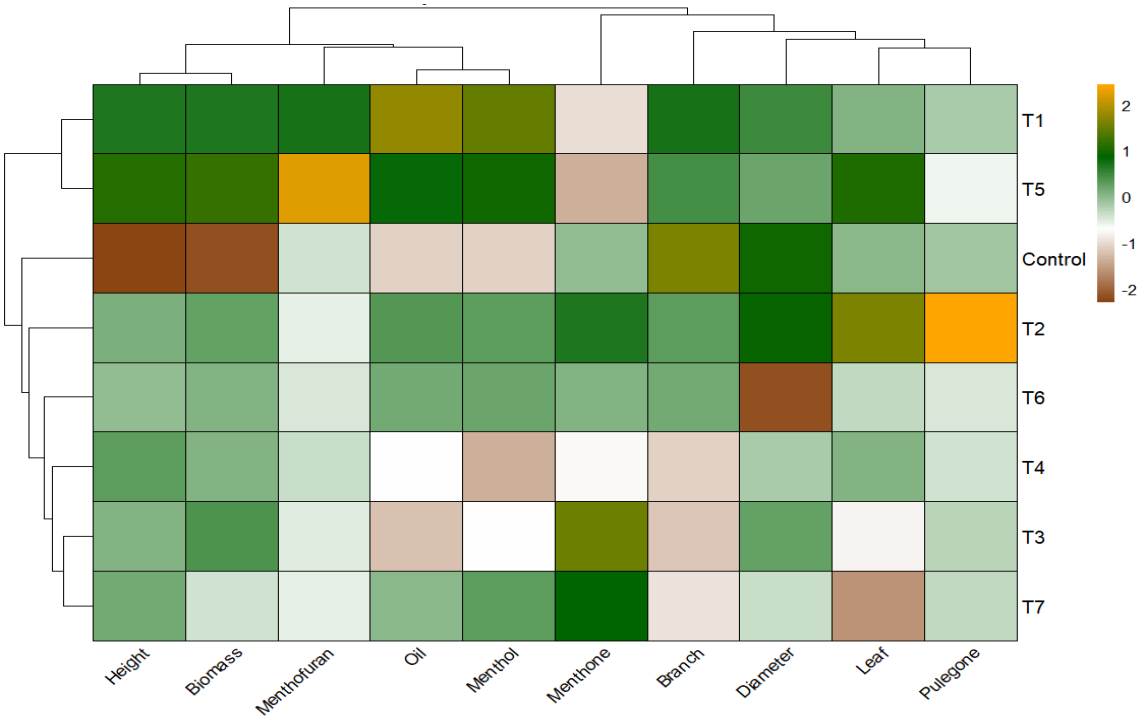

Figure 1. Heat map showing correlation between morpho-chemical attributes along with applied treatments over peppermint variety CIM-Suras.

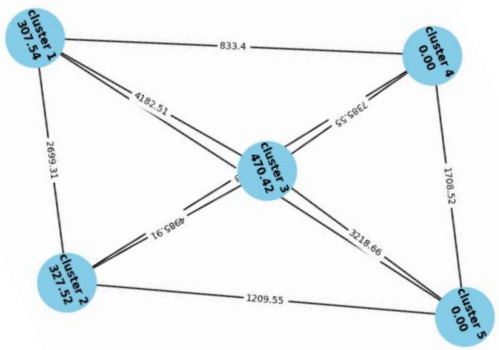

Figure 2. Average inter and intra cluster distances (Mahalanobis Euclidean distance) among 8 treatments of peppermint variety CIM-Suras.