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Essential oil production by *Mentha piperita* grown using aeroponics

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ABSTRACT:

Aeroponics is a form of soilless cultivation system with controlled conditions for growth of plants. Growth and production of essential oil was studied in the economically important *Mentha piperita* variety using aeroponics system. Chemical composition of the essential oil was compared with plants grown in soil. Effect of biostress on production and chemical composition of essential oil was studied in aeroponics system. This system is suitable for growing peppermint plants and resulted in significant increase in oil production by the plant with enhanced menthol content

***Keywords**:* Aeroponics, soilless cultivation, peppermint, biostress, essential oil

INTRODUCTION:

Peppermint, botanically known as *Mentha piperita L.*, an aromatic perennial herb belongs to the Lamiaceae family. The plant grows from 45 to 80 cm tall, with long petiolated opposite lanceolate leaves. The plant is cultivated throughout the world for peppermint oil produced in the leaves. The leaf lamina (4–14 cm) possesses hair and glandular trichomes on both the surfaces which contain the essential oil. Peppermint is grown as an annual crop in India. The commercial cultivation of peppermint is concentrated in the Indo- Gangetic plains i.e. in the states of Uttar Pradesh, Punjab and Haryana of which about 95 per cent of the crop is grown in Uttar Pradesh. India is a leading exporter contributing about 80% (40,000 tons) of essential oil, menthol crystal and allied products. Today, mint cultivation occupies about 3,00,000 hectares in India. (Chaturvedi et al, 2021)

However, the cultivation and the production of essential oil is limited by agricultural and environmental factors, the presence of specific pathogens, and by differences in comparative costs. Some of the factors affecting essential oil production of *M. piperita* in India include type of soil, climate, altitude, fertilizers and drying conditions of the leaves. (Soltanbeigi et al, 2021)

Highly acidic or alkaline soils are challenges for cultivation and the crop requires near neutral soil pH. The crop initially requires lower temperatures and later, a mean temperature of 20–40°C is suitable for its vegetative growth. Ample sunshine is required during most part of the growing period; shade is undesirable as it induces higher ester and menthone content in the oil.

The plants are propagated in the spring, when the young shoots from the crop of the previous year attain a height of about 10 cm.

Varieties of planting material having herb yield potential between 200–225 q/ha and oil content between 0.40–0.45% are available. A plantation lasts about four years, the best output being the second year. The fourth-year crop has lower yield.

Under Indian conditions, rust, powdery mildew, wilt, leaf blight and stolon rot are the five major fungal diseases in regions with high humidity. Of these, the recurrence of leaf blight and rust is more frequent. Harvest occurs between 145 to 160 days for first harvest and 97 to 111 days for second harvest of the crop. The content of oil and its chemical constituents vary with the growth and developmental stage of the plant.

On suitable soil and with proper cultivation, yields of 15 to 17 tons of peppermint herb per hectare may be expected.

Soilless cultivation of plants (Hussain et al, 2014)

Soilless culture is the technique of growing plants under soil-less condition with nutrients provided at the roots. This includes hydroponics and aeroponics. Aeroponics is a form of hydroponic plant cultivation in which plant roots are suspended in a closed chamber and misted with a complete nutrient solution. Aeroponics requires no solid or aggregate growing medium and allows for easy access to roots. The chamber and misting system provide complete control of the root zone environment, including temperature, nutrient level, pH, humidity, misting frequency and duration, and oxygen availability. Advantages of growing plants using aeroponics in comparison to soil as a medium and even hydroponics are many. Round the year cultivation, increased plant growth, easier system maintenance, reduced demand for space, nutrients and water, easier and multiple harvesting, disease free produce, reduced demand for energy. Aeroponics systems can reduce water usage by 98 per cent, fertilizer usage by 60 per cent, and pesticide usage by 100 per cent, all while maximizing crop yields (Kumari and Kumar 2019). In the present work, soilless cultivation of peppermint plants is reported with increased levels of secondary metabolite production.

Materials and Methods

Plant material and preparation of cuttings

Peppermint plant cuttings (Kukrail variety) were procured from CIMAP, Pantnagar, India and maintained in the herbal garden of the Innovation Center.

The multi nodal cuttings were harvested regularly from the herbal garden-maintained plants for experiments. Average length of the cuttings used for the study was 18 cm with 2-3 nodes bearing atleast six leaves.

The cuttings were dipped in a solution of 0.1 % hydrogen peroxide for 5 min, washed with RO treated water at pH 6 and subsequently dipped in a solution containing 5 ppm IBA and 0.1% Calcium chloride for 30 minutes.

The cuttings were then placed in aeroponics system for experiments.

Aeroponics system

The system consisted of vertical towers with 40 plant slots per tower in a polyhouse with controlled environment. Nutrition was provided using Hoagland's solution with cycle time of 30 seconds per hour. The humidity was maintained in the range of 55-65%RH and temperature between 25-30°C.

Conditions of adventitious rooting

For adventitious rooting combination of IBA and Calcium chloride in Hoagland's solution was provided.

Measurement of Yield.

Fresh weight of each plant was measured on harvest and average weight of 10 plant was noted.

The plants were dried using solar drying and essential oil was extracted using Clevenger apparatus.

The essential oil extracted was analysed using Shimadzu make GCMS-TQ8040, with auto sampler Column: RTX-20, Carrier gas – helium a1ml/min flow rate, injection 1 µL with split ratio100:1, injector temperature 280°C , Detector temperature: 260°C, Program: 40°C for 0.5 min hold, increase temperature at 5°C/min to 290°C, hold for 3 minutes.

Essential oil content was compared with garden grown plants.

Parameters studied for maximum oil production included leaf size, harvest time, weight of plant (average of 10 plants). Leaf size values were calculated as area from length x breath of at least 10 leaves and average as well as mean SD values were calculated.

Materials: Chemicals used for preparation of nutrient solution were purchased from SRL labs, Seaweed extract was a gift sample from C6 solutions, bioconsortium containing microorganisms including spores of the fungi *Trichoderma* was purchased from Bloomberg Agro as tablets of 4 g. the biofertiliser was dispersed in water (1g/liter) and mixed with cocopeat in the ratio 1:5 of water. Cocopeat was placed at the roots of the plants (1g/plant). Seaweed extract was sprayed on foliage as per instructions from the manufacturer; 1g/Liter water sprayed to give approximately10ml per plant with frequency of 15 days

GC-MS: the GC-Ms profile of essential oils was analysed using Agilent 7000E Triple Quadrupole GC-MS. The method followed was as follows:

Column Oven Temp.	50.0 °C
Injection Temp.	230.00 °C
Injection Mode	Split
Flow Control Mode	Linear Velocity
Pressure	26.7 kPa
Total Flow	10.5 mL/min
Column Flow	0.68 mL/min
Linear Velocity	30.0 cm/sec

Purge Flow	3.0 mL/min	
Split Ratio	10.0	
Splitter Hold	OFF	
Rate	Temperature(°C)	Hold Time(min)
-	50	1
5	210	1

Results and discussion

1. **Increase in leaf size with time**

The increase in leaf size was measured over 40 days.

As seen in table 1, the leaf size of bioconsortium treated plants was greater than control plants indicating faster growth as well as healthy leaves. The comparative increase in leaf size was constant with a 35% bigger leaf in bioconsortium treated plants after 40 days of growth.

Growth period days	Control area Av sq cm (LxB)	Mean SD	Bioconsortium treated area Av sq cm (LxB)	Mean SD	Average increase in leaf size with Bioconsortium treatment %
0	8.93	0.01	12.69*	0.01	11.69
15	16.82	0.04	21.19	0.04	20.19
30	28.37	0.07	34.58	0.24	33.58
40	31.39	0.31	36.81	0.10	35.81

***zero day was measured after 5 days of inoculation with bioconsortium**

The average weight of plants was measured after 60 days of growth (table 2)

It was observed that the weight of plants treated with bioconsortium was significantly higher than control plants. After seaweed treatment the plants maintained the weight at the time of treatment.

Table 2 :average weight of plant (g)after 60 days

Control plant	Bioconsortium treated plants	Bioconsortium treated + seaweed treated plants
20	84	75.4

2. Effect of stress on essential oil content of foliage

Rooted plants were divided into 2 sets of 20 plants each. One set was treated with bioconsortium containing Trichoderma spores while the second set was not treated with the bioconsortium. Nutrients and water cycle was the same for both sets. After a definite period of growth, leaves were harvested, and essential oil extraction was carried out using Clevenger apparatus. Oil content was reported as % on dry weight basis.

Table 3: oil content of plants treated with bio stress

Growth period days	**% Oil on dry weight basis**	
	Control	**Bioconsortium treated plants**
15	2.18	3
30	2.8	3.18
60	2.66	3.26

Further the plants were grown in a new cycle with the optimized protocol. This included rooting protocol, optimum water cycle and treatment with bio stress

The upper portion of the plants were harvested after 60 days such that 2 internodes remained with roots. Thereafter 2 rattoon harvests were carried out after 45 and 30days each. No biostress was given after first harvest.

The oil content of the harvested foliage was determined by hydro distillation using Clevenger apparatus. Results are presented in table 3.

Table 3:Essential oil production by peppermint plants (optimum protocol)

Growth cycle	**Growth period days**	**Oil content (% dry weight)**
First crop	60	3.26
Ratoon crop 1	45	3.07
Ratoon crop 2	30	2.8

3. GCMS profile of peppermint plants grown using aeroponics

The GC-MS profile of essential oil from peppermint plants grown using aeroponics was compared with that from soil grown plants

Peak No.	**A (plant grown using aeroponics)**			**C (soil grown plant essential oil)**			**Difference**
	RT	**% area**	**Name**	**RT**	**% area**	**Name**	
1.	7.964	0.41	alpha-Pinene				+0.41
2.	9.45	0.52	beta-Pinene	9.459	4.04	beta.-Pinene	-3.52
3.	10.317	0.99	3-Carene	10.324	0.37	3-Carene	+0.62
4.	10.77	0.21	Isocineole				+0.21
5.	10.924	0.58	D-Limonene	10.931	2.23	D-Limonene	-1.65
6.	11.198	0.65	o-Cymene				+0.65
7.	11.409	4.72	Eucalyptol	11.429	0.56	Eucalyptol	+4.16
8.	12.918	0.18	(+)-4-Carene				+0.18
9.	15.559	**20.37**	**l-Menthone**	15.569	**37.40**	**l-Menthone**	-17.03
10.	**16.031**	**41.18**	**DL-Menthol**	16.041	**30.52**	**DL-Menthol**	+10.66
11.	16.6	4.67	L-alpha terpineol	16.606	3.12	L-alpha terpineol	+1.55
12.	17.616	0.45	L-Menthyl chloroformate				+0.45
13.	18.495	2.38	Pulegone	18.504	0.49	Pulegone	+1.89
14.				19.052	0.5	p-Menth-1-en-3-one	
15.	19.115	11.7	Menthyl acetate	19.125	1.11	Menthyl acetate	+10.59
16.				19.797	0.68	Anethole	
17.	21.976	0.19	Menthofurolactone				+0.19
18.				22.368	10.14	Caryophyllene	
19.				22.612	0.44	Chloroxylenol	

20.				23.363	1.7	Isogermacrene D	
21.				23.867	0.49	gamma. - Amorphene	
22.				23.973	0.61	alpha-ylangene	
23.	26.597	7.67	Isomintlactone				+7.67
24.	27.246	0.59	Globulol				+0.59
25.	27.485	0.83	Isomintlactone $				+0.83
26.	27.95	1.7	Hydroxymintlactone				+1.7
27.				28.448	5.59	Santalol, E-cis, epi- beta	
Total		100			100		

As seen in table, Menthol content in essential oil from aeroponics grown plants was significantly higher than in soil grown plants by 10.66%. Menthyl acetate concentration was also higher in aeroponics grown plants. Pulegone was produced in higher concentrations in plants grown through aeroponics. On the other hand, menthone content was significantly lower in aeroponics grown plants. Few new peaks were observed such as Isomint lactone, Globulol, Iso mint lactone **$**, Hydroxy mint lactone in significant levels only in aeroponics grown plants. The lactones (-)-mintlactone and (+)-isomintlactone were identified as trace components of Mentha piperita oil grown in mid-west US by Takahashi et.al in 1980. The synthesis of these metabolites have been studied because of their significance as flavor components in the essential oil. (Ferraz et al, 2002)

In peppermint, the intermediate pulegone is reduced to menthone and isomenthone that is subsequently reduced, resulting in the four stereoisomers of menthol found in peppermint oil. (Croteau et al., 2005). In the present studies, increase in pulegone levels were observed with significant decrease in methone levels while menthol levels have significantly increased. It can be suggested that menthone has been further metabolized to menthol. Higher concentrations of menthol results in increase in economic value for the oil.

According to studies by Croteau et al (2005), the organization of menthol biosynthesis is complex in involving four subcellular compartments, and regulation of the pathway appears to reside largely at the level of gene expression. The aeroponics system with availability of minerals in simple form seems to have enhanced the expression of the enzymes in the biosynthetic pathway to result in significant increase in menthol concentration. Scanning electron micrograph of an upper peppermint leaf surface showed peltate glandular trichomes in which menthol and related monoterpenes are produced and accumulated, that are interspersed with several smaller capitate trichomes and non-glandular hairs that do not produce monoterpenes

Some peaks that were seen in soil grown plants such as Caryophyllene, Chloroxylenol, Isogermacrene D, gamma. -Amorphene, alpha-ylangene and Santalol were not produced in aeroponics grown plants.

The GC-MS profile of essential oils from aeroponics grown plants with different stress conditions was compared.

Peak #	Retention Time	Name	% Area		
			Aeroponics Control	Bioconsortium treatment	Bioconsortium + seaweed treatment
1	7.964	Alpha-Pinene	0.41	0.39	0
2	9.45	Beta-Pinene	0.52	0.6	0.21
3	10.317	3-Carene	0.99	0.9	4.85
4	10.77	Isocineole	0.21	0.23	0.71
5	10.924	D-Limonene	0.58	0.69	0.76
6	11.198	o-Cymene	0.65	0.62	0.66
7	11.409	Eucalyptol	4.72	5.83	8.95
8	12.918	(+)-4-Carene	0.18	0.19	0.66
9	13.386	Linalool	0	0.15	0
	15.559	L-Menthone	20.37	47.14	20.51
10	16.031	DL-Menthol	41.18	12.06	7.28
11	16.6	L-Alpha terpineol	4.67	2.66	1.57
12	17.616	L-Menthyl chloroformate	0.45	0.47	1.07
13	18.495	Pulegone	2.38	1.82	5.47
14	19.115	Menthol acetate	11.7	15.65	30.1
	19.137	Neomenthol acetate	0	0	1.27
15	19.635	Isomenthol acetate	0	0.95	0

16	21.976	Menthofurolactone	0.19	0.63	0
17	26.597	(+)-Isomintlactone	8.5	7.05	13.67
18	27.246	(-)-Globulol/ Epiglobulol *	0.59	0.54	0
19	27.95	7α-Hydroxy mintlactone	1.7	1.43	1.53
			100	100	100

***Epiglobulol was produced in Trichoderma treated plants at the same Retention Time**

In a study on the effect of salinity on essential oil production in peppermint plants, it was observed that stress in the form of NaCl resulted in slight increase in production of limonene, isomenthone menthol, menthyl acetate and 1,8 -cineol with slight decrease in menthone levels. (Aktsoglou et al 2021).

In the present studies, effect of infection by the microorganisms in the bioconsortium in aeroponics system resulted in significant increase in menthone as well as menthol acetate concentrations in the oil while the concentration of menthol was reduced considerably to approximately 1/3rd value. Combination of seaweed extract treatment along with bioconsortium infection resulted in significant increase in concentration of menthol acetate (3-fold increase), 3-carene as well as 1,8 cineole (2 fold), slight increase in concentrations of isocineole, D-Limonene, and +4, carene. The concentration of menthol was further reduced to 7.28%. The stress induced by microbial infection as well as combination of seaweed extract with microbial infection has resulted in increase in menthyl acetate content with considerable decrease in menthol concentration. In a study conducted by Scavroni *et. al.* to study effect of biosolids on essential production in peppermint plants, it was reported that increase in menthyl acetate concentration consisting of 40.55% of total oil content was observed with decrease in menthol content. The authors suggested that due to rehydration, elements were probably made available at levels above the necessary requirements of the plants and resulted in menthyl acetate as a major component of the oil.

In another study (Clark and Menary, 1979) it was reported that shade is undesirable for peppermint essential oil production as it induces higher ester and menthone content in the oil. Essential oil in plants grown under short day conditions had lower menthol content with higher menthyl acetate content.

The increase in menthyl acetate with decrease in menthol content reduces the economic value of the oil. Menthyl acetate is used in perfumery, emphasizing floral notes, especially of roses, and is used in toilet waters, having a lavender odor. It has also been used to flavor caraway extracts or for mint flavors. Menthol on the other hand is valued for its medicinal effects. Higher menthol content is directly related to higher economic value for the oil.

On a molecular level, as proposed by Croteau *et. al.,* the last step of the menthol pathway is the reduction of the C3- carbonyl of (-1R,4S)-menthone to (−1R,3R,4S)-menthol. Infection by the fungus *Trichoderma* present in the bioconsortium seems to have affected this conversion step in the present studies either by enhancing the availability of nutrients or production of the enzymes responsible for catalysing the reaction.

CONCLUSION

Growing peppermint plants using aeroponics resulted in significant increase in oil production by the plant with increased menthol content. Increased menthol content enhances the economic value of the essential oil. Stress in the form of fungal infection results in increase in oil production and leaf size but negatively affects the menthol content of the essential oil. Aeroponics without biostress is suitable for improved cultivation and essential oil production in peppermint plants.

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Author contribution statement

Corresponding author - Dr Malathy Venkatesan has contributed towards the following:

study conception and design, analysis and interpretation of results, and manuscript preparation.

Co-author – Mr Bhargav Meshiya has contributed towards the following:

study design, data collection, analysis and interpretation of results

Declaration of conflict of interests

The authors Dr. Malathy Venkatesan and Mr. Bhargav Meshiya declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper