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Original article

# Response of growth, essential oil composition, endogenous hormones and microbial activity of *Mentha piperita* to some organic and biofertilizers agents

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## ABSTRACT

The effect of organic (poultry and cattle manures) and biological (effective microorganisms, EM) fertilizers on growth, essential oil yield and its compositions, endogenous phytohormones content and antibacterial activity of peppermint plants grown in pot over 12 weeks was studied. Application of organo- and bio-fertilizers greatly affected on growth, essential oil production and other estimated parameters of peppermint plants. Slight stimulation effect was happened due to soil application of organic manures. Soil application of EM alone or in combination with organic fertilizers significantly increased growth, yield and components of essential oils, endogenous hormones of peppermint as compared to other treatments. Using disc diffusion method, the extracted oil of peppermint plants amended with organic and biofertilizers recorded the highest antibacterial activity against tested pathogenic bacteria like *Klebsiella pneumoniae* and *Staphylococcus aureus*.

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## 1. Introduction

Peppermint plant is a curatively significant plant belongs to the family Lamiaceae. It is extensively grown in self-controlled areas of the world (Briggs, 1993). The therapeutic amounts of peppermint are the essential oil mined from the plant, the dried leaves, the fresh flowering plant and the whole plant (Hopkins and Huner, 2004). Furthermore, Peppermint is very unique and beneficial plant (Kline et al., 2001; Szymanski and Patterson, 2003). Peppermint oil may also increase the flow of bile from the gall bladder (Al-Qudah et al., 2018; Mimica-Dukic et al., 2003).

In chicken manure, about 90% of the total N and P are available for plant uptake in the year of application (Javanmardi and Ghorbani, 2012). Also, Cattle manure is the source of N and other

nutrients for plants (Najm et al., 2012). In addition. Biofertilizer treatments like actual Microorganisms (EM) contain man species of valuable microbes (Higa and Chinen, 1998). These microorganisms multiply and develop beneficial effects when applied to agricultural soils. Szymanski and Patterson (2003) named the Effective Microorganisms as EM Technology.

Peppermint oil and menthol have modest pure effects touching both pathogenic gram-positive and gram negative bacteria (Diaz et al., 1988; Alkofahi et al., 1990; Ebrahimghochi et al., 2018).

The aim of the existing work is to investigate the outcome of different fertilizers organic fertilizers as (poultry manure and cattle manure) in absence or presence of biofertilizers as EM (Effective Microorganisms) on aerial growth, essential oil productivity and its components, chemical composition, endogenous phytohormones contents and antibacterial activity of peppermint plant.

## 2. Materials and methods

## 2.1. Fertilizer materials

Recommended doses of chemical fertilizers (N, P, K) were applied for control plants as provided by Ministry of Agriculture in Saudi Arabia at the rates of 150 kg/fed as a mixture of ammonium sulphate (20.5% N), Calcium super phosphate (15.5 %P<sub>2</sub>O<sub>5</sub>)

Abbreviations: CT, Cattle manure; EM, Effective microorganisms; IAA, Indole acetic acid; GA3, Gibberellic acid; PM, Poultry manure; MIC, Minimum inhibitory concentrations; HPLC, High performance chromatograph.

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and Potassium sulphate (48% K<sub>2</sub>O) as a source of N, P and K, respectively; Calcium super phosphate, ammonium sulphate and Potassium sulphate.

### 2.1.1. Organic manures

Ripe cattle fertilizer and poultry compost were taken from the station of animal and chicken production of Al-Riyadh, Saudi Arabia. Each treatment of organic manure consisting of 0.5 g/kg soil (based on previous studies). All doses of organic manures were mixed with soil during preparation of soil for planting. Chemical and physical properties of organic manures are analyzed (unpublished data).

### 2.1.2. Bio-fertilizers

The active of microorganisms contains of N<sub>2</sub>-fixing, phosphate and microorganisms, VA mycorrhiza, Lact bacillus, budding fungi, and actinomycetes. EM was obtained from Ministry of Agriculture of Japanese EMRO institution (Research Organization) (Okinawa Japan) was added to soil at planting.

### 2.2. Plant material and growth conditions

Healthy and homogenized seeds of peppermint (*Mentha Piperita* L.), obtained from the Experimental Farm of Aromatic and Medicinal Research Center, were surface sterilized in 7% sodium hypochlorite for 5 min. and rinsed thoroughly with sterilized distilled water. The sterilized seeds were left to germinate in petri dishes for five days on wetted filter paper under dark conditions. Identical germinated seedlings were planted into sterilized plastic pots (20 cm width × 25 cm height) containing 6 kg of clay sandy soil (2: 1, v/v). All plastic pots were divided into seven sets according to the type of fertilizers. These seven treatments were replicated ten times (two plant per pot) to give a total of 70 pots. These treatments were arranged as follow: Control (recommended dose of NPK), Poultry manure (0.5 g/kg soil), Cattle manure (0.5 g/kg soil), Effective microorganisms, EM (4 ml/kg soil), Poultry manure (0.5 g/kg soil) + EM (4 ml/kg soil), Cattle manure (0.5 g/kg soil) + EM (4 ml/kg soil) AND Poultry manure (0.5 g/kg soil). + Cattle manure (0.5 g/kg soil) + EM (4 ml/kg soil). All doses of organic and biofertilizers were applied according to the primary experiments and previous studies. All pots were irrigated by tap water by equal amount of water near field capacity. Plants were grown in a glass-house under natural day/night condition (minimum/maximum temperature, relative humidity and day length were 16/18°C, 60/70% and 10/12 h, respectively). Plants were harvested (ten plants per each treatment) at 12 weeks after planting.

### 2.3. Measurements

#### 2.3.1. Growth parameters

At harvest, the plant height was scaled with tape. Fresh and dry weights of plants for each treatment were determined. Number of branches was also recorded. Leaf area was measured using a leaf area meter.

#### 2.3.2. Essential oil productivity and its components

The essential oil percentage was gritty in the air-dried herb by subjecting to hydro distillation in Clevenger apparatus. The resulted oil was dried by anhydrous sodium sulphate and kept in dark tight bottles at a refrigerator until Gas Liquid Chromatography Analysis (GLC). The essential oil percentage was calculated as the following equation:

$$\text{The essential oil (\%)} = \frac{\text{Volume of oil in graduated tube}}{\text{Weight of the sample}} \times 100$$

#### 2.3.3. Chromatographic analysis of the essential oil (GLC)

Samples of plant plants for each treatment were quantitative analyzed using gas liquid chromatograph to determine their main oil constituents according the methods described by Hoftman (1967).

#### 2.3.4. Estimation of endogenous phytohormones content

**2.3.4.1. Extraction.** Ten grams of fresh tissue were normalized with 70% methanol and stirred immediate at 4 °C. The extract was filtered through a Whatman filter paper and the methanol evaporated under vacuum to give an aqueous residue. The aqueous phase was adjusted to pH 8.5 with a 0.1 M phosphate buffer and then partitioned three times with ethyl acetate. After removal of the basic ethyl acetate fraction, the aqueous phase was adjusted to pH 2.5 with 1 N HCl and partitioned three times with ethyl acetate. The acidic ethyl acetate soluble fraction was used for determination of acidic hormones such as IAA, ABA, GA<sub>3</sub> and JA by using the method of Chen (1990).

**2.3.4.2. HPLC analysis of phytohormones.** HPLC analysis was used to determine the phytohormones in the extracts dissolved in 1 ml of analytical grade methanol. Analysis was performed on a model Gas Chromatograph (5890). Samples were identified and determined using GC-MS.

#### 2.3.5. Antibacterial activity

The antibacterial activity was determined by measuring the clear zone of inhibition against the indicator organisms in comparison to a control of reference standards.

### 2.4. Statistical analysis

All the collected data were tabulated and statistically analyzed using the technique of analysis variance (ANOVA) at the significance level of  $P \leq 0.05$  method using the software (costat) according to Snedecor and Cochran (1980).

## 3. Results

### 3.1. Growth parameters

Generally, growth parameters (fresh and dry masses, leaf area, plant height and number of branches of peppermint were highly affected by different types of fertilizers when compared with the control treatments (Table 1). In this connection, Application of organic fertilizer like cattle and poultry manures encouraged a slight increase in the growth levels as comparing to control plants. Combination treatments of organic fertilizer and biofertilizer (EM) accomplished the highest studied growth parameters when compared to other treatments and control.

Of particular interest in this study that the application of EM on peppermint plants alone or in combination with other organic fertilizers gave highest values of all studied growth parameters as comparing to other fertilizers. In this esteem, the highest values in growth levels potency be due to the efficacy of microorganisms in the soil which convert the ability of mobilizing the unobtainable forms of nutrient to available forms easily utilized directly by plants.

### 3.2. Essential oil production

results recorded in Table 2 showed that the percentage of essential oil, essential oil yield per plant and fed of tested plant leaves was affected, to varied degree, by the application of the different fertilizers. Application of poultry manure and biofertilizer

**Table 1**Effect of different fertilizers on growth parameters of *Mentha piperita*.

Fertilization Treatments	Fresh weight/plant (g)	Dry weight/plant (g)	Plant height (cm)	Number of branches Per plant	Leaf area (cm <sup>2</sup> /plant)
Control	96.59 ± 3.45	24.02 ± 1.63	65.92 ± 2.56	20.54 ± 0.96	1205 ± 20.38
Poultry manure	80.54 ± 2.20	22.61 ± 1.49	56.60 ± 2.92	17.51 ± 1.82	1230 ± 22.47
Cattle manure	61.71 ± 4.43	19.58 ± 2.29	49.58 ± 2.55	14.21 ± 1.08	1220 ± 20.66
EM	115.68 ± 5.60	27.66 ± 2.13	68.28 ± 2.70	26.74 ± 1.04	1458 ± 23.55
Cattle manure + EM	120.45 ± 5.19	34.58 ± 2.30	64.68 ± 2.89	20.39 ± 1.00	1524 ± 24.41
Poultry manure + EM	136.85 ± 6.09	40.56 ± 2.27	80.33 ± 2.03	32.59 ± 1.83	1660 ± 24.32
Poultry manure + cattle manure + EM	142.38 ± 6.33	41.51 ± 2.40	86.66 ± 2.54	36.21 ± 1.84	1855 ± 25.02
LSD at 5%	8.47	2.06	4.73	2.48	105

**Table 2**Effect of different fertilizers on essential oil percentage and yield of *Mentha piperita* leaves.

Fertilization Treatments	Volatile oil (ml /100gm DW.)	Essential oil yield / plant (ml)	Essential oil yield /Fed (L)
Control	2.25 ± 0.126	0.765 ± 0.057	12.848 ± 1.20
Poultry manure	2.07 ± 0.186	0.674 ± 0.064	11.324 ± 1.07
Cattle manure	1.61 ± 0.076	0.475 ± 0.024	7.983 ± 0.981
EM	2.52 ± 0.095	0.950 ± 0.033	15.963 ± 1.55
Cattle manure + EM	2.05 ± 0.126	0.825 ± 0.016	13.865 ± 1.33
Poultry manure + EM	2.71 ± 0.101	1.099 ± 0.035	18.461 ± 1.871
Poultry manure + Cattle manure + EM	2.76 ± 0.136	1.147 ± 0.068	19.277 ± 1.88
LSD at 5%	0.22	0.078	0.880

EM significantly augmented the percentage of oil when compared to control plants. In difference, no significant differences were observed between plant fertilized with cattle manure and control plants. In this connection, the combination of organic fertilizers and biofertilizer EM realized significant increased the essential oil production of peppermint plants equating with control and other fertilizers treatments.

The combination treatment of organic and biofertilizers attained the superior averages of essential oil yield of peppermint leaves over the control and all other treatments may be due to increased cell division, cell elongation, differentiation of buds, synthesis of IAA, GA<sub>3</sub> and aeration of the soil, which all associated useful relationship leading to more synthesis of essential oil.

### 3.3. Chromatographic analysis of the essential oil

Analysis of essential oil samples for plants received different rates of fertilizers are presented in Table 3 and illustrated in Fig. 1 A & B. Such identified components namely were menthol (free alcohols) menthone (ketones), beside other constituents which were traces and more lowest such as iso-menthone, menthol

acetate (ester), β-caryophyllene, B-pinene, 1,8-cineole, Sabonine and α-pinene. It is evident from the results that the main components of the essential oil were menthol and menthone. These components diverse in response to the different fertilization treatments. The highest percentage of menthol obtained in response to combination treatment of organic and biological EM fertilizers (Fig. 1 B). In contrast, the lowest percentage of menthol was gained in peppermint plants fertilized with cattle and poultry manures as organic fertilizers, respectively.

### 3.4. Endogenous phytohormones content

Gibberellins, indole acetic acid, abscisic acid (ABA) and cytokinins were sedate in plant leaves. Organic and biofertilizers significantly affected on the endogenous phytohormones content of peppermint plants (Table 4). It is apparent from the results that combination of the organic manures and biological fertilizers significantly augmented IAA, GA<sub>3</sub> and cytokinin content plants (Fig. 2). On contrary, mixed treatments recognized lowermost content of (ABA) as compared to other treatments.

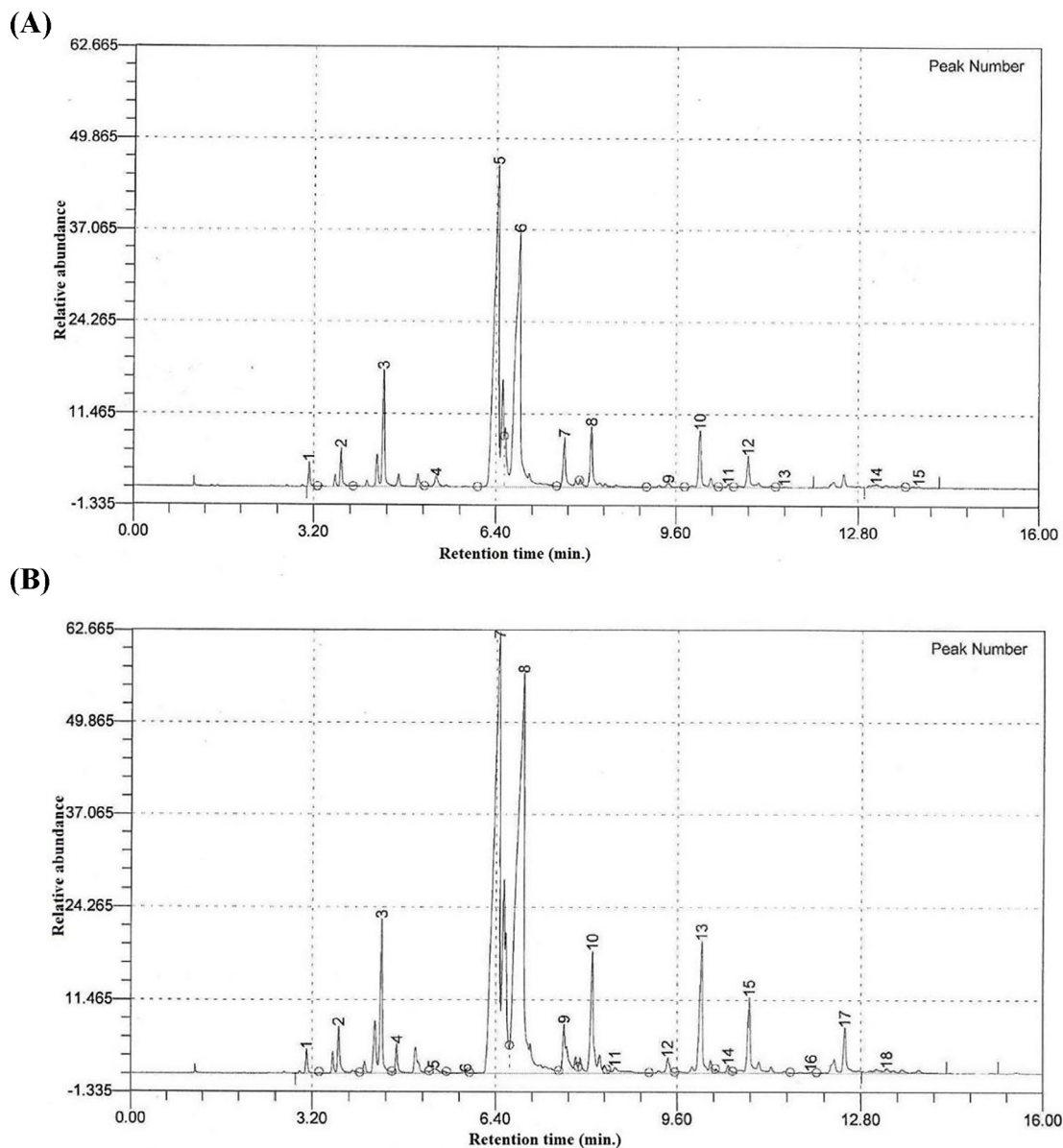
The increases in the amounts of endogenous phytohormones could be qualified to the increase their biosynthesis of plants. Furthermore, increasing IAA and GA<sub>3</sub> in peppermint plants parallel with the increase in growth rate.

### 3.5. Antibacterial activity

Data in Table 5 shown that combination between organic manures and biofertilizers presented the highest reserve zone in all tested pathogenic bacteria as compared to other treatments. *Klebsiella pneumoniae* and *Staphylococcus aureus* are more susceptible to essential oils than other strains at all types of fertilizers and control treatments.

**Table 3**Effect of different fertilizers on essential oil percentage and yield of *Mentha piperita*.

Oil Components (%)	Fertilization Treatments							LSD at 5%
	Control	Poultry manure	Cattle manure	EM	Cattle manure + EM	Poultry manure + EM	Poultry manure + Cattle manure + EM	
α-Pinene	1.09	1.99	1.75	0.77	1.99	0.87	1.15	0.150
Sabonine	2.15	2.95	2.10	1.95	2.05	2.20	2.60	0.214
1,8-Cineole	3.40	4.88	3.47	1.47	1.56	2.50	3.50	0.244
Limonene	3.88	5.58	4.50	2.70	2.75	3.00	4.10	0.312
B -Pinene	2.50	2.75	2.70	1.15	1.20	1.50	2.79	0.199
Menthone	32.70	38.42	33.50	33.15	34.25	33.90	40.25	5.200
Menthol	38.20	39.63	39.10	38.00	39.10	39.10	41.52	4.100
Iso-menthol	9.30	7.17	5.30	8.78	9.11	9.88	10.11	1.200
Menthol acetate	5.50	6.20	5.10	5.60	5.70	5.90	6.20	0.240
β-Caryophyllene	0.56	0.45	0.33	0.50	0.56	0.76	1.04	0.090



**Fig. 1.** (A). Chromatogram of peppermint oil extracted from plants grown in soil amended with recommended dose of NPK (control). Oil constitutions is: (1)  $\alpha$ -pinene, (2) Sabinene, (3)  $\beta$ -pinene, (8) 1,8 cineole, (7) Limonene, (5) Menthol (6) Menthone, (10) Iso-menthone, (12) Menthyl acetate, (13)  $\beta$ -caryophyllene. (B). Chromatogram of peppermint oil extracted from plants grown in soil amended with mixture of Poultry manure + Cattle manure + EM. Oil constitutions is: (1)  $\alpha$ -pinene, (2) Sabinene, (3)  $\beta$ -pinene, (9) 1,8 cineole, (10) Limonene, (7) Menthol (8) Menthone, (12) Iso-menthone, (15) Menthyl acetate, (18)  $\beta$ -caryophyllene.

**Table 4**

Effect of different fertilizers on endogenous phytohermones contents ( $\text{mg.g}^{-1}\text{f.w}$ ) of *Mentha piperita*.

Fertilization Treatments	Indole acetic acid (IAA)	Gibberellin (GA3)	Absciscic acid (ABA)	Cytokinin (CK)
Control	20.15	400.8	30.24	360.7
Poultry manure	21.15	399.5	36.90	365.8
Cattle manure	17.30	289.5	40.17	320.2
EM	29.5	470.2	32.20	481.8
Poultry manure + cattle manure	30.2	520.5	30.50	501.7
Poultry manure + EM	28.20	410.5	33.24	420.6
Poultry manure + cattle manure + EM	39.4	625.6	38.7	530.6
LSD at 5%	1.99	20.80	2.54	18.77

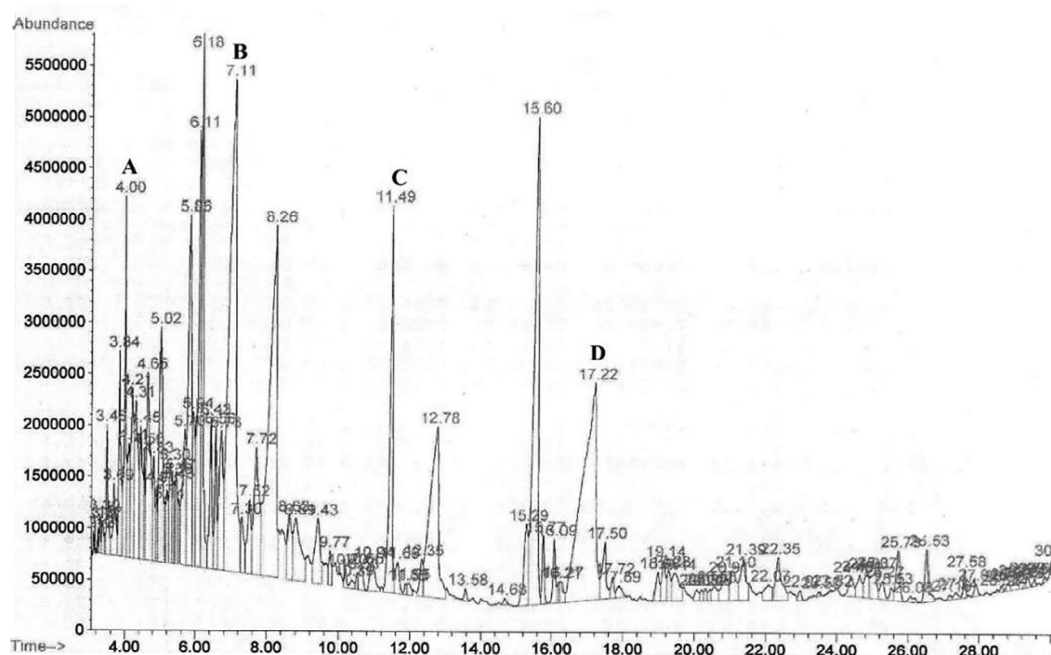
#### 4. Discussion

Organic fertilizer (cattle and poultry manures) induced a slight increase in the growth parameters as comparing to control plants. Combination treatments of organic fertilizer and biofertilizer (EM)

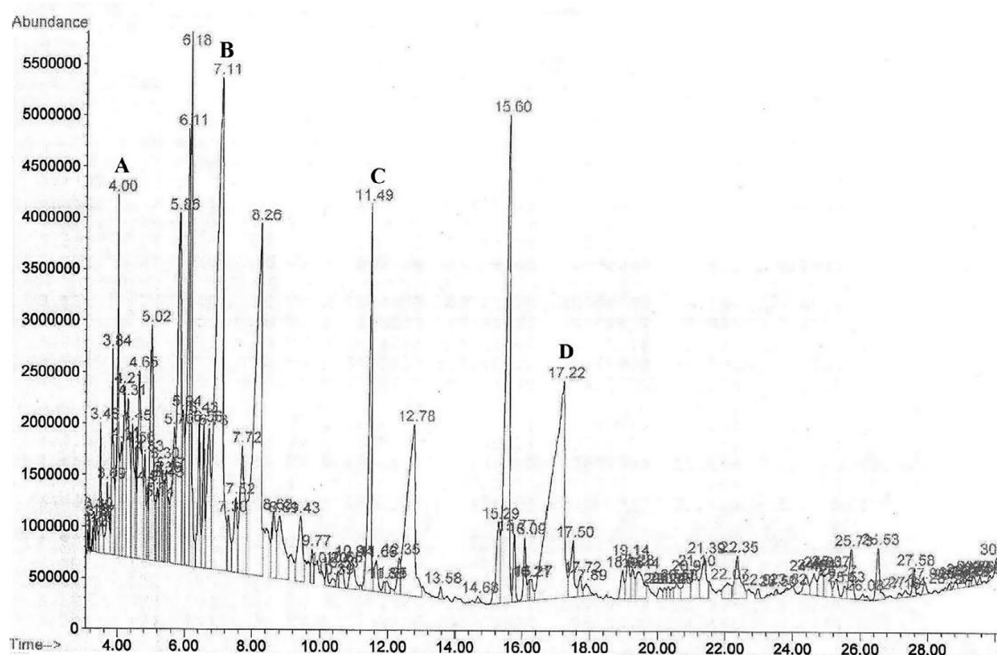
achieved the highest studied growth parameters when compared to other treatments. These results were in agreement with the previous findings (Baddour, 2010, Ebrahimgochi et al., 2018) who reported that poultry manure is rich in nutrients than cattle manure. The richer the food is in protein and the composition of man-



(A)



(B)



**Fig. 2.** Contents of endogenous phytohormones (mg. g<sup>-1</sup> f.w) in *Mentha piperita* grown under control conditions (A) and mixture of Cattle manure + Poultry manure and EM. Where: A = IAA; B = GA3; C = ABA and D = Cytokinin (B).

ure depends on the quality of the feed of animal eats (Ram and Kumar, 1997). In this respect, the highest values in growth criteria might be due to the efficiency of microorganisms in the soil which convert the ability of mobilizing the unavailable forms of nutrient to available forms easily utilized by plants (Naguib, 2011). Moreover, the microorganisms produce growth promoting substances which increase the plant growth (Jayasri and Anuja, 2010, Akhzari et al., 2018)

Application of poultry manure and biofertilizer EM significantly increased the percentage of oil when compared to control plants. In contrast, no significant differences were detected between plant

fertilized with cattle manure and control plants. In this connection, the combination of organic fertilizers and biofertilizer EM realized significant increased the essential oil production of peppermint leaves comparing with control and other fertilizers treatments. These results are in accordance with those reported by Ram and Kumar (1997) on *Mentha arvensis*, El-Sanafawy (2007) on *Ocimum basilicum* and Al-Fraihat et al. (2011) on *Majorana hortensis* and Al-Qudah et al. (2018) on *Phlomis* species.

These components varied in response to the different fertilization treatments. The highest percentage of menthol obtained in response to combination treatment of organic and biological EM

**Table 5**

Antibacterial activity of extracted oil of peppermint plants grown under different fertilizers.

Fertilization Treatments	Diameter of inhibition zone (mm)			
	Gram + ve		Gram -ve	
	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumoniae</i>
Control	19.05 ± 1.05	16.20 ± 0.98	18.80 ± 0.90	20.00 ± 1.11
Poultry manure	19.55 ± 1.18	16.00 ± 0.66	19.00 ± 1.07	21.22 ± 1.07
Cattle manure	18.00 ± 1.07	15.00 ± 0.54	18.99 ± 0.88	20.14 ± 0.98
EM	22.42 ± 1.95	18.99 ± 0.93	20.11 ± 1.56	22.81 ± 1.56
Cattle manure + EM	22.98 ± 2.12	19.25 ± 1.11	20.41 ± 1.53	20.41 ± 1.33
Poultry manure + EM	22.66 ± 1.19	19.63 ± 1.35	20.20 ± 1.87	22.20 ± 1.77
Poultry manure + Cattle manure + EM	28.14 ± 2.49	22.14 ± 1.68	25.44 ± 1.99	27.44 ± 1.99
LSD at 5%	0.994	0.896	0.980	1.150

fertilizers. In contrast, the lowest percentage of menthol was obtained in peppermint plants fertilized with cattle and poultry manures as organic fertilizers, respectively. These results are in contract with those gotten by [Jacoub \(1999\)](#) on *Ocimum basilicum* and [Sakr \(2001\)](#) on peppermint and [Al-Qudah et al. \(2018\)](#) on *Phlomis* species. The constructive effect of biofertilization treatments on the main components of oil may be attributed to stimulating mineral nutrition in addition to release plant promoting substances like IAA, GA<sub>3</sub> and cytokinin ([Jayasri and Anuja, 2010](#), [Al-Frihat et al., 2011](#), [Akhzari et al., 2018](#)).

It is obvious from the results that combination of the organic manures and biological fertilizers significantly increased IAA, GA<sub>3</sub> and cytokinin content plants. On the other hand, combination treatments realized lowest content of (ABA) as compared to other treatments. These results are confirmatory to those reported by [Al-Frihat et al. \(2011\)](#) and [El-Sanafawy \(2007\)](#). The increases in the amounts of endogenous phytohormones could be qualified to the increase their biosynthesis of plants. Furthermore, increasing IAA and GA<sub>3</sub> in peppermint plants parallel with the increase in growth rate due to the role of these engogenous hormones in stimulating cell division, cell elongation and subsequently growth ([Taiz and Zeiger, 1998](#)).

The antibacterial activity of extracted and purified peppermint oil had been examined using some pathogenic Gram positive and negative bacteria using agar diffusion method. Susceptibility of tested strains to peppermint oil varied with respect to their genera and species and also and according different types of fertilizers. Combination between organic manures and biofertilizers gave the highest inhibition zone in all tested pathogenic bacteria as compared to other treatments. The antimicrobial activity of peppermint oil is due to the presence of terpenoides menthol, menthyle acetate ([Gupta et al. \(2008\)](#)). The volatile extracted from peppermint plant has powerful antimicrobial properties ([Deans and Baratta, 1998](#), [Akhzari et al., 2018](#)). *Klebsiella pneumoniae* and *Staphylococcus aureus* are more susceptible to essential oils than other strains at all types of fertilizers and control treatments.

## 5. Conclusion

Based on the obtained results here, growth and production of essential oils in leaves of peppermint plants affected greatly by different types of fertilizers. Combination treatment of organic and biological fertilizers gave the highest growth, yield and its components of essential oil and endogenous phytohormones of peppermint as comparing to other treatments. In addition, purified of extracted essential oil of peppermint leaves could be used to inhibit some of pathogenic bacteria. It was proposed that EM fertilizer play an important role for stimulating phytohormones production and decomposition of organic matter to be easily for utilization by the plants. Additional trainings should be required to explain the other different mechanisms of the essential oil produced.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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