Influence of Organic-Biofertilizers and Foliar Spray with Different Biostimulants on Increasing Yield and Essential Oil of sweet marjoram *(Marjorana hortensis* L.*)* Plant

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ABSTRACT

Given the importance of medicinal and aromatic plants in various medical and food industries, it is important to apply the organic production methods. A field trail was performed at the research farm of Medicinal and Aromatic Plants Department, Horticulture Research Institute (HRI), Agriculture Research Center (ARC), Dokki, Giza, Egypt, throughout two consecutive growing seasons 2020/2021 and 2021/2022. Aims to investigate the effect of spraying by various biostimulants (seaweed extract, fulvic acid and humic acid at 10 ml/L.) on vegetative growth, yield, essential oil content plus several chemical compositions of marjoram plant fertilized with organic-biofertilizers. Soil health indicator was also studied by determining the biological activity of soil enzymes. The results showed that, plants fertilized with organic-biofertilizers and sprayed with the biostimulant seaweed extract recorded the highest significant values for all studied traits, followed by plants sprayed with fulvic acid extract compared to control treatment (mineral fertilizers alone). So, the application of these biostimulants could be promoted organic marjoram production, which is compatible with sustainable development goals and food security.

Keyword: Marjoram plant, Organic-biofertilizers, Seaweed extract, Fulvic and Humic acid.

INTRODUCTION

Sweet marjoram (*Majorana hortensis* L.) is an important source of bioactive compounds; it has been famous since the ancient Egyptians and successfully cultivated in Egypt and other Mediterranean countries. Its aerial parts have an abundance of gray-green leaves and stems with small white or red flowers (El-Khateeb et al., 2017). Being a bushy vigorous plant, it could be harvested more than twice/year at full bloom stage, which is important process to increase the value of the leaves, dry leaves can be used as a food flavoring or in extract form to treat coughs and colds (AL-Qzwini and Alobady, 2019). In addition, it is rich in yellow essential oil which is widely used in many pharmacological applications as antimicrobial, antioxidant, anti-inflammatory, antidiabetic, digestive and other medical industries due to its high volatile chemical components of α-Pinene, β-Pinene, α-Terpinene. Limonene, linalool, terpinen-4-ol, p-cymene, trans-sabinene hydrate and thymol. (Khater, 2020).

The organic production of marjoram is required to fulfill the goals of food security and sustainable development. The organic agriculture system is natural and safe for human and the environment, as well as mitigating the harmful effects of overuse of chemical fertilizers. Organic fertilizers as compost can be applied to the soil to improve soil quality, provide essential macro and micronutrients and improve crop growth and quality (Tursun, 2022).Plant growth promoting rhizobacteria (PGPR) as biofertilizers contain beneficial microorganisms that fix-nitrogen, dissolve phosphorus and solubilize potassium, they also produce phytohormones such as indole-3- acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), salicylic acid and siderophore that enhance the nutrients availability in the soil and improves plant growth (Hindersah et al., 2020).

The great response to foliar spraying with seaweed extract, fulvic acid and humic acid as biostimulators attrbuitted to their quick absorb by plant leaves (Schreel and Steppe, 2020). *Ascophyllum nodosum*, seaweed extracts a natural and organic plant growth stimulant contains plant growth regulators as gibberellins, auxin, cytokinins, ethylene, abscisic acid. In additions, it is rich in polysaccharides, minerals, vitamins and bioactive compounds like polyphenols, lipids and proteins (Tursun, 2022). Besides, it contains bioactive compounds like organic matter, vitamins, amino acids, sterols and trace nutrients as Fe, Cu, Zn, Co, Mo, Mn and Ni (Ali et al., 2021).

Humic and fulvic acids are produced via the biodegradation of organic matter resulting a mixture of acids including phenolate and carboxyl groups (Maraei et al., 2019). Fulvic acid is a valuable portion of soil organic matter, an important part of the dissolved organic carbon pool in soils, has higher chemical and physicochemical activity compared to humic acid, has significant functions as soil's ability to buffer acids to maintain an acid–base balance. It supports in retention and release of mineral ions and organic compounds into soil and enhances bioavailability and ion movement (Mohamed et al., 2021).

Hence, the application of organic-biofertilizers along with biostimulants to mitigate the extreme use of mineral fertilizers and promote organic productions, giving more opportunities for export. Therefore, the goal of the current research is to improve the vegetative growth efficiency, yield quality, productivity of the organic essential oil as well as nutrients content of sweet marjoram through the application of organic-biofertilizer and foliar spray with different biostimulants (seaweed extract, fulvic acid and humic acid).

MATERIALS AND METHODS

Field experiment was performed at the research farm of Medicinal and Aromatic Plants Research department, HRI, ARC, Dokki, Giza, Egypt, during two successive growing seasons 2020/2021 and 2021/2022. With preliminary season (2019/2020) to investigate the influence of organic-biofertilizers and foliar spray with various biostimulators namely seaweed extract (SWE), fulvic acid (FA) and humic acid (HA) on sweet marjoramyield and essential oil chemical composition.

Five treatments with three replicates were designed as follow:

1- Compost-biofertilizers + foliar spray with SWE (10 ml/L).
2- Compost-biofertilizers + foliar spray with FA (10 ml/L).
3- Compost-biofertilizers + foliar spray with HA (10 ml/L).
4- Compost-biofertilizers + foliar spray with tap water.
5- Mineral fertilization + foliar spray with tap water (as control).

Experimental design: Seedlings were obtained from the experimental station of El-Qanater El-Khairia, Qalyubia Governorate, ARC, chosen to be uniform and healthy. The experiment consists of 4 ridges (30cm×4m long), 16 seedlings/ridge (25cm between plants). Three soil samples were taken randomly before planting, mixed into one homogeneous sample. Physical and chemical analysis (Jackson, 1973) were determined, total counts of fungi, bacteria and actinomyces (Allen, 1959; Difco, 1985 and Jensen, 1930) were enumerated (Table A).

Nitrogen-fixing bacteria, phosphate solubilizing bacteria and potassium releasing bacteria were enumerated in the marjoram rhizosphere using semi-solid malate medium (Dobereiner et al., 1976), Pikosvkey's medium (Pikovskaya, 1948) and Aleksandrov's agar medium (Parmar and Sindhu, 2013) respectively.

The strains *Azospirillum brasilense*, *Bacillus megaterium* and *Bacillus circulans* were obtained from central laboratory of organic agriculture (CLOA).

Table (A). Physical, chemical analysis and microbial counts of the experimental soil before planting.

Mechanical analysis		**Cations and anions mmq/L.**		**Total count (CFU/ml.)**	
Coarse sand	3.20	pH (1:2.5)	7.52	Fungi	0.01×10^{5}
Fine sand	17.30	EC(1:5) dS/m^3	1.47	Bacteria	1.00×10^{6}
Silt	19.80	Ca^{++}	7.30	Actinomycetes	0.10×10^{7}
Clay	59.70	Mg^{++}	1.30	*B. megaterium*	1.50×10^{6}
Texture class	**Clay**	Na^{+}	3.10	*B. circulans*	0.43×10^{6}
Available nutrients (ppm)		K^{+}	2.80	**Most probable number in the rhizosphere/ml. media**	
Nitrogen	14.90	CO_3^{--}	--		
Phosphorus	11.70	HCO_3^{-}	1.40		
Potassium	31.20	Cl^{--}	2.40	*A. brasilense*	0.20×10^{6}
		SO_4^{--}	10.70		

CFU: colony-forming unit

- **Organic fertilizer:** Compost (gathering a mixture of animal residues 50% + basil and marjoram wastes 50%) was obtained from the Egyptian Company for Solid Waste Recycling, Minya Governorate, Egypt. Compost was blended with the soil at average of 15 m^3/fed during tillage except the control (mineral fertilizer). Physical and chemical properties were determined as described by (Page et al., 1982), Table B.

Table (B). Some physical and chemical analysis of the compost used.

Physical and Chemical properties	**Values**
Bulk density Kg/m^3	620
Moisture content%	20.5
pH (1:10)	7.6
EC (1:10) ds/m	4.6
Organic matter%	43.1
Organic carbon%	29.1
C/N ratio%	15:01
Ash%	66.93
Total nitrogen%	1.92
Total phosphorus%	0.99
Total potassium%	1.77
Available N(NH_4) ppm	98
Available N(NO_3) ppm	225
Seed weed	Not detected
Nematode (worm)	Not detected
Total coliform (cfu/g)	Not detected

- **Biofertilizers:** The three isolates were grown individually (x10^8 CFU/ml), mixed in equal quantities and used as a biofertilizer, all the seedlings roots except for the control treatment (mineral fertilizers) were dipped in the biofertilizer mixture immediately before planting and added once a month during the growing seasons (5 liters/fed) (Afifi et al., 2014).
- **Seaweed extract (SWE):** was obtained from National Research Center. It applied individually as foliar spray once a month (10 ml/L) Table C

Fulvic acid (FA) and humic acid (HA): were obtained from Microbiology department, Soils, Water and Environ. Res. Inst., (ARC), Giza, Egypt. They were applied individually as foliar spray once a month (10 ml/L) Table C

Table (C). Main properties of seaweed extract, fulvic acid and humic acid.

Seaweed extract							
Color	**Density**	**pH**	**Organic substance**	**Free amino acids**	**N%**	**P%**	**K%**
Dark brown	0.65 (g/cm^3)	4.5	48%	24 ($mg\ Kg^{-1}$)	15	10	15

Chemical analysis	Fulvic acid	Humic acid
pH	3.3	7
EC (ds/m)	58.1	55.5
O.M%	14.6	22.1
C%	8.6	12.7
C/N	11.5	7.5
N%	0.88	1.66
P%	0.2	0.15
K%	9.5	9.1
Total acidity (mmol/100g)	270	590
COOH group (mmol/100g)	210	300
Phenolic group (mmol/100g)	300	630

- **Mineral fertilizers:** The recommended NPK doses according to the Egyptian Ministry of Agriculture and Land Reclamation are 300 kg/fed ammonium sulphate (20.5% N); 300 kg/fed calcium superphosphate (15.5% P_2O_5) and 100 kg/fed potassium sulfate (48% K_2O) (Yousef et al., 2008)

- **Experiment date:** it was started on 15 November in the both seasons. Three cuts were taken (cutting 5cm over the soil surface) as follow:

- First cut was taken on 2 April, the second cut was taken on 15 July and the third cut was taken on 20 November for both seasons.-

Vegetative growth characteristics:

- Plant height (cm,), numbers of branches/plant, fresh weight (g/plant), total yield (kg/fed), dry weight (g/plant) and total yield (kg/fed) were estimated.

Microbiological estimations

- *Azospirillum brasilense* were enumerated by using the method of most probable numbers MPN (×10^5 cfu/g rhizosphere), while, *B. megaterium* and *B. circulans* were determined by agar plate counts method (Society of American Bacteriologists, 1957) in biofertilizers and minerals fertilizers treatments during the growing seasons.
- Soil enzymes activity: Nitrogenase (μmole C_2H_4 g Soil/day), Dehydrogenase (μg TPF/g dry Soil/day) and total phosphatase (acid and alkaline) (mg PNP/g dry soil) were determined in the rhizosphere according to (Somasegaran and Hoben, 1994, Skujins, 1976 and Tabatabai, 1982) respectively.

The chemical composition of herbage

- Endogenous phytohormones content (mg/g f.w.): Indole acetic acid IAA, gibberellic acid GA_3 (Sadeghian, 1971) were determined.
- Total phenolic compounds (mg/100g f.w.) (Shahidi and Naczk, 1995), total flavonoids content (mg/100g f.w.) (Mabry et al., 1970), total free amino acid content (mg/100g) (Carpena-ruiz, et al., 1989), total antioxidant % (Chrysargyris et al., 2016), total carbohydrates% (A.O.A.C. 1990) in the dried leaves and photosynthetic pigments content (mg/100g f.w.) (Sankar, et al., 2013).
- Total nitrogen%, phosphorus%, potassium% were estimated in dried leaves (Piper, 1950; Chapman and Pratt, 1961).
- Essential oil%, plant oil yield (ml/plant) and total oil yield (L/fed).
- Essential oil constituents were determined by GLC apparatus (British Pharm., 1980).
- Soil chemical analysis and microbial counts of the soil were determined at the end of the experiment.

Statistical analysis: The obtained results were analyzed with analysis of variance (ANOVA) procedure according to Snedecor and Cochran (1990). In addition, significant variances among means were differentiated according to the Duncan's multiple test range (Duncan, 1955).

RESULTS AND DISCUSSIONS

The combined impact of compost-biofertilizers with/without various biostimulators sprays on vegetative growth parameters of marjoram plant.

- Plant height and number of branches

Applying compost-biofertilizers with foliar spraying by different biostimulators enhancing the vegetative growth parameters in terms of plant height and number of branches of marjoram during the three cuts presented in Table 1. The maximum effects were found when plants were fertilized with organic-biofertilizers in the presence of beneficial SWE (T_1) as foliar spray, which improved all vegetative characteristics as the tallest plant and the highest number of branches compared with the mineral fertilizers for the three cuts in both seasons. Followed by compost-biofertilizers + FA in this respect. On the other hand, insignificant differences due to (T_2) compost-biofertilizers + FA and (T_3) compost-biofertilizers + HA which were noticed in different cuts during the both seasons.

Table (1). Impact of compost-biofertilizers with/without various biostimulators sprays on vegetative growth parameters of marjoram plant.

Treatments	Plant height (cm)			Number of branches/plant		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
First season						
T_1	55.00[a]	75.67[a]	92.00[a]	6.33[a]	8.33[a]	11.67[a]
T_2	51.33[b]	72.00[b]	86.67[b]	5.33[b]	7.33[b]	10.67[ab]
T_3	50.00[b]	69.00[c]	85.00[bc]	5.00[b]	7.00[b]	10.00[bc]
T_4	47.00[c]	67.00[d]	80.67[cd]	4.00[c]	6.67[b]	10.00[bc]
T_5	45.00[c]	63.33[e]	76.67[d]	3.67[c]	5.67[c]	9.33[c]
Second season						
T_1	58.33[a]	82.00[a]	94.33[a]	11.67[a]	13.33[a]	15.33[a]
T_2	54.67[ab]	76.00[b]	86.00[b]	9.00[b]	13.00[a]	14.33[b]
T_3	49.67[bc]	73.00[b]	81.00[c]	8.33[b]	11.00[b]	14.00[c]
T_4	43.33[c]	67.67[c]	77.67[c]	7.33[c]	10.33[bc]	14.00[c]
T_5	30.67[d]	63.67[c]	74.00[d]	6.33[d]	9.67[c]	12.00[d]

T_1- Compost-biofertilizers + foliar spray with seaweed extract, T_2- Compost-biofertilizers + foliar spray with fulvic acid, T_3 Compost-biofertilizers + foliar spray with humic acid, T_4- Compost-biofertilizers+ foliar spray with tap water and T_5- Mineral fertilizers+ foliar spray with tap water (as control)

- Fresh weight (g/plant), total fresh yield (kg/fed), dry weight (g/plant) and total dry yield (kg/fed.).

Data illustrated in Table (2) confirmed that, all treatments were affected significantly by using compost-biofertilizers, with various sprayers as the biostimulators, the maximum values for the heaviest fresh and dry weights (g/plant) beside total yield (kg/fed) were recorded by (T_1) compost-biofertilizers + SWE, compare to the mineral fertilizers treatment without any sprays, which gave the highest values in both seasons. While compost-biofertilizers + FA came in the second order in this respect. Also, these applications have the same effect for fresh weight in the 2nd cut for the 1st season only and the 3rd cut for the second one.

Table (2). Impact of compost-biofertilizers with/without various biostimulators sprays on fresh weight (g/plant), total yield (kg/feddan), dry weight (g/plant) and total yield (kg/feddan) of marjoram plant.

Treatments	Fresh weight (g/plant)			Total yield fresh weight (kg/feddan)			Dry weight (g/plant)			Total yield dry weight (kg/feddan)		
	1^{st}cut	2^{nd}cut	3^{rd}cut	1^{st}cut	2^{nd}cut	3^{rd}cut	1^{st}cut	2^{nd}cut	3^{rd}cut	1^{st}cut	2^{nd}cut	3^{rd}cut
First season												
T_1	153.73^{a}	291.30^{a}	419.00^{a}	7.68^{a}	14.56^{a}	20.95^{a}	23.97^{a}	24.20^{a}	18.67^{a}	1.19^{a}	1.2^{a}	0.933^{a}
T_2	102.60^{b}	265.67^{a}	376.00^{b}	5.13^{b}	13.28^{ab}	18.80^{ab}	22.70^{ab}	23.60^{b}	17.07^{b}	1.13^{ab}	1.18^{ab}	0.853^{b}
T_3	95.73^{c}	253.10^{ab}	347.67^{b}	4.78^{c}	12.56^{b}	17.38^{b}	21.63^{bc}	23.40^{b}	16.30^{bc}	1.08^{bc}	1.17^{ab}	0.815^{bc}
T_4	82.2^{d}	221.9^{b}	298.17^{c}	4.11^{cd}	11.09^{c}	14.90^{c}	20.8^{bc}	22.73^{c}	15.40^{cd}	1.04^{cd}	1.13^{c}	0.770^{c}
T_5	72.87^{e}	144.67^{c}	231.00^{d}	3.64^{d}	7.23^{d}	11.55^{d}	20.00^{c}	21.90^{d}	14.77^{d}	1.00^{d}	1.09^{d}	0.738^{d}
Second season												
T_1	150.33^{a}	321.30^{a}	437.67^{a}	7.51^{a}	16.06^{a}	21.88^{a}	27.90^{a}	30.67^{a}	21.87^{a}	1.39^{a}	1.53^{a}	1.09^{a}
T_2	115.40^{b}	286.90^{b}	398.00^{a}	5.77^{b}	14.34^{b}	19.90^{b}	25.20^{b}	29.27^{b}	21.27^{b}	1.26^{b}	1.46^{bc}	1.06^{b}
T_3	101.33^{bc}	260.77^{c}	372.10^{ab}	5.06^{b}	13.03^{bc}	18.60^{b}	23.97^{bc}	28.27^{bc}	21.07^{b}	1.19^{c}	1.41^{c}	1.05^{b}
T_4	94.80^{bc}	221.60^{d}	308.97^{b}	4.74^{bc}	11.08^{c}	15.44^{c}	23.40^{bc}	27.30^{c}	20.40^{c}	1.17^{c}	1.36^{cd}	1.02^{c}
T_5	82.70^{c}	160.90^{e}	143.10^{c}	4.13^{c}	8.04^{d}	7.15^{d}	21.90^{c}	26.27^{d}	19.57^{d}	1.09^{d}	1.31^{d}	0.978^{d}

T_1- Compost-biofertilizers + foliar spray with seaweed extract, T_2- Compost-biofertilizers + foliar spray with fulvic acid, T_3 Compost-biofertilizers + foliar spray with humic acid, T_4- Compost-biofertilizers+ foliar spray with tap water and T_5- Mineral fertilizers+ foliar spray with tap water (as control)

These results are in harmony with Abd El-Wahab et al. (2016) proved that the highest vegetative growth and total yield of marjoram plants were recorded by the effective combinations of compost-biofertilizers and sprayed with algae extract.

The organic-biofertilizers play a vital role in stimulating the vegetative growth, through its richness of organic matter which affected positively in enhancing soil structure and have the ability to increase the soil activity of microorganisms by increasing their biodegradation, the plant cells division, the meristematic activity, improving the efficiency of photosynthesis process and enzymatic performance which is reflected in providing the nutritional substances thus it will finally leads to boosting the overall plant growth and productivity (Khater 2021 and Attia *et al.,* 2022).

Moreover, the stimulatory features of seaweed extract as foliar application proved to have a great influence in promoting growth and yield quality, these were due to its content of valuable nutrients as macro- and microelements and enhancing their availability. Also, the additional active ingredients such as plant growth regulators, amino acids, phytohormones and vitamins (Karthikeyan and Shanmugam, 2016). In addition, seaweed extract is responsible for vascular growth elongation and nutrient mobilization in vegetative and reproductive sections of plants, so it have levels of cytokinin, auxin and gibberellins, these wide range of hormones may work for promoting nutrient uptake and improving various growth processes in plant (Nassar et al., 2020).

The combined impact of compost-biofertilizers with/without various biostimulators sprays on microbes counts of marjoram plant rhizosphere.

- Bacterial counts in organic-biofertilizers and mineral fertilizers rhizosphere.

Fig (1). Showed that the effects of mineral fertilizers and compost-biofertilizers on bacterial counts in the rhizosphere are presented in Fig1, results showed that the total bacterial counts for *Azospirillum brasilense*, *Bacillus megaterium* and *Bacillus circulans* in the rhizosphere of plants treated with compost-biofertilizers were higher than those found in plants applied with mineral fertilizers. Additionally, the highest bacterial numbers were obtained in the second cut in all applications, then decreased in the third cut, which may be due to the reduction in root exudates of older plants, these results are in agreement with Gao et al. (2020) who indicated that, the effectiveness of biofertilizer depends on beneficial soil microorganisms. Biofertilization aims to speed up microbial processes that provide nutrients to that plant, which can easily absorb nutrients and increase the number of beneficial microorganisms in the soil.

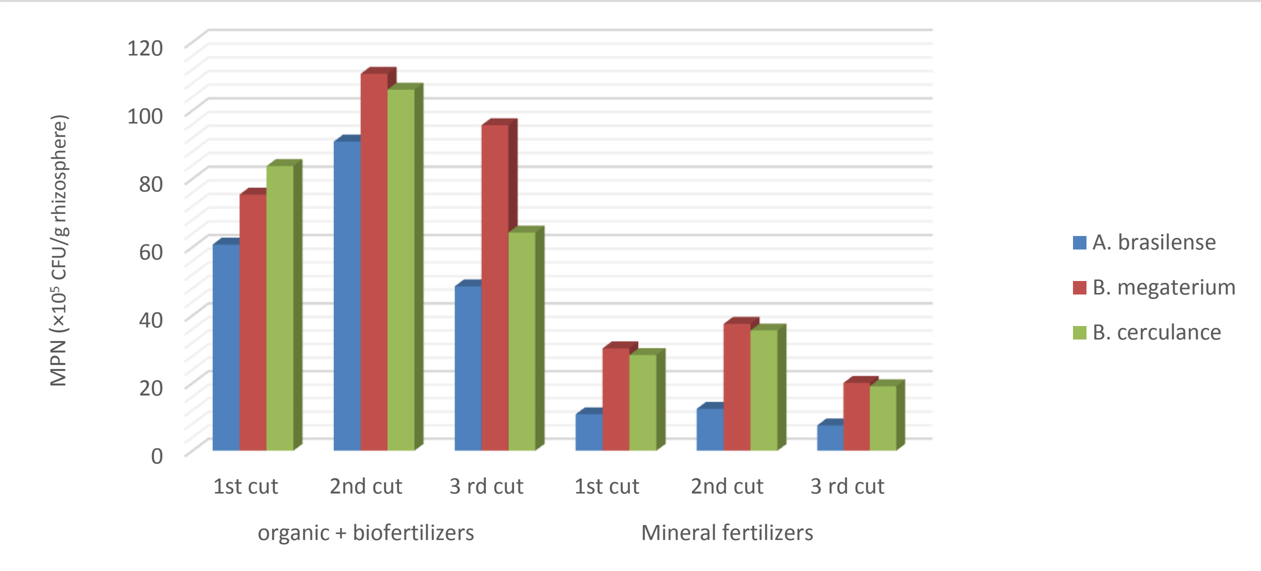

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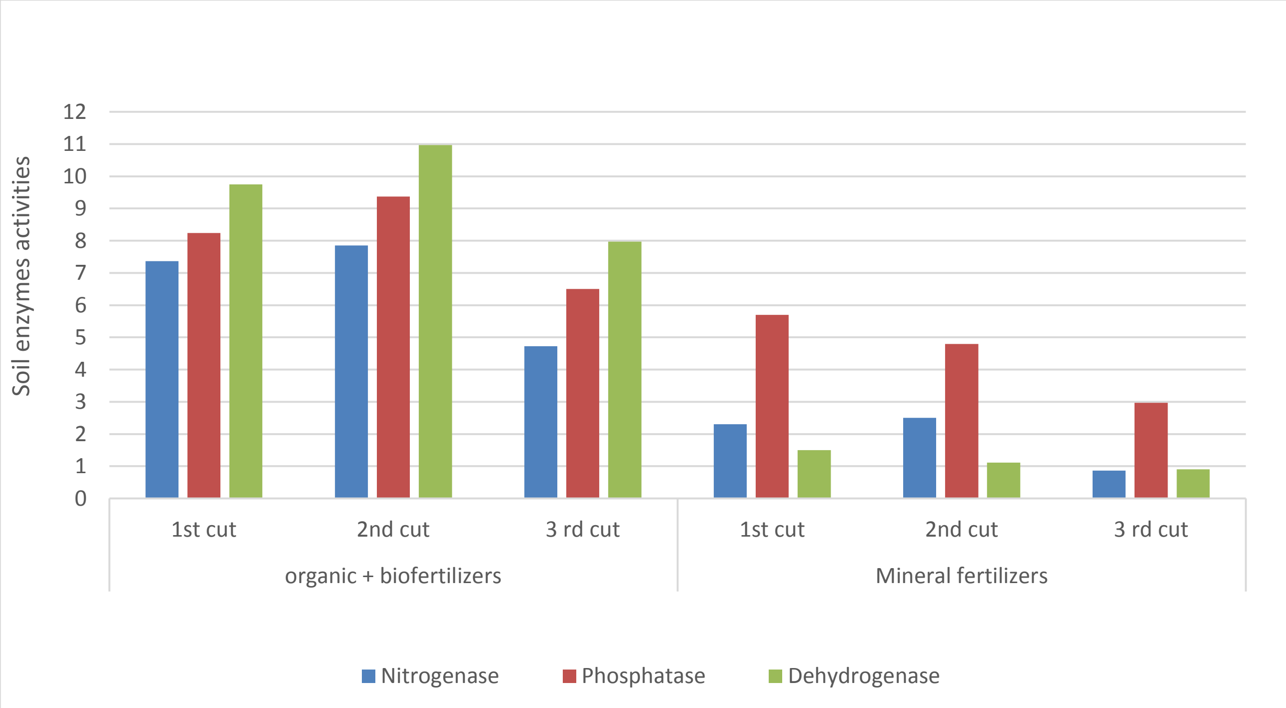
Fig 1. Bacterial counts in both biofertilizers and mineral fertilizers rhizosphere

- Soil enzymes activities: Nitrogenase, dehydrogenase and total phosphatase

The effects of mineral fertilizer and compost-biofertilizers on soil enzymes activity in the rhizosphere are shown in Fig (2), results documented that the activities of nitrogenase, phosphatase and dehydrogenase in the rhizosphere of plants treated with compost-biofertilizers were higher than those found in plants treated with mineral fertilizer. In addition, the highest enzymes activities were recorded in the second cut in all applications, and then decreased in the third cut, which may be due to decrease the microbial counts. Key soil enzymes, namely nitrogenase, phosphatase and dehydrogenase were induced from microbial activities in the rhizosphere, playing an important role for organic matter decomposition and nutrient availability in rhizosphere soil (Gao et al., 2020). The amount of soil enzymes increases with the microbial count and the amount of organic fertilizers. These enzymes activity can be a good indicator of the quality and quantity of organic matter that promote microbial growth in soils. Also, indicates for healthy and fertility soil (Dewi et al., 2021).



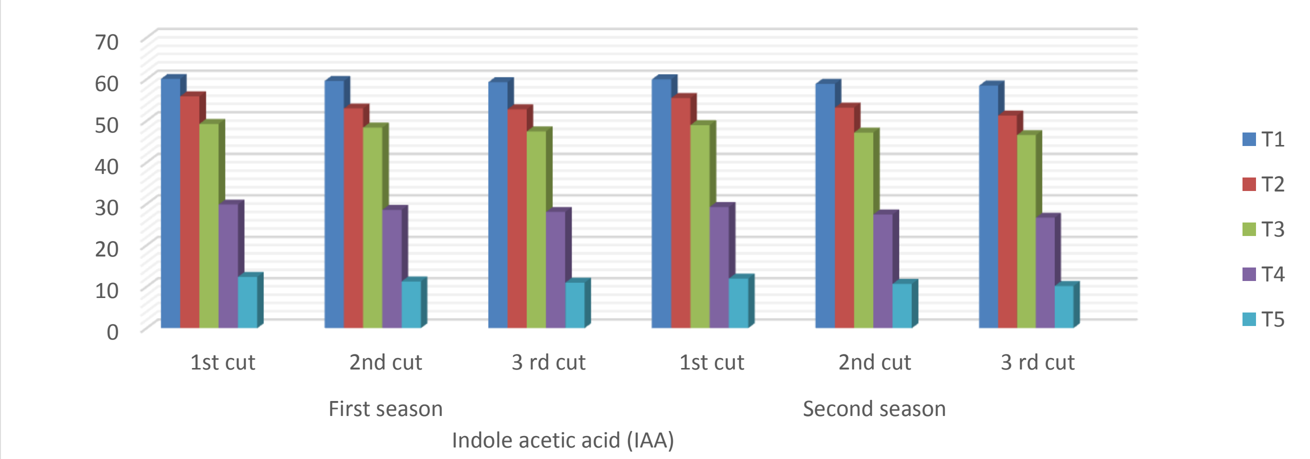
T_1- Compost-biofertilizers + foliar spray with seaweed extract, T_2- Compost-biofertilizers + foliar spray with fulvic acid, T_3 Compost-biofertilizers foliar + spray with humic acid, T_4- Compost-biofertilizers+ foliar spray with tap water and T_5- Mineral fertilizers+ foliar spray with tap water (as control)

Fig 2. Soil enzymes activities in the treatments of biofertilizers and mineral rhizosphere

The chemical composition of herbage

- Endogenous phytohormones content of leaves

Fig (3) shows the content of endogenous phytohormone of marjoram leaves such as auxine (indole acetic acid IAA and gibberellic acid GA3) as growth stimulating hormones. Data showed that, the foliar spraying with SWE, FA and HA were significantly differed in their effect on the endogenous phytohormone content of leaves in both growing seasons. Also, results showed that the highest contents of IAA and GA3 were detected in plants sprayed with SWE followed by FA compared to the other treatments. While, untreated plants recorded the lowest values, in both seasons. These results agree with (Abou El- Hassan and El- Batran, 2020) indicated that, algae extract is rich in macro and microelements and natural hormones as Auxins, Gibberellins and Cytokinins that stimulate cell division and increase cell enlargement, they work to balance between physiological and biological processes in plant cells, also increase photosynthesis processes which enhance plant growth. In addition, Wang et al. (2019) explained that, fulvic acid is similar to the auxin hormone in plant, it enhances antioxidants, IAA, GA3 and Cytokines hormones and vitamins that improve plant growth, it has a good role in absorbing potassium, which is responsible for starch metabolism, magnetizing of water molecules and facilitating the movement of nutrients as Ca, Mg, Fe, Cu, Mn and Zn to plants.



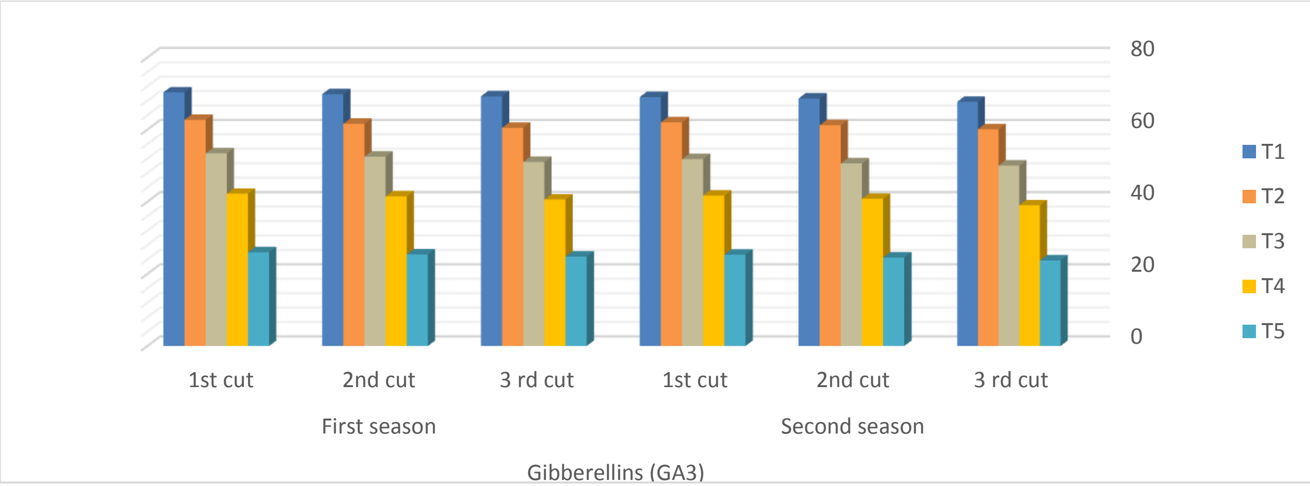


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Fig 3. Endogenous phytohormones content of leaves

Total phenols, flavonoid, free amino acid, antioxidant, total carbohydrates and total pigment.

The phytochemicals compounds i.e. total phenols, flavonoids, free amino acid, antioxidant contents, total carbohydrates and total pigment accumulation in marjoram are shown in Table (3). The highest results were obtained when plants were sprayed with SWE during both successive seasons especially in the second cut followed by spraying with FA, compare to the control. The obtained results are in line with Mahmoud et al. (2019) who found that, the effect of foliar application of SWE gave significant highest values of antioxidant activity phenolics and flavonoids contents in plant leaves, which enhances the nutritional, crude protein values of the treated plants, Also Abou El Magd (2019) who explained, the positive response in increasing the amino acids and antioxidants content in plants maybe because seaweed extract contains macro and micronutrients, amino acids, vitamins, IAA, IBA, Gibberellins, cytokinins and abscisic acid as plant growth stimulants. Fulvic acid act as phytohormones stimulators and secondary compounds such as phenols, protein synthesis and antioxidant in plants, so the use of fulvic acid has positive effects on most fruit characteristics (length and diameter of fruits), vegetative growth and chemical properties (Li et al., 2021 and Masoud et al., 2023).

(Meng et al., 2022) who explained that the seaweed extract can increase the photosynthetic rate by reducing stomatal closure and increasing gas exchange, Also, it contains a large amount of cytokinin, which has a protective effect on chloroplasts and promotes endogenous cytokinin synthesis in treated plants. The other side, (Abd El-Basir et al., 2020) found that, FA plays important roles as increased chlorophylls content in plants and photosynthesis rate due to it may increase N and Mg (structural component of Chl.) uptake and enhanced Chl accumulation causing greater rate of photosynthesis as well as retarding senescence. That may be contributed to the favorable role of FA that easily chelate minerals (Ca, Mn, Fe, Zn & Cu) and it can directly provide plants with these elements (Maraei et al. 2019).

- **Total macronutrients %, essential oil%, oil (ml/plant) and total oil yield (L/fed) of marjoram plant.**

The total macronutrients in dry leaves, essential oil%, oil content (ml/plant) and total oil yield (L/fed) of Marjoram plant were evidently altered by the various used treatments in this trial as compare to the control (T_5) in both seasons. As clear from Table (4). Compost-biofertilizers with SWE foliar spray motivated significantly the values percentages of the same ascribed parameters were influenced positively as compared with control plants during the three cuts in both seasons. Insignificant results were due to compost-biofertilizers + foliar spray with FA treatment, which have the same oil% in both 1^{st} and 3^{rd}cuts for the 1^{st} season only. While fertilizing the plant with the same previous application showed insignificantly different for oil content (ml/plant) in the 2^{nd}cut for the 1^{st}season only and 3^{rd} cut for both seasons, respectively.

The results obtained were agree with (Okasha et al., 2023) who found that, the highest values of N, P, K, Fe, Zn, total carbohydrates, crude protein were recorded in leaves with seaweed spraying. Also, Harhash et al. (2021) indicated that, foliar spraying with SWE improves vegetative growth, yield, leaf macronutrients content (nitrogen, phosphorous and potassium) and fruit quality because seaweed contains a high percentage of mineral elements as N, P,K, Mg, Ca, S, Cu, Fe, Mn, B and Mo. Fulvic acid is an organic fertilizer that chelates minerals (non-toxic) and binds water thus increasing its absorption, macronutrient such as N, P, K and micronutrients through the leaves, thus increases plant productivity (Maraei et al. 2019).

In addition, Massoud et al., (2017) on marjoram plants, suggested that using different biofertilizers had a stimulated impact in enhancing the essential oil/plant and oil %, which attributed to the improvement in vegetative growth parameters and an increasing in glands number / plant in comparison with the mineral fertilized.

Also, the results of Khater (2021) and Sun et al., (2021) clarified that, the application of organic-fertilizers had a direct effect on increasing oil parameters and production of marjoram plants since they facilitating and providing organic matter, various nutrients and microorganisms as well as modifying the soil structural, stability and pH around the roots, which is reflecting on boosting the total growth and oil yield as compared with the control herbs.

Moreover, (Jafr et al., 2022) supported that, improving the productivity of essential oil was attributed to seaweed-treated plants. Similarly, improving yield of the essential oil through the foliar application of seaweed extract might be related to its enrichment content of important nutrients sources, polysaccharides, plant hormones, betaines, auxins, cytokinins, and sterols, which affected positively many changes such as nutrient uptake, plant metabolism, increasinggrowth and oil yield (Tursun, 2022).

Table (3). Impact of compost-biofertilizers with/without various biostimulators sprays on phenols (mg/100g fw), flavonoids (mg/100g fw), free amino acids (mg/100g), antioxidants, total carbohydrates and total pigments of marjoram plant.

Treatments	Phenols content			Flavonoid content			Free amino acids			Total antioxidant			Total carbohydrates%			Total pigment		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
First season																		
T_1	1.37a	2.17a	1.27a	0.17a	0.21a	0.14a	9.79a	10.64a	7.70a	77.74a	82.28a	74.73a	4.10a	4.53a	4.33a	1.33a	1.70a	1.17a
T_2	1.23b	1.93ab	1.10b	0.15b	0.18b	0.13b	9.19b	9.66b	6.51b	76.69a	74.52b	72.59b	3.73b	4.30a	3.77b	1.23ab	1.47ab	1.00b
T_3	1.17b	1.77b	0.97bc	0.14bc	0.18b	0.12c	8.60c	8.90c	6.06c	69.25b	71.29bc	67.21c	3.60b	3.90b	3.50bc	1.13b	1.37bc	0.90b
T_4	1.03c	1.47c	0.87cd	0.13c	0.17bc	0.11cd	7.68d	8.25d	5.93c	67.39b	67.70cd	65.67cd	3.23c	3.53c	3.23cd	0.97c	1.23bc	0.67c
T_5	1.00c	1.03d	0.80d	0.12d	0.16c	0.10d	6.92e	7.83d	5.29d	64.48c	63.09d	64.06d	2.90d	3.30c	3.07d	0.77d	1.17c	0.60c
Second season																		
T_1	2.3.0a	2.63a	2.23a	0.20a	0.21a	0.16a	10.31a	15.88a	7.60a	78.14a	85.74a	75.71a	6.40a	8.30a	5.23a	1.64a	1.80a	0.99a
T_2	2.00b	2.33b	1.93b	0.19a	0.18b	0.15b	9.83b	11.49b	6.84b	71.98b	82.89a	69.55b	5.70b	7.33b	4.63b	1.45b	1.67b	0.88b
T_3	1.80c	2.07c	1.77bc	0.18b	0.18b	0.13c	9.18c	10.99c	6.33c	69.64bc	74.36b	67.21bc	4.80c	6.67c	4.17c	1.36c	1.60b	0.82c
T_4	1.67cd	1.87cd	1.67c	0.16c	0.17b	0.13c	8.68d	9.83d	5.82d	66.95bc	70.50c	64.52bc	4.10d	6.17cd	3.73cd	1.24d	1.50c	0.75d
T_5	1.57d	1.77d	1.57c	0.07d	0.14c	0.12c	8.31d	9.13e	5.63d	64.38c	64.61d	61.95c	3.87d	5.77d	3.47d	1.17d	1.50c	0.69e

Table (4). Impact of compost-biofertilizers with/without various biostimulators sprays on total N,P,K%, on essential oil%, oil (ml/plant) and total oil yield (L/fed) of marjoram plant.

Treatments	Total nitrogen%			Total phosphorus%			Total potassium%			essential oil %			Oil (ml/plant)			Total oil yield(L/fed)		
	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
First season																		
T_1	1.60a	1.74a	1.35a	0.43a	0.43a	0.33a	1.57a	1.70a	1.60a	0.40^{a}	0.57^{a}	0.40^{a}	0.57^{a}	1.47^{a}	1.43^{a}	28.50^{a}	73.50^{a}	71.50^{a}
T_2	1.40b	1.56b	1.20b	0.37ab	0.40a	0.30ab	1.33ab	1.33b	1.47a	0.40^{a}	0.38^{b}	0.36^{a}	0.40^{b}	1.37^{ab}	1.20^{ab}	20.00^{b}	68.50^{b}	60.00^{b}
T_3	1.50bc	1.42b	1.10c	0.30bc	0.40a	0.27b	1.00b	1.20bc	1.33a	0.33^{b}	0.34^{bc}	0.33^{ab}	0.37^{b}	1.20^{b}	1.13^{b}	18.50^{b}	60.00^{c}	56.50^{c}
T_4	1.30cd	1.12c	1.01cd	0.30bc	0.33b	0.20c	0.50c	1.00cd	0.93b	0.30^{b}	0.32^{c}	0.26^{b}	0.30^{b}	0.90^{c}	0.77^{c}	15.00^{c}	45.00^{d}	38.50^{d}
T_5	1.25d	0.98c	0.94d	0.27c	0.30b	0.20c	0.50c	0.83d	0.60c	0.13^{c}	0.30^{c}	0.09^{c}	0.17^{c}	0.40^{d}	0.2^{d}	8.50^{d}	20.00^{e}	10.00^{e}
Second season																		
T_1	1.72a	1.67a	1.37a	0.53a	0.63a	0.53a	1.44a	1.28a	1.22a	0.95^{a}	0.60^{a}	0.40^{a}	1.07^{a}	1.63^{a}	1.50^{a}	53.50^{a}	81.50^{a}	75.00^{a}
T_2	1.49b	1.57b	1.10b	0.50ab	0.57b	0.50ab	1.35b	1.09b	1.02b	0.74^{b}	0.50^{b}	0.30^{b}	0.80^{b}	1.03^{b}	1.33^{ab}	40.00^{b}	51.50^{b}	67.50^{a}
T_3	1.38bc	1.50bc	1.07b	0.47b	0.50c	0.47b	1.31bc	1.03bc	0.95bc	0.54^{c}	0.47^{b}	0.30^{b}	0.60^{c}	0.90^{bc}	1.07^{b}	30.00^{c}	45.00^{b}	53.5^{b}
T_4	1.30bc	1.47c	0.97b	0.40c	0.50c	0.40c	1.26c	0.98cd	0.85c	0.45^{c}	0.37^{c}	0.30^{b}	0.47^{cd}	0.70^{cd}	0.60^{c}	23.50^{cd}	35.00^{c}	30.00^{c}
T_5	1.20c	1.27d	0.73c	0.40c	0.40d	0.40c	1.06d	0.91d	0.61d	0.31^{d}	0.30^{d}	0.13^{c}	0.30^{d}	0.57^{d}	0.30^{c}	15.00^{e}	28.50^{cd}	15.00^{d}

T_1- Compost-biofertilizers + foliar spray with seaweed extract, T_2- Compost-biofertilizers + foliar spray with fulvic acid, T_3 Compost-biofertilizers + foliar spray with humic acid, T_4- Compost-biofertilizers+ foliar spray with tap water and T_5- Mineral fertilizers+ foliar spray with tap water (as control)

- Essential oil constituents of sweet marjoram by GLC analysis:

Table (5). Illustrated the effect of compost-biofertilizer and different bio-stimulators application on the active components of *Majorana hortensis* essential oil identified 15 compounds in the 2^{nd} cut of the second season were provided by using Gas Liquid Chromatography analysis. It was clear that spraying plants with seaweed extract gave more richness in Terpinen-4-ol, which is the major compound of marjoram oil followed by FA spraying compared with the control treatment (mineral fertilizers) which recorded the lowest content. In addition, Linonene and Linalyl acetate compounds came in the second and third rank of marjoram oil content when sprayed the plants with SWE followed by FA.

Table (5). Impact of compost-biofertilizers with/without various biostimulators sprays on oil compounds (GLC) of marjoram plant

S. No	Compounds	T_1	T_2	T_3	T_4	T_5
1	α-pinene	4.29	5.34	4.31	5.03	1.61
2	Sabinene	0.70	0.49	6.22	5.86	2.37
3	β-phellandrene	6.03	5.48	13.74	6.30	3.75
4	Linonene	17.75	15.22	4.21	8.25	4.57
5	Unidentified	0.62	2.22	2.80	12.50	7.58
6	Linalool	2.31	7.06	2.67	4.67	4.41
7	Unidentified	5.89	1.87	1.80	2.03	6.57
8	Linalyl acetate	8.01	8.64	3.94	0.80	2.04
9	Unidentified	1.44	2.03	1.37	8.34	0.78
10	Terpinen 4, ol	44.34	41.82	40.42	36.88	11.53
11	Thuyanol	1.09	0.93	0.99	3.53	44.82
12	α-terpineol	3.62	0.36	2.54	3.80	4.21
13	Piperttol	3.25	3.49	2.35	1.09	5.67
14	Thuyan 4, ol	0.53	4.23	11.74	0.87	--
15	B-Caryophylene	--	0.80	0.89	--	--

T_1- Compost-biofertilizers + foliar spray with seaweed extract, T_2- Compost-biofertilizers + foliar spray with fulvic acid, T_3 Compost-biofertilizers + foliar spray with humic acid, T_4- Compost-biofertilizers+ foliar spray with tap water and T_5- Mineral fertilizers+ foliar spray with tap water (as control)

These results were in harmony with Khater (2021) found that, there were differences in oil composition as affected by different treatments. The dominant compound found under all treatments was Terpinen-4-ol as a major compound in marjoram oil. Recently, Attia et al., (2022) confirmed that marjoram plants treated with organic fertilizers gave the highest constituent of Terpinen-4-ol as a main component of the herb oil, which is responsible for the spicy taste and it gives the essential oil its antioxidant properties as distinctive characteristic in addition to the antimicrobial activities.

- The impact of organic-biofertilizers on chemical analysis and microbial counts of the soil at the end of the experiment:

Table (6), discussed that compost-biofertilizers provided a positive modification of soil physical properties, especially soil structure, soil aggregation and drainable pores. It is found that the soil pH tends to decrease slightly and moderate after the applied of compost-biofertilizer more than mineral fertilizers. These results are in agreement with (Mohamed et al., 2019) indicated that the applied of biofertilizer and compost reducing the soil pH due to presence of various acids (amino acids and humic acid), in addition, the activity of microorganisms producing organic acid released from biofertilizers. In addition, the EC values tend to decrease when soil treated with biofertilizers compared to mineral fertilizers soil.

Table (6). Chemical analysis and microbial counts of the experimental soil at the end of the experiment

Cations and anions (mmq/L)	Organic-biofertilizers treatments	Chemical treatment
pH (1:2.5)	7.37	7.59
EC 1:5 (dS/m^3)	1.52	1.41
Ca^{++}	7.50	7.10
Mg^{++}	1.80	1.10
Na^+	2.60	3.30
K^+	3.60	2.80
CO_3^{--}	--	--
HCO_3^-	1.90	1.40
Cl^{--}	3.30	2.40
SO_4^{--}	10.30	10.50
Available nutrients (ppm)		
Nitrogen	15.38	15.00
Phosphorus	12.10	11.80
Potassium	33.70	31.50
Bacterial counts (CFU/ml.)		
Fungi	2.0×10^5	1.0×10^5
Bacteria	3.0×10^6	1.0×10^6
Actinomycetes	2.0×10^7	1.0×10^7
B.megaterium	11×10^7	30×10^6
B.circulance	12×10^7	30×10^6
Most probable number(MPN) in the Marjoram rihzosphere		
A.barasilense	90×10^6	10×10^6

The highest values of N, P and K contents in soil were noticed in soil treated by compost-biofertilizers compared to mineral fertilizers. These results are in agreement with (Abdel-Azeem, 2020) who indicated that the addition of biofertilizers led to increased N, P , K content and the availability of soil organic matter which may be due to enhanced microbial activities. The numbers and diversity of microbial populations as a sign of soil fertility are affected by the amount and type of plant litter, root exudates and mineral or organic fertilizers. This in turn affects crop production and sustainable soil health. The highest microbial population in the rhizosphere of (*Majorana hortensis* L.) plants recorded with organic-biofertilizer, whereas the lowest count was recorded at mineral fertilizers. Similar results reported by fetyan et al., (2015), who revealed that, organic material significantly increased the microbial population. In addition, Li et al., (2023) found that, the soil health index value (Soil enzymatic activities, microbial biomass and bacterial community composition) was higher during the use of organic-biofertilizer application while the lowest value was in mineral fertilizer which failed to improve soil health.

Conclusion

Due to the natural stimulating effects of Seaweed extract and fulvic acid which can be applied at a rate of 10 ml/I foliar spray once a month to improve the vegetative growth, yield and nutritional quality and essential oil yield of marjoram plants. Moreover, it was believed to be used as a promising agricultural practice for the production of medicinal and aromatic plants.

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تأثير التسميد العضوي -الحيوي والرش الورقي بالمنشطات الحيوية المختلفة على زيادة المحصول والزيت العطري لنبات البردقوش الحلو (*Marjoram hortensis* L.)

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نظراً لأهمية النباتات الطبية والعطرية في مختلف الصناعات الطبية والغذائية، فمن المهم زيادة الإنتاج العضوي دون الإفراط في استخدام المواد الكيميائية الضارة. أجريت تجربة حقلية في المزرعة البحثية لقسم النباتات الطبية والعطرية بمعهد بحوث البساتين، الدقي، الجيزة، مصر خلال موسمي نمو متتاليين ٢٠٢١/٢٠٢٠ و٢٠٢٢/٢٠٢١ بهدف دراسة تأثير الرش الورقي باستخدام المنشطات الحيوية المختلفة (مستخلص الطحالب البحرية، حامض الفولفيك، حامض الهيوميك، ١٠ مل/ لتر) على خصائص النمو الخضري، المحصول والإنتاجية، محتوى الزيت العطري وبعض التركيبات الكيميائية لنبات البردقوش الذي تم معاملته بتطبيقات التسميد العضوي - الحيوي. كما تمت دراسة مؤشرات صحة التربة من خلال تحديد النشاط البيولوجي لإنزيمات التربة. لذا أثبتت النتائج أن الإنتاج الناجح لنبات البردقوش العضوي يتوافق مع أهداف التنمية المستدامة والأمن الغذائي، ويمكن التوصية بأن النباتات التي تم تسميدها بالتسميد العضوي-الحيوي والتي تم رشها بمستخلص الطحالب البحرية المحفزة حيوياً سجلت أعلى القيم المعنوية لجميع الصفات المدروسة، تليها النباتات التي تم رشها بمستخلص حامض الفولفيك مقارنة بمعاملة الكنترول (الأسمدة المعدنية منفردة).