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EFFECT OF ORGANIC AND BIO-FERTILIZERS ON SOME QUALITY AND QUANTITY CHARACTERS OF *Mentha piperita* L. PLANTS

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ABSTRACT

A nutritional experiments were carried out on Peppermint plants (*Mentha piperita* L.) at the experimental Baramoon Research Farm, Dakahlya Governorate, Egypt, during the two successive seasons, 2010-2011 and 2011-2012, aiming to investigate the effect of (FYM), humic acid, microbein and phosphorein, as well as active dry yeast on vegetative growth, herb yield, essential oil production and chemical composition of plants.

The results showed that in both seasons, all fertilizer treatments achieved significantly better growth and yield than the untreated plants (control). Treatments included that plant biomass responded linearly to (FYM) application and most values of all measurements were realized at the rate of 30 m^3/fed. than obtained at both 20, 10 m^3/fed. in comparison with those of control plants in both seasons, respectively. Also, it is evident that the application of (FYM) at rates of 10, 20 and 30 m^3/fed., interacted with combinations of both (phosphorein + microbein), (phosphorein + yeast) and (phosphorein + humic acid) improved significant increments in the all growth characters compared with control plants, and the highest one was realized with combination of (FYM) at rate 30 m^3/fed. + phosphorein + humic acid.

These findings clearly indicate that phosphorein, humic acid and 30 m^3/fed. of (FYM) could be used as traditional fertilizers and may consequently minimized pollution of agricultural environments.

INTRODUCTION

Utilization and demand for medicinal and aromatic plant is interest demand for herbal medicine applications. Other purposes included food flavorings, cosmetics such as fragrances, skin, oral and hair care preparations and industrial chemicals.

Peppermint (*Mentha piperita* L.) plants belongs to family Lamiaceae (Labiateae) and it has been used since the eighteenth century on account of its aromatic compound contents, especially menthol. Peppermint tea has long been a domestic remedy for indigestion, relieving stomach aches and menstrual cramps due to its anti-spasmodic effect, and alleviating tension headaches and tiredness. Peppermint also stimulates gastric secretions, which promote digestion. Peppermint oil is a familiar ingredient of many brands of toothpaste, and combines an antiseptic action with a refreshing flavour. Menthol, with its cooling and anaesthetic action, is also found in rubs or massage lotions for aching muscles and rheumatic joints. Applying the fresh leaves eases muscular aches and pains. Menthol, too, makes an

effective inhalation for clearing up catarrh and nasal congestion. Aromatherapies recommended a footbath of diluted peppermint oil for tired feet. Commercially, peppermint is widely used as a flavoring in the confectionery industry, Kruger (1992).

Chemical nutrients especially nitrogen, phosphorus and potassium are very important for plants, because they partake in structure of several components of the whole plants (protein, hormones, amino acids, enzymes, nucleic acids, fats and regulation of water conditions). However, using intense chemical fertilization cause serious problems on human health by pollution of the whole environmental conditions (soil, air and drainage water).

Farmyard manure (FYM) is added to soils to improve their physical and chemical properties. It increases the soil fertility owing to its composition from macro and micro elements, amino acids, organic acids, sugars and organic matter. Also, it is a considerable useful habitat for several beneficial microorganisms.

Humic substances are organic compounds that result from the decomposition of plant and animals waste materials. Humic acid and their salts which derived from coal and other sources may provide a viable alternative to liming, to ameliorate soil acidity and improve soil structural stability. It is the humic fractions (humic acid, fulvic acid and humin) of the soil organic matter that are responsible for the generic improvement of soil fertility and productivity, Kononova (1966) and Fortun *et al*., (1989). On other hand, Russo & Berlyn (1990), Sanders *et al*., (1990) and Pioncelot (1993) stated that increasing the permeability of plant membranes due to humate application resulted in improving growth of various groups beneficial microorganisms which accelerate cell division, increased root growth and all plant organs for a number of horticultural crops and turf grasses, as well as, the growth of some trees.

Bio-fertilizers are the most reliable tools to reduce the rate of chemical fertilizers applied for medicinal plants production in all types of soil and hence decreasing environmental pollution, EL-Mahrouk (2000). Bio-fertilizers were provided by the General Organization for Agriculture Equalization Fund (G.O.A.E.F.), Ministry of Agriculture, Egypt. The applied bio-fertilizers were microbein which contains live cells of efficient bacteria strains for N-fixation and phosphate solubilizing (*Azotobacter* sp., *Azospirillum* sp., *Pseudomonas* sp., *Rhizobium* sp. and *Bacillus megatherium*) and phosphorein which contains live cells of efficient bacteria strains as phosphate solubilizing bacteria (*Bacillus megaterium*).

All the bacterial growth media were used at a rate of 400 g/fed. (0.2 gm/plot/season).

The significant effect of bio-fertilizers may be due to the effect of different strain groups and nutrients mobilizing microorganisms which help in availability of metals and their forms in the composted material and increased levels of extractable minerals,

Yeast as a natural biostimulator is very safe to human, animals and environments, EL-Araby (2004). It is natural source of many growth substances (thiamine, riboflavin, cholin, niacin, pyridoxine, folic acid and vitamin B_{12}) and most of nutrient elements (Na, Ca, Fe, K, P, S, Zn and Si),

as well as, organic compounds i.e. protein, carbohydrate, nucleic acids and lipids, Nagodawithana, (1991). The various positive effects of applying dry yeast were attributed to its content of different nutrients, higher percentage of proteins, large amount of vitamin B and natural growth hormones, namely, cytokinins, in addition, application of yeast is very effective in releasing CO_2 which improved photosynthesis, Idso *et al*., (1995). The plant height, number of branches and N, P and K contents as well as the volatile oil composition of black cumin were highest as affected by the treatment of 2 mg yeast, Naguib and Khalil (2002).

The final goal of this search was to investigate the effect of different levels of both farmyard manure, biofertilizers (phosphorein, microbein and yeast) and organic manure (humic acid) and their interactions on growth, essential oil productivity and chemical components of peppermint plants.

MATERIALS AND METHODS

This study was conducted at the experimental Baramoon Research Farm, Dakahlya Governorate, Egypt during the two successive seasons 2010/2011 and 2011/2012, aiming to investigate the effect of farmyard manure (FYM), humic acid, microbein, phosphorein and yeast as well as their interactions on vegetative growth, herb yield, essential oil production and chemical composition of peppermint plants.

Uniform cuttings of *Mentha piperita*, L. (peppermint) plants were taken from symmetry mother plants and planted in the nursery for rooting on Oct. 15th 2010 and 2011 seasons. The growing seedlings were transplanted on Feb. 15th at 60 cm apart on the eastern side of row in an irrigated soil. The soil of the experimental location was clay in texture.

Randomized soil samples were obtained from the field to determine the physical and chemical contents according to the standard method described by Wild *et al*. (1985). Soil properties are presented in Table (A).

Table (A): Some physical and chemical characteristics of the experimental soil in the two seasons.

Season	Sand (%)	Silt (%)	Clay (%)	$CaCO_3$ (%)	pH	Available nutrients (ppm)		
						N	P	K
2010	24.60	30.20	42.60	3.20	7.40	13.90	11.60	32.70
2011	15.32	34.96	45.12	3.10	7.75	15.10	11.90	35.60

The all plots received chemical NPK fertilizers as an activation dose at the recommended rate of Ministry of Agriculture, Egypt.

The experimental field received (FYM) which consists of equal quantities from poultry and cattle manures, at three doses as 10, 20 and 30 m^3/fed. during soil preparation for planting. The (FYM) samples were analyzed at the Water and Soil Laboratory in both seasons as presented in table (B).

Table (B): Chemical analysis of the added (FYM) in both seasons (2010/2011 & 2011/2012).

Properties	2010/2011	2011/2012
Organic matter	65.45	68.90
Total carbon	37.06	32.51
Total nitrogen	1.76	1.80
C/N ratio	1:21.25	1:21.50
Total phosphate	1.04	0.89
Total potassium	0.98	0.87
pH (in 1:5)	8.20	8.15

Humic acid is an organic material was added as 6 L/fed., drench with irrigation water divided in four equal doses. The first dose was applied after one week from planting of seedlings followed with other doses at intervals of two weeks.

Biofertilizers applied in this respect were microbein(*Azotobacter sp*., *Azospirillum sp*., *Pseudomonas sp*., *Rhisobium sp*., and *Bacillus megatherium*), phosphorein(*Bacillus megaterium*) and yeast (*Saccharomyces cerevisiae*). Microbien and phodphorein applied 4kg/fed one time before planting while yeast was done as foliar spray with concentration of 3gm/l at three times through the growing seasons.

The experimental design :

The experimental design was split-plot with 3 replicates. (FYM) treatments were main plots at rates 10, 20 and 30 m^3/fed., while microbein, yeast and humic acid interacted with phosphorein were sub-main plots.

The treatments :

1- Control (NPK) recommended doses
2- FYM 10 m^3/fed.
3- FYM 20 m^3/fed.
4- FYM 30 m^3/fed.
5- Microbein + phosphorein.
6- Microbein + phosphorein + FYM 10 m^3/fed.
7- Microbein + phosphorein + FYM 20 m^3/fed.
8- Microbein + phosphorein + FYM 30 m^3/fed.
9- Yeast + phosphorein.
10- Yeast + phosphorein + FYM 10 m^3/fed.
11- Yeast + phosphorein + FYM 20 m^3/fed.
12- Yeast + phosphorein + FYM 30 m^3/fed.
13- Humic acid + phosphorein.
14- Humic acid + phosphorein + FYM 10 m^3/fed.
15- Humic acid + phosphorein + FYM 20 m^3/fed.
16- Humic acid + phosphorein + FYM 30 m^3/fed.

Harvesting :

In both seasons, the plants were harvested twice yearly by cutting the aerial parts of each plant (10 cm) above the soil surface. The first cut was carried out in the second week of June and the second cut was in the second week of September.

Five plants were randomly chosen from each treatment at each cut, in both seasons. The vegetative growth parameters (plant height, number of branches, plant fresh and air dried weight as well as herb yield) were recorded.

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The essential oil percentages were determined from dry leaves using 100 gm, samples for each cut. The distillation of essential oil and the content determination were as described in the Egyptian Pharmacopoeia (1984). The oil content was calculated by multiplying oil percentage by weight of dry plants (ml/plant) and per feddan (Liter/fed.). The essential oil obtained from the second cut of the second season was analyzed using Gas Liquid Chromatography technique (GLC), which carried out at the Laboratory of Aromatic and Medicinal Plants Research Department, Cairo, Egypt.

The use of GLC in the quantitative determination was performed using the methods described by Bunzen *et al*., (1969) and Hoftman (1967).
Obtained data was subjected to the statistical analysis of variance (ANOVA) in split-plot design as mentioned by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

1- Plant growth characters :

Data presented in Tables (1, 2, 3 and 4) indicated that providing peppermint plants with different levels of (FYM), (phosphorein + microbein), (phosphorein + yeast), (phosphorein + humic acid) and their interactions, exerted significant differences concerning vegetative growth characters expressed as plant height, number of branches, herb fresh and dry weights compared with control. The highest vegetative growth characters resulted from fertilized plants with the interaction between (FYM) 30 m^3/fed., and (phosphorein + humic acid).
The pre-mentioned increases may be due to the essential and vital role of (FYM), biofertilizers phosphorein, microbein and yeast as well as humic acid, either of them fertilized solely or in combinations. The stimulatory effect of (FYM) as a result of improving physical, chemical, biological, texture and drainage of the soil which in turn, positively influence the growth, Salem (1986). The increases in vegetative growth characters owing to yeast may be due to its content of tryptophan, Abd EL-Latif (1987) and precursor of IAA, Moor (1979). Increments resulted from biofertilized treatments may be due to the supplements of biofertilizers to plants with nitrogen which is considered a precursor of protein synthesis and a vascular osmoticum. The osmotic compounds in the cell sap are important in order to allow cell enlargement. These results are supported by Hamza *et al*., (2007) on *Plantago ovata* Forsk plants and Massoud (2007) on marjoram plants. On the other hand, Kononova (1966) and Fortun *et al*., (1989) performed that humic acids are known to posses many beneficial agricultural properties, they participate actively in the decomposition of organic matter, improve soil texture, enhancement of photosynthesis which resulted in greater plant growth. The results of this study are in harmony with those obtained with Massoud *et al*., (2010) on marjoram plants.

The interactions showed that there were significant differences as plant growth characters were concerned. These results were similar in the two seasons.

Table (1): Plant height (cm) of peppermint as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters / Treatments	First season 2010/2011									
	Plant height									
	First cut					Second cut				
	0	FYM 10 m³/fed.	FYM 20 m³/fed.	FYM 30 m³/fed.	Mean (A)	0	FYM 10 m³/fed.	FYM 20 m³/fed.	FYM 30 m³/fed.	Mean (A)
Control(NPK)	60.2	70.8	74.9	78.8	71.2	66.9	73.2	77.6	82.4	75.0
Phosphorein+Microbein	79.6	82.3	85.9	89.7	84.4	80.3	81.3	86.2	90.5	84.6
Phosphorein+Yeast	76.3	79.9	82.3	85.6	81.1	77.1	79.6	84.3	88.2	82.3
Phosphorein+Humic acid	82.7	85.9	89.6	91.8	87.5	86.9	88.7	90.3	93.3	89.8
Mean (B)	74.7	79.7	83.2	86.5	----	77.8	80.7	84.6	88.6	----
	A		B		A x B	A		B		A x B
L.S.D at 0.05	4.57		4.08		8.15	5.78		5.62		11.64
L.S.D at 0.01	6.42		5.17		10.14	7.34		6.33		12.45
	Second season 2011/2012									
	First cut					Second cut				
Control(NPK)	64.9	69.2	75.3	79.6	72.3	71.6	75.6	79.3	85.3	77.9
Phosphorein+Microbein	80.6	83.1	86.7	89.9	85.1	82.7	83.7	87.9	91.7	86.5
Phosphorein+Yeast	75.3	80.6	84.3	87.2	81.8	78.8	81.1	84.2	88.6	83.2
Phosphorein+Humic acid	86.9	89.7	92.2	95.7	91.1	87.6	89.1	90.1	93.9	90.2
Mean (B)	76.9	80.7	84.6	88.1	----	80.2	82.4	85.4	89.9	----
	A		B		A x B	A		B		A x B
L.S.D at 0.05	3.67		4.52		7.62	4.61		4.80		9.67
L.S.D at 0.01	4.29		5.63		10.24	6.33		6.15		12.11

Table (2): Number of branches/plant of peppermint as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters / Treatments	First season 2010/2011									
	Number of branches / plant									
	First cut					Second cut				
	0	FYM 10 m³/fed.	FYM 20 m³/fed.	FYM 30 m³/fed.	Mean (A)	0	FYM 10 m³/fed.	FYM 20 m³/fed.	FYM 30 m³/fed.	Mean (A)
Control(NPK)	11.2	13.6	15.80	19.20	14.95	12.80	14.60	16.90	19.25	15.90
Phosphorein + Microbein	14.60	16.20	19.30	22.60	18.18	14.80	16.90	19.60	23.40	18.68
Phosphorein + Yeast	13.90	15.80	18.30	21.70	17.43	13.00	15.40	18.30	21.20	16.98
Phosphorein + Humic acid	16.80	18.80	21.20	23.40	20.05	17.20	19.60	21.90	23.80	20.63
Mean (B)	14.13	16.10	18.65	21.73	----	14.45	16.63	19.18	21.91	----
	A		B		A x B	A		B		A x B
L.S.D at 0.05	2.02		1.09		2.80	2.01		1.08		2.06
L.S.D at 0.01	3.01		2.80		4.60	3.00		2.60		4.01
	Second season 2011/2012									
	First cut					Second cut				
Control(NPK)	12.50	13.90	15.60	18.90	15.20	13.6	15.20	17.30	19.70	16.50
Phosphorein + Microbein	14.90	16.30	18.90	21.80	17.98	15.3	16.70	18.60	20.90	17.88
Phosphorein + Yeast	13.20	15.10	17.20	20.60	16.53	14.1	15.00	17.30	19.10	16.38
Phosphorein + Humic acid	16.4	18.90	20.9	23.60	19.95	18.1	19.90	22.30	24.60	21.23
Mean (B)	14.25	16.050	18.15	21.23	----	15.28	16.70	18.88	21.08	----
	A		B		A x B	A		B		A x B
L.S.D at 0.05	1.06		2.03		2.60	1.09		2.01		2.20
L.S.D at 0.01	2.10		3.01		4.32	2.30		3.50		4.30

Table (3): Herb fresh weight/plant (g) of peppermint as affected by (FYM), phosphorein, microbein, yeast, humic acid and their interactions during 2011 and 2012 seasons for two cuts.

	First season 2010/2011									
	Herb fresh weight / plant									
	First cut					Second cut				
	0	FYM 10 m^3/fed.	FYM 20 m^3/fed.	FYM 30 m^3/fed.	Mean (A)	0	FYM 10 m^3/fed.	FYM 20 m^3/fed.	FYM 30 m^3/fed.	Mean (A)
Control(NPK)	510.3	606.9	646.8	693.6	614.4	536.6	616.3	653.6	702.3	627.2
Phosphorein + Microbein	695.6	720.3	736.9	760.8	728.4	701.3	727.9	745.3	765.2	734.9
Phosphorein + Yeast	670.1	703.6	710.7	750.2	708.7	680.6	708.3	715.8	753.7	714.6
Phosphorein + Humic acid	734.7	760.7	771.9	791.3	764.6	750.3	765.8	780.3	806.8	775.8
Mean (B)	652.7	697.9	716.6	749.0	----	667.2	704.6	723.8	757.0	----
	A		**B**		**A x B**	**A**		**B**		**A x B**
L.S.D at 0.05	28.9		33.6		78.7	32.2		39.6		87.6
L.S.D at 0.01	45.6		57.3		93.6	47.3		59.1		99.2
	Second season 2011/2012									
	First cut					**Second cut**				
Control(NPK)	516.7	620.3	653.6	699.5	622.5	541.2	626.6	660.8	710.6	634.8
Phosphorein + Microbein	698.3	725.4	740.2	766.7	732.7	709.6	735.9	750.7	772.9	742.3
Phosphorein + Yeast	673.6	710.7	712.7	756.6	713.4	690.1	715.8	719.6	758.7	721.1
Phosphorein + Humic acid	746.9	766.2	779.9	798.3	772.8	760.6	773.6	782.2	810.6	781.8
Mean (B)	658.9	705.7	721.6	755.3	----	675.4	713.0	728.3	763.2	----
	A		**B**		**A x B**	**A**		**B**		**A x B**
L.S.D at 0.05	36.2		42.6		88.3	38.7		47.8		95.2
L.S.D at 0.01	52.3		66.7		98.2	57.6		69.7		103.3

Table (4): Herb dry weight/plant (g) of peppermint as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

	First season 2010/2011									
	Herb dry weight / plant									
	First cut					Second cut				
	0	FYM 10 m^3/fed.	FYM 20 m^3/fed.	FYM 30 m^3/fed.	Mean (A)	0	FYM 10 m^3/fed.	FYM 20 m^3/fed.	FYM 30 m^3/fed.	Mean (A)
Control(NPK)	56.08	66.69	71.08	76.22	67.52	58.10	67.73	71.82	77.18	68.71
Phosphorein + Microbein	76.44	79.15	80.98	83.60	80.04	77.07	79.99	81.90	84.09	80.76
Phosphorein + Yeast	73.64	77.32	78.10	82.44	77.89	74.80	77.84	78.66	82.82	78.53
Phosphorein + Humic acid	80.74	83.59	84.82	86.10	83.81	82.45	84.15	85.75	88.66	85.25
Mean (B)	71.73	76.69	78.75	82.09	----	73.11	77.43	79.53	83.19	----
	A		**B**		**A x B**	**A**		**B**		**A x B**
L.S.D at 0.05	14.2		19.6		45.7	18.6		26.1		56.6
L.S.D at 0.01	20.6		30.2		73.2	26.7		39.2		69.7
	Second season 2011/2012									
	First cut					**Second cut**				
Control(NPK)	56.78	68.16	71.71	76.87	68.38	59.47	68.86	72.62	78.09	69.76
Phosphorein + Microbein	76.74	79.71	81.34	84.25	80.51	77.98	80.87	82.49	84.93	81.57
Phosphorein + Yeast	74.02	78.10	78.32	83.14	78.40	75.84	78.66	79.08	83.37	79.24
Phosphorein + Humic acid	82.08	84.20	85.70	87.73	84.93	83.58	85.01	85.96	89.08	85.91
Mean (B)	72.41	77.54	79.27	82.10	----	74.22	78.35	80.04	83.87	----
	A		**B**		**A x B**	**A**		**B**		**A x B**
L.S.D at 0.05	18.3		22.7		50.6	20.6		29.9		63.6
L.S.D at 0.01	29.7		39.9		79.8	30.4		45.7		79.8

2- Essential oil yield :

The essential oil percentage and content in the dried leaves of peppermint plants varied from one treatment to other (Tables 5 and 6). The highest increas in oil percentage were obtained from plants fertilized with (FYM) 30 m^3/fed. combined with interaction of (phosphorein + microbein) which were (1.33, 1.70 and 1.76, 1.82 %) for two cuts in both two seasons, respectively, whereas same treatment achieved the most increments of essential oil content (cc/plant) in the two cuts for both two seasons and the differences were significant in comparison with those obtained by control plants, (1.41, 1.43 and 1.47, 1.55 cc/plant).

Similar results of positive effects of both (FYM) or biofertilizers (phosphorein and microbein) on the essential oil productivity were obtained by Shalan *et al*., (2001) on chamomile, Sakr (2001) on peppermint, Abd EL-Latif *et al*., (2000) on *Matricaria chamomilla*, Hamed (2004) on *Salvia officinalis*, Massoud (2007) on marjoram and EL-Sanafawy (2007) on *Ocimum basilicum* and *Majorana hortensis* plants.

Table (5): Essential oil percentage of peppermint as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters / Treatments	Control (NPK)	FYM 10 m^3/fed.	FYM 20 m^3/fed.	FYM 30 m^3/fed.	Mean (A)	Control (NPK)	FYM 10 m^3/fed.	FYM 20 m^3/fed.	FYM 30 m^3/fed.	Mean (A)
	First season 2010/2011									
	Essential oil percentage									
	First cut					Second cut				
Control(NPK)	0.66	1.32	1.19	1.04	1.05	0.69	1.35	1.22	1.09	1.09
Phosphorein + Microbein	0.69	1.38	1.57	1.69	1.33	0.72	1.39	1.55	1.70	1.34
Phosphorein + Yeast	1.09	1.29	1.26	1.21	1.21	1.10	1.32	1.27	1.20	1.22
Phosphorein + Humic acid	1.60	1.36	1.21	1.18	1.34	1.50	1.38	1.23	1.17	1.32
Mean (B)	1.01	1.49	1.31	1.28	----	1.00	1.36	1.32	1.29	----
	A		B	A x B		A		B	A x B	
L.S.D at 0.05	0.09		0.05	0.11		0.09		0.06	0.12	
L.S.D at 0.01	0.14		0.07	0.14		0.14		0.08	0.17	
	Second season 2011/2012									
	First cut					Second cut				
Control(NPK)	0.67	1.35	1.24	1.07	1.08	0.71	1.38	1.26	1.09	1.11
Phosphorein + Microbein	0.70	1.42	1.64	1.76	1.38	0.73	1.45	1.63	1.82	1.41
Phosphorein + Yeast	1.15	1.36	1.30	1.22	1.26	1.18	1.39	1.32	1.24	1.28
Phosphorein + Humic acid	1.65	1.41	1.29	1.20	1.39	1.54	1.43	1.28	1.16	1.35
Mean (B)	1.04	1.39	1.37	1.31	----	1.04	1.41	1.37	1.33	----
	A		B	A x B		A		B	A x B	
L.S.D at 0.05	0.02		0.05	0.11		0.05		0.06	0.12	
L.S.D at 0.01	0.04		0.07	0.14		0.08		0.08	0.16	

Table (6): Oil content of peppermint cc/plant as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters / Treatments	First season 2010/2011									
	Oil content									
	First cut					Second cut				
	Control (NPK)	FYM 10 m³/fed.	FYM 20 m³/fed.	FYM 30 m³/fed.	Mean (A)	Control (NPK)	FYM 10 m³/fed.	FYM 20 m³/fed.	FYM 30 m³/fed.	Mean (A)
Control(NPK)	0.37	0.88	0.85	0.79	0.72	0.40	0.91	0.88	0.84	0.76
Phosphorein + Microbein	0.53	1.09	1.27	1.41	1.08	0.55	1.11	1.27	1.43	1.09
Phosphorein + Yeast	0.80	1.03	1.02	1.00	0.96	0.82	1.03	1.00	0.99	0.96
Phosphorein + Humic acid	1.29	1.14	1.02	1.02	1.12	1.24	1.16	1.05	1.04	1.12
Mean (B)	0.75	1.03	1.03	1.06	----	0.75	1.05	1.05	1.08	----
	A		B		A x B	A		B		A x B
L.S.D at 0.05	0.09		0.05		0.11	0.09		0.06		0.12
L.S.D at 0.01	0.14		0.07		0.14	0.14		0.08		0.17
	Second season 2011/2012									
	First cut					Second cut				
Control(NPK)	0.38	0.92	0.89	0.82	0.75	0.42	0.95	0.92	0.85	0.79
Phosphorein + Microbein	0.54	1.13	1.33	1.47	1.12	0.57	1.17	1.34	1.55	1.16
Phosphorein + Yeast	0.85	1.06	1.02	1.01	0.99	0.89	1.09	1.04	1.03	1.01
Phosphorein + Humic acid	1.35	1.19	1.09	1.03	1.20	1.29	1.22	1.10	1.03	1.16
Mean (B)	0.78	1.08	1.08	1.08	----	0.79	1.11	1.10	1.10	----
	A		B		A x B	A		B		A x B
L.S.D at 0.05	0.12		0.11		0.21	0.12		0.09		0.20
L.S.D at 0.01	0.18		0.15		0.29	0.18		0.13		0.27

These increases might be attributed to the enhancing effect of organic and biofertilizers on vegetative growth, in terms of fresh yield besides increasing uptake of nutrients especially phosphorus element which linked by phosphate bounds which is adenosine triphosphate (ATP). In this form, the energy can be undergoing processes such activation uptake and the synthesis of various organic compounds such as essential oil, EL-Ghadban *et al*., (2003) and Heikal (2005).

3- Herb and oil yield :

Data reported in Table (7) revealed significant differences in dried herb yield of peppermint plants due to different levels of FYM fertilizer (10, 20 and 30 m^3/fed.) solely or combined with interactions of both (phosphorein + microbein), (phosphorein + yeast) and (phosphorein + humic acid).

Fertilization with the highest level of (FYM) 30 m^3/fed., combined with interaction (phosphorein + humic acid) produced the heaviest dried herb yield (2.935 and 2.971 ton/fed.) as a total for two cuts in both two seasons, respectively, compared with control plants.

Concerning the effect of different levels of fertilizers in this respect, on essential oil yield, data in Table (7) showed that the highest yearly essential oil production (Liter/fed.) have achieved when plants fertilized with (FYM) 30 m^3/fed. combined with interaction of (phosphorein + microbein) such as data recorded (47.71 and 50.74 Liter/fed.) as a total essential oil yield in both cuts for two seasons, respectively, when compared with control.

Table (7): Yearly dry weight yield (ton/fed.) and yearly essential oil yield (Liter/fed.) of peppermint plants as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters / Treatments	Yearly dry weight (ton/fed.)						Yearly essential oil (L/fed.)					
	First season 2010/2011			Second season 2011/2012			First season 2010/2011			Second season 2011/2012		
	1st cut	2nd cut	Total	1st cut	2nd cut	Total	1st cut	2nd cut	Total	1st cut	2nd cut	Total
Control(NPK)	0.942	0.976	1.918	0.954	0.999	1.953	6.22	6.72	12.94	6.38	7.06	13.44
(FYM) 10 m^3/fed.	1.120	1.139	2.259	1.145	1.157	2.302	14.78	15.29	30.07	15.46	15.96	31.42
(FYM) 20 m^3/fed.	1.194	1.207	2.401	1.205	1.220	2.425	14.28	14.78	29.06	14.95	15.46	30.41
(FYM) 30 m^3/fed.	1.280	1.297	2.517	1.291	1.312	2.603	13.27	14.11	27.38	13.78	16.63	30.41
Microbein + Phosphorein	1.284	1.295	2.579	1.289	1.310	2.599	8.90	9.24	18.14	9.07	9.58	18.65
Mic. + Phos. + (FYM) 10 m^3/fed.	1.330	1.344	2.674	1.339	1.359	2.698	18.31	18.65	36.96	18.98	19.66	38.64
Mic. + Phos. + (FYM) 20 m^3/fed.	1.360	1.376	2.736	1.367	1.386	2.753	21.34	21.34	42.68	22.34	22.51	44.85
Mic. + Phos. + (FYM) 30 m^3/fed.	1.404	1.413	2.817	1.415	1.427	2.842	23.69	24.02	47.71	24.70	26.04	50.74
Yeast + Phosphorein	1.237	1.257	2.494	1.244	1.274	2.518	13.44	13.78	27.22	14.28	14.95	29.23
Yeast + Phos. + (FYM) 10 m^3/fed.	1.299	1.308	2.607	1.312	1.321	2.633	16.80	17.30	34.10	17.81	18.31	36.12
Yeast + Phos. + (FYM) 20 m^3/fed.	1.312	1.321	2.633	1.316	1.329	2.645	17.14	16.80	33.94	17.14	17.47	34.61
Yeast + Phos. + (FYM) 30 m^3/fed.	1.385	1.391	2.776	1.397	1.401	2.798	16.80	16.63	33.43	17.05	17.30	34.35
Humic acid + Phosphorein	1.363	1.385	2.748	1.379	1.404	2.783	21.67	20.83	42.50	22.68	21.67	44.35
Hum. acid + Phos. + (FYM) 10 m^3/fed.	1.404	1.414	2.818	1.415	1.428	2.843	19.15	19.49	38.64	20.09	20.50	40.59
Hum. acid + Phos. + (FYM) 20 m^3/fed.	1.425	1.441	2.866	1.440	1.444	2.884	17.14	16.80	33.94	18.31	18.48	36.79
Hum. acid + Phos. + (FYM) 30 m^3/fed.	1.446	1.489	2.935	1.474	1.497	2.971	17.14	17.47	34.61	17.30	17.30	34.60

Phos. = Phosphorein. Mic. = Microbein. Hum. = Humic

The highest increase in oil yield/fed. was obtained from combinations between both of organic and biofertilizers, may be due to the increase in herb yield as well as the increment in the essential oil percentage. On other hand, this increment may be due to that mineral nutrition exerted some effects either directly on the enzyme system dealing with this conversion or indirectly by its effects on photosynthetic process.

4- Essential oil components :

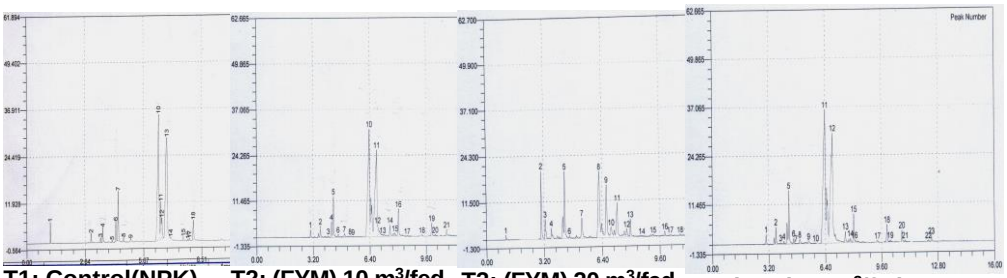
Data presented in Table (8) and illustrated in Figure (1) identified (10) compounds separated from peppermint herb oil samples produced from plants fertilized with (FYM) or inoculated with phosphorein, microbein, yeast, humic acid and their interactions. The obtained chromatograms revealed the presence of (22) components from which (10) components were identified by the retention times obtained from pure authentic substances. The (10) main components are illustrated in Table (8).

It is evident from the results that the compound of menthol recorded the highest values (48.51, 46.02, 45.63 and 45.00 %) when plants treated with (yeast + phosphorein + FYM 30 m^3/fed.), (humic acid + phosphorein + FYM 20 m^3/fed.), (yeast+phosphorein + FYM 10 m^3/fed.) and (yeast + phosphorein + FYM 20 m^3/fed.) interaction, respectively- followed by menthone compound which recorded (35.70 %) which was the best when plants treated with (yeast+phosphorein) interaction, in comparison with control and other treatments in this respect. It is clear that co-operation between both of biofertilizers and organic manure activated biosynthesis reactions of peppermint plants resulting the most values of main components.

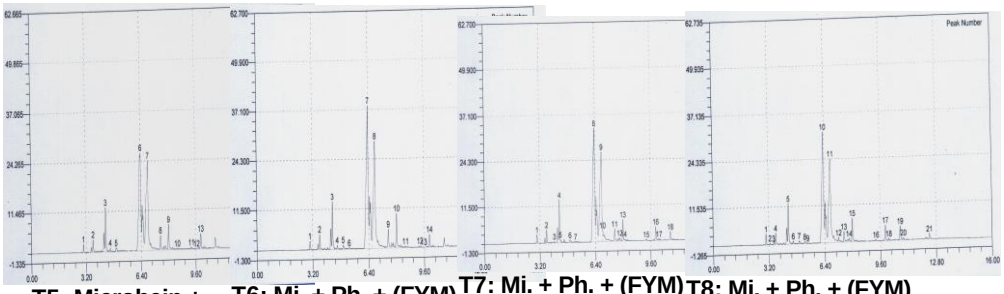
Table (8): GLC of peppermint plants oil as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Main components / Treatments	α-pinene	Sabinene	β-pinene	Limonene	1,8 cineole	Menthone	Menthol	Iso-menthone	Menthyl acetate	β-caryophyllene
Control(NPK)	0.97	0.98	0.57	1.74	2.98	32.89	35.82	0.55	3.14	2.14
(FYM) 10 m^3/fed.	0.73	7.96	3.05	2.47	6.69	14.84	23.37	13.74	10.07	4.03
(FYM) 20 m^3/fed.	0.85	1.95	0.43	2.04	4.95	28.97	40.90	3.41	4.96	2.30
(FYM) 30 m^3/fed.	0.88	2.05	0.19	0.25	7.02	33.60	42.80	0.83	2.69	2.07
Microbein + Phosphorein	0.73	2.20	0.71	1.41	7.15	33.29	42.21	2.22	4.97	2.45
Mi. + Ph. + (FYM) 10 m^3/fed.	0.67	2.13	0.65	0.75	6.69	30.25	44.30	3.82	4.72	2.30
Mi. + Ph. + (FYM) 20 m^3/fed.	0.77	2.26	0.18	0.79	6.67	28.45	44.40	2.75	4.33	2.18
Mi. + Ph. + (FYM) 30 m^3/fed.	1.14	0.22	0.04	0.70	7.50	31.36	40.77	1.60	4.06	2.37
Yeast + Phosphorein	1.36	0.40	1.09	1.03	5.55	35.70	36.83	2.03	2.60	5.01
Yeast + Ph. + (FYM) 10 m^3/fed.	0.82	0.23	2.26	0.50	1.05	27.50	45.63	1.21	3.55	1.67
Yeast + Ph. + (FYM) 20 m^3/fed.	0.85	2.03	0.18	0.55	7.13	28.23	45.00	1.09	3.58	2.47
Yeast + Ph. + (FYM) 30 m^3/fed.	0.28	0.42	0.67	1.74	3.70	24.22	48.51	0.98	4.00	2.36
Humic acid + Phosphorein	0.68	1.76	0.16	0.17	5.92	34.81	41.18	3.50	4.19	2.16
Hu. acid + Ph. + (FYM) 10 m^3/fed.	0.81	1.97	0.32	0.79	7.92	28.85	41.34	1.34	4.92	2.61
Hu. acid + Ph. + (FYM) 20 m^3/fed.	0.77	2.19	0.21	0.79	7.29	29.53	46.02	0.94	3.22	2.25
Hu. acid + Ph. + (FYM) 30 m^3/fed.	4.33	5.08	0.81	1.24	5.77	22.71	27.98	2.47	21.80	2.71

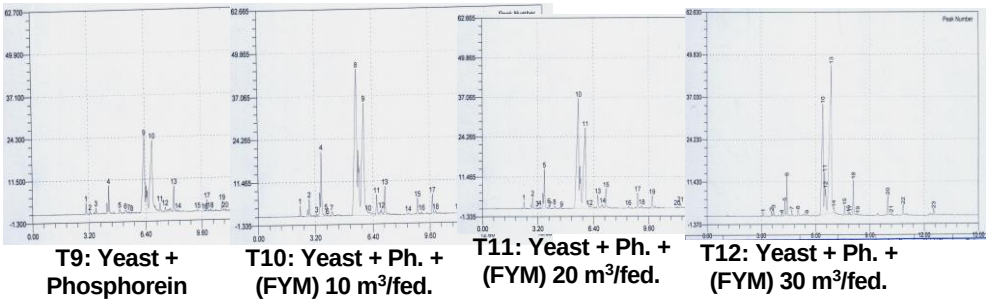
Farmyard manure = FYM, Phosphorein = Ph., Microbein = Mi., Humic acid = Hu., Feddan = Fed.



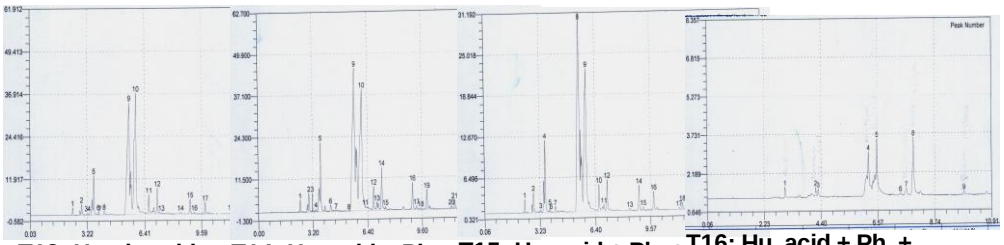
T1: Control(NPK) **T2: (FYM) 10 m³/fed.** **T3: (FYM) 20 m³/fed.** **T4: (FYM) 30 m³/fed.**



T5: Microbein + Phosphorein **T6: Mi. + Ph. + (FYM) 10 m³/fed.** **T7: Mi. + Ph. + (FYM) 20 m³/fed.** **T8: Mi. + Ph. + (FYM) 30 m³/fed.**



T9: Yeast + Phosphorein **T10: Yeast + Ph. + (FYM) 10 m³/fed.** **T11: Yeast + Ph. + (FYM) 20 m³/fed.** **T12: Yeast + Ph. + (FYM) 30 m³/fed.**



T13: Humic acid + Phosphorein **T14: Hu. acid + Ph. + (FYM) 10 m³/fed.** **T15: Hu. acid + Ph. + (FYM) 20 m³/fed.** **T16: Hu. acid + Ph. + (FYM) 30 m³/fed.**

Figure (1): Effect of FYM, Microbein, phosphorein, yeast, humic acid and their interactions on the essential oil components (%) of peppermint plants during 2012 season.

(1) α-pinene / (2) Sabinene / (3) β-pinene / (4) Limonene / (5) 1,8 cineole / (6) Menthol / (7) Iso-menthone / (8) Menthone / (9) Menthyl acetate / (10) β-caryophllene.

RECOMMENDATION

It can be recommended to fertilize *Mentha piperita* L. plants grown in a clay soil with interaction of (30 m^3 FYM/feddan added during preparing the soil, biofertilizers of phosphorein 400Gm/Fed which it is bacteria (*Bacillus megaterium*) dissolving phosphate, microbein which it is a mixture of (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Pseudomonas* sp., *Rhizobium* sp. and *Bacillus megatherium*) as a nitrogen fixing bacteria) at the rate of 30 ml/plant added one month after transplanting and repeated after each cut as soil drench to obtain the best growth and essential oil yield and reduce the pollution resulting from the expensive use of chemical fertilizers.

REFERENCES

Abd EL-Latif, S.H. (1987). Study on utilization of some food industries wastes in the production of single cell protein. M.Sc. Thesis, Fac. of Agric., Moshtohor, Zagazig Univ., Egypt.

Abd Elatif. T. A., Amal G. Salem and N. G. Ghaly (2000). Effect of chemical and bio-nitrogen fertilizers on chamomile plants (*Matricaria Chamomilla, L*). j. Agric. Sci. Mansoura Univ., 27(3):1757-1771.

Bunzen, J.; Guichard, N.; Labbe, J.; Prevot, P.; Sperpinet, J. and Trenchant, J. (1969). Practical Manual of Gas Chromatography. El-Seivier Pubi. Comp., Amsterdam.

EL-Araby, S.M. (2004). Effect of foliar application of yeast and boron on growth characteristics, yield potentials and yield quality of globe artichoke (*Cynara scolymus*, L.). J. Adv. Agric. Res., 9(1): 69-85.

El-Ghadban, E.A.; Ghallab, A. M. and Abdel-Wahab, A. F. (2003). Effect of organic fertilizer and bio-fertilization on growth, yield and chemical composition of marjoram plants under newly reclaimed soil conditions. J. Agric. Sci. Mansoura Univ., 28(9): 6957-6973.

El-Mahrouk, E. (2000). Using biofertilizers for production of the flowers and ornamental plants. Review Article, Fac. of Agric., Kafr El-Sheikh, Tanta Univ., Egypt.

El-Sanafawy, S. (2007). Effect of some fertilization treatments on *Ocimum basilicum* L. and *Origanum majorana* L. Ph. D. Thesis, Fac. of Agric., Kafr El-Sheikh Univ., Kafr El-Sheikh, Egypt.

Fortun, C.; A. Fortun and G. Almendros (1989). The effect of organic materials and their humified fractions on the formation and stabilization of soil aggregates. The science of the total environment. 81/82: 561-568.

Gomez, K. H. and Gomez, A. A. (1984). Statistical Procedures for Agriculture Research. John Willy and Sons, Inc., New York.

Hamed, E. S. (2004). Studies on planting of some medicinal plants in the desert.M. Sc. Thesis, Fac. of Agric., Kafr El-Sheikh, Tanta Univ., Egypt.

Hamza, A.M.; Hekmat Y. Massoud; Malaka E. Eid; M.R. Khater and Seham M.A. EL-Gamal (2007). Effect of farmyard manure (FYM) doses and different biofertilizers on vegetative growth, seed yield and active constituents of *Plantago ovata*, Forsk plants. J. Agric. Sci., Mansoura Univ., 32(7): 5583-5600.

Heikal, A. A. M. (2005). Effect of organic and bio-fertilization on the growth, oil production and composition of thyme (*Thymus vulgaris* L.) plants. M. Sc. Thesis, Fac. of Agric., Cairo Univ., Egypt.

Hoftman, E. (1967). Chromatography. Reinhold Publ. Corp., 2nd ed.: 208-515.

Idso, S. B.; Idso, K. E.; Garcia, R.L.; Kimball, B.A. and Hoober, J. K. (1995). Effect of atmospheric CO2 enrichment and foliar methanol application on net photosynthesis for sour orange trees (*Citrus auratium*). Amer. J. Botany, 82(1): 26-30.

Kruger, A. (1992). An Illustrated Guide to Herbs, Their Medicine and Magic. Dragonٰs World, London.

Kononova, M.M. (1966). Soil organic matter, its role in soil formation and in soil fertility. Pergamon press, Oxford.

Massoud, H. Y.A. (2007). Effect of mineral and bio-phosphate fertilization on the growth, essential oil productivity and chemical composition of marjoram plant. J. Agric. Sci. Mansoura Univ., 32(2): 1293-1308.
plant.J.Plant production, Mansoura university, Vol. 1(8):1113-1123,2010.

Massoud, H.Y.A.; M.Y.A. Abdalah; A.A.A. Mosa and E.A.E. Nour Eldeen (2010). Effect of water stress and foliar spray of humic acid on growth and essential oil quality of marjoram (*Majorana hortensis* Moench) plant. J. Plant Production, Mansoura Univ., 1(8): 1113-1123.

Moor, T.C. (1979). Biochemistry and Physiology of Plant Hormones. Pub. by Springer- Verlag. New York, USA.

Nagodawithana, W.T. (1991). Yeast Technology. Foods Corporation Milwaukee, Wisconsin. Published by Van Nostrand Reinhold, New York, USA. P: 273.

Naguib, N.Y. and M.Y. Khalil (2002). Studies on the effect of dry yeast thiamin and biotin on the growth and chemical constituents of black cumin (*Nigella sativa*, L.). Arab Univ. J. Agric. Sci., 10(3): 919-937.

Pioncelot, R.P. (1993). The use of a commercial organic biostimulant for bedding plant production. J. Sustainable Agriculture, 3: 99-110.

Russo, R. O. and Berlyn, G. P. (1990). The use of organic biostimulants to help low input sustainable agriculture. J. Sustainable Agriculture, 1 : 19-42.

Sakr, W. R. A. (2001). Effect of some organic and inorganic fertilizers on *Mentha piperita*. M. Sc. Thesis, Fac. of Agric., Cairo Univ., Egypt.

Salem, N. M. M. (1986). Agro-chemical aspects related to the use of conditioners and organic wastes in soils. Ph. D. Thesis, Fac. of Agric. Sci., Mansoura Univ., 28(4): 3215- 3226.

Sanders, D.; J. Ricotta and L. Hodges (1990). Improvement of carrot stands with plant biostimulant and fluid drilling. HortScience, 25: 181-183.

Shalan, M.N.: E.O.EL Ghawwas: M.M. Dessouky and S.G.I.Soliman (2001). Effect of sources and levels of phosphorus fertilization on polish chamomile (Matricaria chamomilla, L.). J. Agric. Sci. Mansoura Univ., 26(4):2215-2233.

Wild, S. A.; Corey, R. B.; Lyer, J. G. and Voigt, G. K. (1985). "Soil and Plant Analysis for Tree Culture": 93-106, 3rd ed. Oxford and IBM. Publishing Co., New Delhi.

تأثير بعض الأسمدة العضوية والحيوية على إنتاج وجودة الزيت على نبات النعناع الفلفلي

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أجريت هذه الدراسة خلال موسمين ٢٠١١ ، ٢٠١٢ فى مزرعة البرامون – محطة بحوث البساتين – المنصورة – محافظة الدقهلية لدراسة تأثير سماد عضوى الفارم يارد وحمض الهيوميك وبعض الأسمدة الحيوية مثل الخميرة الجافة والفوسفورين والميكروبين والتفاعلات بين هذه العناصر على نمو وإنتاج الزيت الطيار ومكوناته فى نبات النعناع الفلفلي.

تم معاملة عقل نباتات النعناع الفلفى بسماد الفارم يارد بتركيزات ١٠ ، ٢٠ ، ٣٠ م٣/الفدان فى معاملات منفصلة ثم سمدت نباتات أخرى بتفاعلات من الفوسفورين + الميكروبين مع كل من التركيزات الثلاثة للسماد العضوى ومعاملات أخرى سمدت بالفوسفورين + الخميرة مع كل من معدلات السماد العضوى الثلاثة ومعاملات ثالثة سمدت بالفوسفورين + حمض الهيوميك مع كل من معدلات السماد العضوى الثلاثة أيضاً فى موسمى الزراعة المتتابعين كما سمدت نباتات الكنترول بالجرعات الموصى بها من السماد الكيماوى لوزارة الزراعة بجمهورية مصر العربية.

وأظهرت النتائج تحسن وتفوق الصفات الخضرية المختلفة لنباتات النعناع الفلفلى عن نباتات الكنترول مثل إرتفاع النبات وعدد الأفرع الجانبية والوزن الطازج والجاف للنبات وللفدان والنسبة المئوية للزيت فى النبات وإنتاج المحصول للفدان ومكوناته نتيجة لكل المعاملات وكانت أفضل المعاملات هى الفوسفورين + الخميرة + فارم يارد ٣٠ م٣/الفدان بالتبادل مع الفوسفورين + حمض الهيوميك + فارم يارد ٣٠ م٣/الفدان على الصفات الخضرية تحت الدراسة وكانت الزيادات جميعها معنوية.

أظهر التحليل الكروماتوجرافى للزيت أنه يحتوى على عدد (٢٧) من المكونات الرئيسية تم التعرف على عدد (١٠) مركبات منها وكان مركب منثول هو الرئيسى على الإطلاق وقد أدت المعاملات المذكورة كلها إلى زيادات واضحة عن الكنترول.

وبذلك يمكن التوصية للحصول على محصول وافر من زيت النعناع الفلفلى ذو مواصفات أفضل بمعاملة النباتات بالفوسفورين + الميكروبين + فارم يارد ٣٠ م٣/الفدان ذات التأثير الكلى على إنتاج الزيت لنبات النعناع الفلفلى.

قام بتحكيم البحث

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Table (C): Chemical analysis of yeast extract according to Khedr and Farid (2000).

Minerals		Amino acids	Carbohydrates	Enzymes	Vitamins
Macro(g/100gdry weight)	Micro(mg/100g dry weight)	(mg/100g dry weight)	(mg/100g dry weight)	(mg/100g dryweight)	(mg/100g dry weight)
P2O5 7.23	Al 650.2	Arginine 1.99	Carbohydrate23.2	Cytochro	VitaminB1 2.23
K2O 51.68	B 175.6	Histidine 2.63	Glucose 13.33	me 0.35	VitaminB2 1.31
N 34.39	Co 67.8	Isoleucine 2.31		Oxidase	Riboflavin 4.96
	Pb 438.6	Leucine 3.09		0.29	Nicotinicacid 39.88
	Mn 81.3	Lysine 2.95			Panthothenicacid19.56
	Sn 223.9	Methionine 0.72			Biotin 0.09
	Zn 335.6	Phenylalanine 2.01			P-amino benzoic acid 9.23
	NaO 0.35	Thereonine 2.09			VitaminB6 1.25
	Mg 5.76	Tryptophan 0.45			Folicacid 4.36
	Cao 3.05	Valine 2.19			Thiamine 2.71
	SiO2 1.55	Glutamic acid 2.00			Pyridoxine 2.90
	SO2 0.49	Serine 1.59			VitaminB12 153(mg/100g)
	Cl 0.06	Aspartic acid 1.33			Inositol 203(mg/100g)
	FeO 0.92	Cystine 0.23			
	NaCl 0.30	Proline 1.53			
		Tyrosine 1.49			