Research Journal of Agriculture and Biological Sciences, 4(5): 566-574, 2008

Employment of Biotechnology in Recycling of Plant Wastes for Improving Plant Production under Siwa Conditions

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Abstract: In order to study the influence of different biofertilizers types (cellulose decomposing, N-fixing and P-dissolving bacteria) on the composting process of plant residues, weeds and grasses to enrich the compost with nutrients, eliminate plant pathogens and nematodes from resulted compost, overcome the problem of accumulation of plant residues, weeds and grasses with ecological and economical investments and reduce composting time. A field experiment was carried out during two successive seasons on spearmint *Mentha viridis* L at Tagzarti Farm, Siwa Oasis, Matruh, Desert Research Center, to study effect of organic matter type (without or sheep manure or produced compost) and biofertilization (*Azotobacter chroococum*, phosphate dissolving bacteria *Bacillus megatherium* and *Saccharomyces cervisiae* (Yeast)) on vegetative growth, herb yield, volatile oil content and its components. The soil microbial parameters were determined as total microbial counts, CO_2 evolution, azotobacters, phosphate dissolving bacteria and yeast counts. Results revealed that the application of compost as an organic matter with mixed treatment with the three biofertilizers used gave the maximum figures for the microbiological activities in plant rhizosphere, fresh and dry weights of the herb and volatile oil percentage and volatile oil yield compared with all other treatments. Treatments including phosphate dissolving bacteria showed increase in oil contents and oil yield whereas treatments received *Azotobacter chroococum* showed increase in fresh and dry weights thus the three tested biofertilizers showed a synergistic effect when mixed together.

Key words: recycling, plant wastes, biotechnology, Siwa, Egypt

INTRODUCTION

Composting is the natural process of rotting or decomposition of organic matter by microorganisms under controlled conditions. Compost is the completely decayed organic matter, dark, odorless, rich nutrients and is the end product of a complex feeding pattern involving hundreds of different organisms, including bacteria, fungi, worms and insects[6]. Also, compost is a rich source of organic matter which plays an important role in sustaining soil fertility and hence in sustainable agricultural production. In addition to being a source of plant nutrient, it improves the physico-chemical and biological properties of the soil. As a result of these improvements, the soil: (i) becomes more resistant to stresses such as drought, diseases and toxicity; (ii) helps the crop in improved uptake of plant nutrients; and (iii) possesses an active nutrient cycling capacity because of vigorous microbial activity. These advantages manifest themselves in reduced cropping risks, higher yields and lower outlays on inorganic fertilizers for farmers[25].

The recent introductions for rapid composting practices include shredding and frequent turning, mineral nitrogen compounds, effective microorganisms, use of worms, cellulolytic organisms, forced aeration and mechanical turnings which leads to reduce the composting period. Most of these methods include a high temperature period and this adds further value to the product by eliminating pathogens, nematodes and weed seeds[25]. The use of biocompost of organic wastes is considered to be a promising alternative way to mineral fertilizers as it reduces the amount of applied mineral fertilizers and at the same time improves the chemical and microbiological properties of desert under less polluted environment. From the economical point of view, such application reduces the agricultural costs, increase the yield of inoculated crops by providing them with an available nitrogen source and growth promoting substances[13,15,22].

Sinha *et al*.,[33] found that in the compost, the temperature reach 70-75 °C within 24-36 h, killing many harmful pathogens and repelling birds, stray animals, flies and mosquitoes from the dump site. El-

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Sharawy *et al*.,[19] studied the effect of the composts of some plant residues as rice straw and cotton stalks on some physical and chemical properties of the sandy soil. Application of these composts significantly improved the physical properties of the tested soil as bulk density hydraulic conductivity and moisture constants. Also, increase the available N, P and K in the cultivated soil, rice straw was better than cotton stalks.

Adedrain, *et al*.,[4] studied the influence of different rates of compost and inorganic fertilizers on maize. The compost and inorganic fertilizer application led to a fairly high population of *Azotobacter* (N-fixing bacteria) and thus its biomass, but the application of inorganic fertilizer caused a sharp reduction in microbial biomass in soil. Also the organic fertilizer improved aggregate stability index and permeability of the soil. On the whole, the positive effects obtained from both compost and inorganic fertilizer favor the recycling of wastes for sustainable crop production.

Mint plant has great importance among medicinal and aromatic plants. It is perennial herb belongs to family Lamiaceae (Labiateae). There are several species of mint such as peppermint Mentha pipertia L., spearmint Mentha viridis L. and Japanese mint Mentha arvensis L. Balbaa *et al*.,[8] mentioned that spearmint herb and its volatile oil are used as flowering agents for many kinds of food products and beverages, carminative, month preparations, gargles, tooth pastes and pharmaceuticals. Carvone is the main constituent (up to 70%)of spearmint.

- Therefore, the aim of this investigation is using bioinocula of highly active cellulolytic microorganisms with or without biofertilizers (*Azotobacter chrococcum,* P. dissolving bacteria and yeast) for the production of high quality compost from plant residue, fallen leaves, grasses and weeds used for increasing the production of mint cultivated under siwa soil conditions.

MATERIALS AND METHODS

Preparation of Compost:

Preparation of substrate: Substrates such as plant residues, weeds and grasses should be chopped. Chopping helps speed up decomposition by increasing the surface area available for microbial action and providing better aeration. The harder or wooden the tissues, the smaller they need to be decomposed rapidly. Woody material should be passed through a grinder.

All existed agricultural wastes were subjected to analysis before composting for C/N ratio which was as follow:

	N%	C/N
Grasses:	4	45
Plant residue:	0.5	90
Fallen leaves:	0.4	20

Sheep manure used as an initiative material had values: O.C: 19.46 %, T.N: 1.4 %, C/N:13.9, O.M: 33.5%, moisture 28.7 and PH 7.6

Composting Method: This method involves digging a pit (360cm long × 180 cm wide ×90 cm deep) in a shaded area (length can vary according to the volume of waste materials available). Farm wastes such as vegetable refuse, weeds, leaves and grasses are spread to a thickness of 15-20 cm. Wet animal dung is spread over this layer to a thickness of 5 cm. Water is sprinkled to moisten the material (50-60 percent of mass). This procedure is repeated until the whole mass reaches a height of 60 cm above ground. It is then covered with plastic sheet. And anaerobic decomposition commences. In four weeks, the mass becomes reduced and the heap flattens. The cover plastic is removed and the entire mass is turned. Aerobic decomposition commences at this stage. Water is sprinkled to keep the material moist. The compost is ready for use after four months Russell[31].

Compost Enrichment: Farm compost is poor in P content (0.4- 0.8 percent).Addition of P makes the compost more balanced, and supplies nutrient to micro-organisms for their multiplication and faster decomposition. The addition of P also reduces N losses[29]. Compost can be enriched by:

Application of Calcium ammonium nitrate (33.3 % N) and calcium super phosphate (15.5 % P_2O_5) were added in concentrations of 20 Kg / ton and 5 Kg / ton as sources of nitrogen and phosphorous, respectively. Calcium carbonate was added in concentration of 20 Kg / ton to neutralize the pH of compost.

- Addition of N-fixer (*Azotobacter chroococcum*) and P solubilizers (*Bacillus megatherium)*, one litre of bacterial suspension is added to 1 liter of molasses and 98 liter of water to obtain 100 litres of ready to use bacterial solution. The bacterial solution functioning as accelerator reduces the composting period from three months to one month.

Soil Inoculation Preparation: Fresh liquid culture medium of 48 hr old at 28 ± °C at the rate of ≈ 10^8 colony forming unit (c.f.u./ml) of pure local strains *Azotobacter chroococcum*, *Bacillus megatherium var. phosphaticum* and *Saccharomyces cervisiae* (Yeast), were used as biofertilizers. The strains were isolated from Egyptian desert soil, purified and identified

according to[9]). The selected isolate were analyzed for: 1- The ability to fix nitrogen was tested by modified Keldahl method after Chapman and Pratl[12].

The capacity of N-fixation was 84 ppm. 2- The ability of P. solubilization were tested qualitatively Nautiyal[26] recorded 3.7cm diameter of halo clear zone around bacterial colonies and quantitavly according to Jackson[23] recorded 7.58 mg P/L soluble phosphate .3- Total protein (23.19 mg/ml) and sum of free amino acids contents (3.87 mg/ml) as essential amino acid (mg/ml) Histidine 0.13, Hydroxylysine+Lysine 0.28, Iso leucine 0.14, Leucine 0.15, Methionine 0.05, Cystine 0.08, Phenyl alanine 0.09, Threonine 0.15, Triptophan 0.06, Valine 0.16 and non essential amino acids (mg/ml) Alanine 0.39, Glycine 0.31, Serine 0.27, Asparagine+ Aspartic acid 0.48, Glutamine+ Glutamic acid 0.62, Tyrosine 0.09, 4. Hydroxy praline+Proline 0.42 of yeast were determined according to method of Lowry *et al*[24] as explained by Pozo-Dengra *et al*[27].

Experiment: The present study was carried out during two successive seasons (2005-2006) at Siwa Experimental station, Matrouh Governorate, Egypt. *Mentha viridis L* rhizomes were obtained from Agriculture Research center, Giza and grown on March for two seasons.

- Biofertilization treatments were applied on planting day and after 30, 60 days from planting as described by Higa[20].
- Organic treatments included three treatments [1- without organic matter 2- with organic matter (sheep manure 20 m^3/feddan). 3- compost 20 m^3/feddan].
- Chemical N fertilizer as ammonium sulphate 60 kg/fed. divided into two portions added after 30 and 60 day after planting date.

The cuts were taken every season after 40, 80 and 160 days from planting date respectively. Plant growth parameters were recorded after every cut were (fresh and dry weights per plant, oil percentage, oil yield per feddan, oil constituents). The volatile oil of air dried leaves was extracted by water distillation for three hours, then dried over unhydrous sodium sulphate and determined according to British pharmacopeia[10]. Identification of volatile oil constituents were carried out using GLC. Mechanical, chemical, and microbiological analyses of the experimental soil were shown in Table (1). The statistical analysis was carried out according to Snedecor and Cochian[34].

Microbiological Determinations: Microbiological analysis of rhizospheric soil included the determination of total microbial counts by planting as described by Taha *et al.*,[36] and determination of *Azotobacter* counts on modified Ashby's medium after Abdel-Malek and Ishac[3]. Carbon dioxide evolution, as indication for microbial activities in rhizosphere, was periodically determined according to Alef and Nannipieri[5].

For counting and growing phosphate dissolving bacteria using Bunt and Rovira medium[11] after addition of 5 ml of sterile solution of 10% K_2HPO_4 following by addition of 10 ml of sterile solution of 10% $CaCl_2$ to each 100 ml of the medium[2]. Counting yeast on Yeast extract Malt extract agar medium Pridham *et al.*[28].

RESULTS AND DISCUSSION

Analysis of Compost: The rapid decomposition can be detected by a pleasant odour, by the heat produced (visible in the form of water vapour given off during the turning of the pile), by the growth of white fungi on the decomposing organic material, by a reduction of volume, and by the materials changing colour to dark brown. As near completion, the temperature drops and finally little or no heat is produced. The compost is then ready to use. Table (2) showed the Physico-chemical and microbiological analysis of resulted compost. It is clear that all macro, micronutrients, heavy metals and organic matter are in the accepted ranges. Both N content and C/N ratio are very close to the reported values by El-Sersawy *et al.*[16]. Microbial examination of obtained compost reveled the increase in numbers of beneficial microorganisms like azotobacters, phosphate dissolving bacteria, aerobic cellulose decomposers and total microbial counts, despite absence of pathogenic microorganisms and nematodes. These results are in compatible with Indira *et al.*[22] and Salem[32].

The quality of compost can be further improved by secondary inoculation of *Azotobacter chroococcum,* and *Bacillus megatherium* (P solubilizers). These microorganisms, can be sprinkled when the decomposing material is turned after one month. By this time, the temperature of the compost has also stabilized at about 35° C. As a result of this inoculation, the N content of compost can be increased by up to 2 percent. In addition to improving N content and the availability of other plant nutrients, these additions help to reduce the composting time considerably[21].

Effect of Organic Matter, Compost and Biofertilizers on Productivity of *Mentha viridis* L. (mint):

Soil Microbial Parameters:

Total Bacterial Counts: Data in Table (3) showed that total microbial counts were higher in the second cut than those recorded in either first or third cut. The type of organic fertilizer applied had a great effect on

Table 1: Mechanical, chemical and microbiological analyses of the experimental soil

Physical analysis		Chemical analysis		Microbiological analysis	
Sand %	75.9	PH	8.1	Total count $\times 10^5$	18
Clay %	10.5	E.C dS/m	6.3	*Azotobacter* count $\times 10^3$	6.2
Silt %	13.6	O.M %	1.12	PDB count$\times 10^2$	3.4
Texture	Loamy Sand	CaCO3 %	10.3	Yeast count$\times 10^2$	0.5

Table 2: Physico-chemical and microbiological analysis of compost:

Physico chemical		Analysis of Heavy metal (ppm)	
PH	7.5	Aluminum	<0.1
E.C (ds/m)	4.2	Boron	9.986
Total dissolved solid (mg/L)	3171	Cadmium	0.045
Nitrogen %	0.84	Cobalt	0.1
Phosphorous (ppm)	237.5	Chromium	<0.007
Potassium (ppm)	1600	Copper	2.351
C%	27.9	Iron	896.28
O.M%	48	Manganese	16.64
C/N	33.2	Molybdenum	0.3357
Calcium(ppm)	228.3	Nickel	0.5052
Sodium (ppm)	480	Lead	1.322
Bicarbonate (ppm)	456.5	Vanadium	0.8535
Sulphate (ppm)	1300	Zinc	28.18
Chloride (ppm)	603.9	Magnesium	147.2

Microbiological analysis	
Microbiological determination (C.F.U/g dry matter)	
Total microbial counts ×105	114
azotobacter count ×103	87
Phosphate dissolving bacteria (PDB)×102	32
Cellulose decomposers ×104	56

microbial behavior and community where compost gave higher values than organic matter or without organic matter. Obtained data generally showed that mixed treatment preferable than single treatments. Thus, the highest counts recorded with mixed treatment with compost application being 79 and 92 $\times 10^5$ cfu/ gm dry soilat second cut and second season of mint plant growth. The result in agreement with the statement of Abd El-Ghany[1].

Yeast counts: Results in Table (3) showed that yeast counts reached their maximum in the mixed treatment with compost application this was followed by mixed treatment with organic matter, Yeast treatment with compost and organic matter in decreasing order respectively Also, result recorded that, yeast counts were higher in the second cut than those recorded in either first or third cut. This trend was almost the same in both seasons. This results in compatible with the findings of[14].

CO_2 Evolution: The rate of CO_2 evolution was measured as a criterion of biological activity in rhizosphere, results in Table (3) showed that highest levels were recorded with mixed treatment with compost application to be about 2 folds as control. The second cut recorded highest values than first and third cut in both seasons Data of CO_2 evolution were almost in harmony with those of total microbial counts discussed before[37].

Phosphate Dissolving Bacteria Counts (PDB): It is obvious from the data recorded in Table (3) that the optimal densities of PDB were recorded in mixed treatment with compost application being 32.5 and 38 $\times 10^2$ cfu. /gm dry soil compared with control being 19.4 and 20.7×10^2 c.f.u. /gm dry soil at second cut and first and second season respectively. This results in accordance with El-Sersawy *et al.*[16], they observed that application of both compost and biofertilizers increased densities of phosphate dissolving bacterial counts, which confirm their importance in supplying the growing plant with available phosphorous via production of organic acids which have the ability to reduce the soil PH.

***Azotobacter* Counts:** The initial count of *A. chroococcum* in soil of Siwa was 4.1×10^4 cfu/g dry soil. Data recorded in Table 3 showed that, the count increased gradually in the first cut and in the second cut in the first season. The same trend was recorded in the second season. The counts under *A. chroococcum* inoculation showed the highest counts all over the experimental periods followed in descending order by Yeast inoculation while PDB (phosphate dissolving

Table 3: Effect of organic matter, compost and biofertilizers on Soil microbial parameters:

	Total microbial counts (× 10^5)																	
	1st season									2nd season								
	OMo			O.M			Compost			OMo			O.M			Compost		
	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
Cont	21	35	30	32	48	41	35	56	44	25	42	32	38	57	48	39	66	59
Az	33	49	38	42	65	57	49	73	62	36	51	38	48	68	61	51	85	71
PDB	28	45	35	39	61	52	42	68	59	33	48	36	45	66	58	47	79	66
Yeast	25	40	31	36	55	43	39	61	57	29	44	35	43	60	55	42	73	62
Mix	36	55	42	47	72	60	53	79	66	41	59	45	54	76	64	58	92	75
L.S.D at 5%	Cut: 0.905			appl.:0.905			Treat.:1.168			Cut: 0.9047			appl.:0.9048			Treat.:0.1.168		
	Yeast count (×10^2)																	
Cont	0.5	0.8	0.7	1.2	1.9	1.5	1.7	2.6	2.4	0.6	0.9	0.7	1.3	2.1	1.6	1.7	2.9	2.5
Az	0.9	1.3	1.1	1.5	2.7	2	2.1	3.2	2.8	0.9	1.4	1.1	1.6	3	2.1	2.3	3.6	2.9
PDB	0.7	1.0	0.9	1.3	2.4	1.9	1.8	3.1	2.6	0.7	1.2	0.9	1.3	2.7	2.2	1.9	3.3	2.8
Yeast	1.1	2.2	1.6	1.8	3.1	2.8	2.4	3.7	3.1	1.2	2.3	1.6	2	3.5	3.2	2.7	4.4	3.8
Mix	1.3	2.5	1.9	2	4.1	3.3	2.9	4.9	4.2	1.4	2.7	2.1	2.6	4.4	3.6	3.3	5.2	4.6
L.S.D at 5%	Cut: 0.1045			appl.:01044			Treat.:0.135			Cut: 0.0916			appl.:0.092			Treat.:0.183		
	CO_2 (mg CO_2/100 g dry soil/24 hr)																	
Cont	7.3	16.4	14.5	20.4	28.7	27.2	29	37	35.1	8.3	16.8	15	20.9	30.5	27.8	30.2	38.1	37.4
Az	16.3	27.5	22.4	30.3	41.9	39.4	37.8	52.6	49.8	17.2	29.1	22.7	31.6	43.8	41.5	38.3	54.6	49.2
PDB	15.6	25.1	20.5	29.6	40.1	37.8	36.5	50.2	38.7	16.4	28.7	22.1	31	42.7	40.4	37.2	51.9	47.1
Yeast	14.9	23.5	19.9	27.6	38	36.2	35.7	49.6	46.4	15.7	26.2	20.8	30.5	41.2	39.8	36	50.4	42.6
Mix	16.9	29.9	24.7	31.5	43.7	39.7	39.8	65	56.6	18.2	32.5	26.2	33.6	46.7	43.9	41.8	71	60.3
L.S.D at 5%	Cut: 0.436			appl.:0.4359			Treat.:0.563			Cut: 0.233			appl.:0.233			Treat.:0.301		
	PDB count (× 10^2)																	
Cont	5.1	12.6	9.9	7.6	15.9	13.2	9.5	19.4	17.5	5.3	12.2	10.3	8.3	16.4	13.3	9.8	20.7	17.7
Az	7.4	15.2	13	10.1	19.3	16.8	12.8	24.5	21.2	7.7	15.5	13.4	10.4	19.5	17.2	13.6	25.2	22
PDB	9.6	20.6	17.1	12.3	24	20.8	15.1	29.8	25.4	10.2	22.3	19.5	12.4	24.6	21.3	15.8	33.9	26.8
Yeast	7.8	15.8	13.2	10.7	20	17.6	13.9	25.3	22.6	8.1	16.3	13.8	11.4	21.2	18.5	14.5	27	23.1
Mix	11.5	23.5	19.8	13.6	27	23.1	15.9	32.5	26.7	1.9	24.8	21.2	15.4	30.2	25.9	16.4	38	27.3
L.S.D at 5%	Cut: 0.26		appl.:0.26		Treat.:0.33		Cut: 0.198		appl.:0.198		Treat.:0.26							
	Azotobacter count (× 10^4)																	
Cont	6.3	14.1	10.9	11.4	19.4	172	13	24	20.3	6.5	15.9	12.7	12.1	21.8	19.7	14	26	21.6
Az	11.2	25.3	21.8	17.5	28.4	26.3	19.4	35	31.2	12	26.8	22.4	19.2	31.5	28.1	218	36.4	32
PDB	7.1	20.6	15.1	16.3	25.1	22.8	18.2.	30.1	27	7.5	21	15.7	16.9	25.8	23	19	31	28
Yeast	6.7	19	13.6	14.5	24.6	21.5	16.8	29.5	26.5	7	19.5	14	15.2	25.1	22.6	17	30	26.8
Mix	12.5	26.2	23	19.4	31.8	29.3	21.3	39	33	13.8	28	24	20.1	33	29.7	21.8	41	35
L.S.D at 5%	Cut: 0.09872			appl.:0.09872			Treat.:0.127445			Cut: 0.102			appl.:0.102			Treat.:0.13171		

OMo: Without organic matter, O.M ; with organic matter (sheep manure), Az: *Aotobacter chroococcum*, PDB: phosphate dissolving bacteria *Bacillus megatherium*, Yeast; *Saccharomyces cervisiae*

Table 4: Effect of organic matter, compost and biofertilizers on Fresh weight and Dry weight (gm/plant):

Treatment	OMo gm/plant 1st cut	OMo gm/plant 2nd cut	OMo gm/plant 3rd cut	OMo T. gm/p	OMo T. Kg/f	O.M gm/plant 1st cut	O.M gm/plant 2nd cut	O.M gm/plant 3rd cut	O.M T. gm/p	O.M T. Kg/f	Compost gm/plant 1st cut	Compost gm/plant 2nd cut	Compost gm/plant 3rd cut	Compost T. gm/p	Compost T. Kg/f
Fresh Weight (1st season)															
Cont	28	51.2	45	124	3607	39	60	53	152	4394	42	69	62	173	5022
Az	35	58	54	147	4254	46	66	59	171	4966	51	75	71	197	5715
PDB	34	57	49	140	4045	43	64	56	163	4730	48	72	68	188	5458
Yeast	31	53	47	131	3802	42	61	55	158	4585	45	70	65	180	5222
Mix	37	62	56	155	4503	48	67	60	175	5074	52	78	73	203	5887
L.S.D at 5%:	Cut: 0.86982					appl.:0.86982					Treat.:1.22936				
Fresh Weight (2nd season)															
Cont	31	54	47	132	3827	40	63	55	158	4583	46	70	66	182	5278
Az	38	61	55	154	4466	47	69	61	177	5134	56	82	74	212	6148
PDB	37	58	53	148	4295	45	66	58	169	4901	55	79	71	205	5953
Yeast	33	56	48	137	3969	44	65	57	166	4816	53	77	68	198	5744
Mix	40	64	59	163	4732	52	72	64	188	5458	59	89	76	224	6496
L.S.D at 5%:	Cut: 0. 8492					appl.:0.84 92					Treat.:1 .0963				
Dry Weight (1st season)															
Cont	3.9	6.8	6.1	16.8	487.2	5.2	7.9	7.2	20.3	588	5.7	8.1	7.4	21.2	614
Az	4.7	8.2	7.6	20.5	294.5	6.1	9.2	8.6	23.9	693	6.8	10.1	9.6	26.5	768
PDB	4.4	8.1	7.3	19.8	5740	5.9	8.9	7.9	22.7	658.7	6.7	9.6	9.1	25.4	736
Yeast	4.1	7.8	6.9	18.8	545.2	5.7	8.6	7.6	21.9	635.1	6.2	9.5	8.8	24.5	711
Mix	5.3	8.6	7.9	21.8	632.2	6.5	9.5	8.7	24.7	716.3	7.2	10.6	9.8	27.6	799
L.S.D at 5%:	Cut: 0.1668					appl.:0.1668					Treat.:0.2154				
Dry Weight (2nd season)															
Cont	4.2	7.2	6.5	17.9	519	5.4	8.2	7.6	21.2	615	5.9	8.6	7.8	22.3	647
Az	5.1	8.8	8.1	22	638	6.3	9.5	8.9	24.7	717	7.1	10.9	10.2	28.2	818
PDB	4.8	8.5	7.9	21.2	615	6.2	9.3	8.7	24.2	710	6.9	10.5	9.8	27.2	789
Yeast	4.6	8.1	7.6	20.3	588	5.9	9.2	8.4	23.5	682	6.7	10.2	9.6	26.5	768
Mix	5.8	8.9	8.3	23	667	6.7	10.3	9.6	26.6	771	7.4	11.4	10.7	29.5	857

L.S.D at 5% : Cut:0.16257 appl.:0.16257 Treat.:0.209878T.gm/P: total gm/plant, T. Kg/f : total Kg/feddan

Table 5: Effect of organic matter, compost and biofertilizers on volatile oil percentage and volatile oil yield

	Oil Yield											
	1st season						2nd season					
	Oil yield%			Oil yield L/ feddan			Oil yield%			Oil yield L/ f		
	O.Mo	O.M	comp	O.Mo	O.M	comp	O.Mo	O.M	comp	O.Mo	O.M	comp
Cont	0.16	0.19	0.2	11.4	11.7	11.8	0.17	0.19	0.21	11.9	12	12.3
Az	0.19	0.21	0.22	11.6	12	12.5	0.2	0.22	0.23	12.1	12.4	14.6
PDB	0.21	0.22	0.23	13.4	12.7	13.9	0.22	0.24	0.26	13.8	15.2	15.9
Yeast	0.18	0.2	0.21	11.4	11.9	12.3	0.19	0.21	0.22	11.9	12.6	13.4
Mix	0.23	0.24	0.25	13.7	14.5	15.6	0.24	0.25	0.27	14.6	17.9	18.5
L.S.D	Treat.:0.01 Appl.: 0.0075			Treat.:0.38 Appl.: 0.292			Treat.:0.028 Appl.: 0.022			Treat.:0.365 Appl.: 0.29		

Table 6: Effect of biofertilization on *Mentha viridis* oil fractions (GLC analysis)

Oil	Control %	Az	PDB	Y	Mix
α-pinene	0.53	1.72	1.45	0.48	0.56
β-pinene	0.67	0.84	1.32	0.62	0.51
Phellandrene	25.37	10.34	6.2	21.5	4.018
Limonene	7.6	8.29	10.3	6.8	5.28
Menthone	3.48	5.14	5.42	4.35	3.2
Menthol	3.9	3.96	2.16	4.62	1.81
Comphene	0.36	0.46	0.13	0.00	0.03
Cintronellol	0.43	0.52	1.61	0.31	0.141
Cineole	0.61	0.94	1.15	0.86	0.311
Linalool	0.29	0.00	0.21	0.00	0.0
Myrcene	1.69	2.43	3.09	2.29	1.24
Pulegone	2.35	2.75	1.53	3.27	1.66
Carvone	52.4	62.61	65.4	54.9	81.24
Caryophyllene	0.34	0.00	0.03	0.00	0.00

bacteria) inoculation caused the least increment of *A. chroococcum* count. Also, mixed applications of *A. chroococcum* + PDB+ Yeast reported highest counts (41×10^2) in second season. The obtained results proved that N_2 fixers *A. chroococcum* enrich the soil by nitrogen fixation which increase soil fertility. The promoting effect due to application of *A. chroococcum* not only due to the nitrogen fixation but also to the production of plant growth promoting substances, production of amino acids, organic acids, vitamins and antimicrobial substances as well, which increase soil fertility, microbial community and plant growth[30].

Effect of Organic Matter, Compost and Biofertilizers on Productivity of *Mentha viridis* L. (mint):

Fresh Weight and Dry Weight (gm/plant): Concerning the effect of different organic fertilizers applied with single and mixed treatments with biofertilizers]*Azotobacter chroococcum*, *Bacillus megatherium* (PDB) and *Saccharomyces cervisiae* (Yeast)[on fresh weight of *Mentha viridis* L. (mint) plant. Data in Table (4) indicated that all organic fertilizers applied with biofertilization treatments influenced the fresh weight per plant and per feddan with highly significant difference compared with control. The maximum fresh weight being (78 and 89 gm/plant) and(5887 and 6496 kg/f) from applying compost with mixed biofertilization treatment in the second cut of first and second seasons, compared with control without organic matter application being (51.2 and 54 gm/ plant) and (124 and 132 Kg/f) respectively.

The same trend was observed in dry weight and the maximum dry weight being (10.6 and11.4gm/plant) and (799 and 857 Kg/f) from applying compost with mixed biofertilization treatment in the second cut of first and second seasons, compared with control without organic matter application being (6.8 and 7.2gm/ plant) and (487.2 and 517 Kg/f) respectively. It could be concluded that, the increase in fresh and dry weight of spearmint herb under applying compost with mixed biofertilization treatment may be due to increase the supply of mineral nutrients to the plant, biological control of plant pathogens, direct plant growth promotion by plant growth regulators production and improve soil characters (increase soil aggregates and water holding capacity) Salem[32].

Volatile Oil Percentage and Volatile Oil Yield: It was evident from Table (5) that the highest oil percentage resulted from applying compost with mixed biofertilization treatment which gave 0.25% in the first season and 0.27% in the second season. Meanwhile, the lowest volatile oil percentages were 0.16% in first season and 0.17% in the second season for control without organic matter application. The same trend was noticed for volatile oil yield as showed in Table (5), the highest oil yield were 15.6 and 18.5 L/f in the first and second seasons respectively for mixed treatment of biofertilizers with compost application while the least

oil yield was obtained from control without organic matter application being 11.4 and 11.9 L/f. Treatments with PDB gave a promotion power toward oil percentage and oil yield due to availability of liberated P. nutrient uptake from soil as a result of organic acids production from PDB. This is in agreement with findings of Attia and Abdel-Azeem[7].

Volatile Oil Constituents (GLC Analysis): Data in Table (6) indicated that Carvone is the main dominant fraction in oil of mint plants treated with mixed treatments compared with control one, followed by Phellandrene. Whereas, Caryophyllene was the lowest one. Different treatments affected the distribution of different oil fractions. These results were similar to those reported by[8] they mentioned that Carvone (mono-cyclic terpene Ketone) the principal constituents of spearmint *Mentha viridis L* up to 70%.

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