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An investigation of rhizobacteria as biofertilizer on *Mentha* L. compounds change

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ABSTRACT

The aim of current research was application of rhizobacteria as native biofertilizers and study of its effect on mint compounds change. Soil samples were collected from rhizosphere zone of Mentha L. and rhizobacteria were isolated on BHI agar, Nutrient agar and Soil extract agar media. The ability of isolated species to utilize nitrate, nitrite and phosphate solubility was assessed by spectrophotometer and Pikovskayas assay respectively and the best bacterial species were selected as biofertilizer. Pots of Mentha L. roots were inoculated by biofertilizer during 20 days at 75% humidity, 25°C temperature and 12 hours lighting and plant morphological was determined. Leaf and stem compounds change was evaluated by ethanolic extraction and GC/MS analysis. The best candidate bacterial species for use as biofertilizers were evaluated by 16S rRNA. 17 species with Bacillus genus as the most bacterial diversity were isolated and three strains were collected for further research. 11.7, 52.9 and 47% of strains had ability to use nitrite, nitrate and phosphorus solubility, respectively. The most size of leaf and internode distance was determined after treating the pots by strains 6 and 3 with average size 1.7 and 3.2cm, respectively. The results of synergetic effect of 3 strains were increased in leaf size and internodes distance, with average 1.8 and 4.4cm, respectively. Propene, Benzofuro benzopyran, Pentanoic acid, Decaborane, chloro ،Heptan1-nitr Methoxycarbonyl and Hepten-1-ol were determined as the significant compounds in leaf plant sample which were inoculated with strains 3, 6, 10 and combination of three strains. Molecular analysis determined Bacillus subtilis, Bacillus endophyticus and Bacillus thuringiensis as strains 3, 6 and 10 respectively.

Keywords: biofertilizer, *Mentha L.*, rhizobacteria, ethanolic extraction

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INTRODUCTION

Biofertilizer is a kind of manure with a specific of large beneficial microorganisms population which ability to enhance the productivity by fixing nitrogenous, solubilising soil phosphorus and synthesis of growth promoting substances such as vitamin and hormones [1].

Many researches showed that the higher population of beneficial microorganism in soil could increase nutrient retention. This led to germination up to 20 percent, yield from 10 to 40 percent, increase the availability and up take of nitrogenous and phosphorus in plants, improve the status of soil fertility maintain good soil health and crop

productivity, suppress harmful and pathogenic soil microorganisms. They are eco-friendly and non-polluting [2]. Nitrogen fixing bacteria, phosphate solubilizing and mobilizing microorganism and organic matter decomposer are the most familiar of biofertilizers groups [3]. The common microorganisms which use as microbial inoculants (biofertilizer) can be divided in two groups, containing symbiotic system such as *Rhizobium* spp., *Frankia* spp. and *Azolla* spp. and non symbiotic system such as *Azotobacter* spp., *Azospirillum* spp. and blue green algae [4].

Many of growth promoting substances are produced by rhizobacteria that are abundant with the ranging form 10^8 to 10^9 per gram in rhizosphere zone. This zone is surrounding the plants root and including complex relations between plant, soil microorganisms and the soil itself [5]. Microbial interactions in the rhizosphere of plants such as syntrophic associations markedly enhance plant growth directly and indirectly through the production of phytohormones, bio-control agents and nitrogen fixation [6].

Pseudomonas spp., *Arthrobacter* spp., *Agrobacterium* spp., *Alcaligenes* spp., *Azotobacter* spp., *Mycobacterium* spp., *Flavobacterium* spp., *Cellulomonas* spp., *Micrococcus* spp. and others have been reported as to be either abundant or sparse in the rhizosphere [7].

Peppermint and spearmint are the most important sources of mint oil contain over 200 chemical compounds including flavonoids, tannins, menthol and menthone which apply in food (as a flavoring in candy, gum, ice cream, syrups and etc), pharmaceutical (as an antiseptic, stimulant, externally for headaches, rheumatism, neuralgia, vomiting, gastritis, cholera, diarrhea, flatulence) and hygiene (as flavor in toothpaste, dental creams, mouth washes, cough drops, soap, household sprays) industries [8].

According to apply the mint composition in different industries, the goal of current research was to use native rhizobacteria as bio-fertilizer for growing of *Mentha. L* and study of its morphology and compounds change during this process.

MATRIALS AND METHODS

Bacterial samples and culture condition:

Soil samples were collected from rhizosphere zone (depth 3-5cm) of *Mentha*. L. from agricultural land in Shahriar-Saleh abad located in south of Tehran for isolating bacteria. Serial dilution of soil samples were prepared in sterile distilled water from 10^{-1} to 10^{-9}. Diluted samples were culture on Soil extract agar medium (soil sample 100g, distilled water 900mL, bacteriological agar 20g), Nutrient agar (peptone 5 g, beef extract/yeast extract 3g, bacteriological agar 15g, NaCl 5g, distilled water 1000mL) and BHI agar (peptone 10g, beef heart infusion 10g, calf brain infusion 7.50g, disodium phosphate 2.5g, sodium chloride 5g, bacteriological agar 15g, distilled water 1000mL) by duplicated method and incubated at 30ºC for 24 hours. For better result to isolate rhizobacteria, Soil extract agar media with different quantities of soil, 20-45% were used [9, 10]. Isolated bacteria were evaluated based on microscopic, macroscopic and biochemical tests according to Bergey's Manual for Systematic Bacteriology [11].

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Bacterial inoculums culture

Pure soil bacteria colonies were cultured in Nutrient broth medium without peptone (beef extract/yeast extract 3g, NaCl 5g, distilled water 1000mL) and incubated at 30ºC in shaking incubator with 120 rpm for 24h. Bacterial cell density was adjusted on 0.8-1 at 600 nm (equal to 5×10^8 CFU/mL) by UV-VIS scanning spectrophotometer, UV 2101 pc, Shimadzu [12].

Phosphate dissolution ability

For screening phosphate dissolution ability of isolated strains, bacteria were cultured on Pikovskays's medium (Glucose 10g, $Ca_3(PO_4)_2$ 5g, $(NH4)_2SO_4$ 0.5g, NaCl 0.2g, $MgSO_4.7H_2O$ 0.1g, KCl 0.2g, yeast extract 0.5g, $MnSO_4.H_2O$ 0.002g, $FeSO_4.7H_2O$ 0.002g, H_2O 1000mL, pH 7.0) by streak culture method and incubated at 30ºC for 24h. Phosphate solubilizers could ability to produce clearing zones around the microbial colonies in medium [13].

Consumption of nitrogenous compounds

Nitrate consumption

Nutrient broth medium which replaced its peptone by sodium nitrate were cultured by 3-5% isolated bacteria inoculums and incubated at 30ºC for 24h. After incubating, each sample was centrifuged (Teppich Rot in 380) at 4000rpm for 10min. Optical density of supernatant was measured at 220nm and was compared with standard curve.

HCl 1N was used as blank sample. For preparation of calibration curve, Different concentrations of sodium nitrate salt (0-24mg/L) were prepared. 1% of HCl 1N, was added to each concentration, optical density of nitrate was measured at 220nm by UV spectophotometry and calibration curve was drawn [14].

Nitrite consumption

All steps were similar to determine nitrate consumption with the difference that sodium nitrite instead of sodium nitrate was used.

Plant roots preparation

Mint roots were washed three to four times with sterile distilled water for 5 to 10 minutes. Dip in 95% ethanol for 3 to 5 seconds and wash once again with sterile distilled water for 5 minutes [15]. Plant samples were maintained in isolated bacterial inoculum for 2h and transferred singly to sterile soil. Each pot was inoculated by 5% bacterium suspension per week. One pot was considered as control and inoculated by water. Combination of bacterial inoculululum was used to determine the synergism role. Pots were transferred to greenhouse room, growth profile and plant morphology were evaluated during 20 days at 25°C and 70% relative humidity with 12h exposure period.

***Mentha L.* extraction**

Leaves and roots of *Mentha L.* were washed, dried, chopped and extracted by 96% ethanol (1:10 w/v). The samples were maintained at 4°C for 24h and flittered by Watman filter paper No 1. Clear liquid was analyzed by GC/MS, Agilent USA, GC68 goN, Network GC system 5973, Hp5-MS [16].

Molecular identification of bacteria

Total DNAs of strains were extracted by the method of Cline *et al.* and DNA extraction kit (Metabion) [17]. The 16S rRNA gene of the isolate was amplified using universal primers with the following forward and reverse primers for bacteria [5'-AGAGTTTGATCCTGGCTCAG-3' (8F) and 5'-GACTACCAGGGTATCTAATC-3' (805R)]. The amplification was performed by initial denaturation at 94°C for 5 min followed by 35 cycles of 94 °C for 30 sec, 55 °C for 30 sec, 72 °C for 30 sec; and a final extension at 72 °C for 15 min [18].

RESULTS AND DISCUSSION

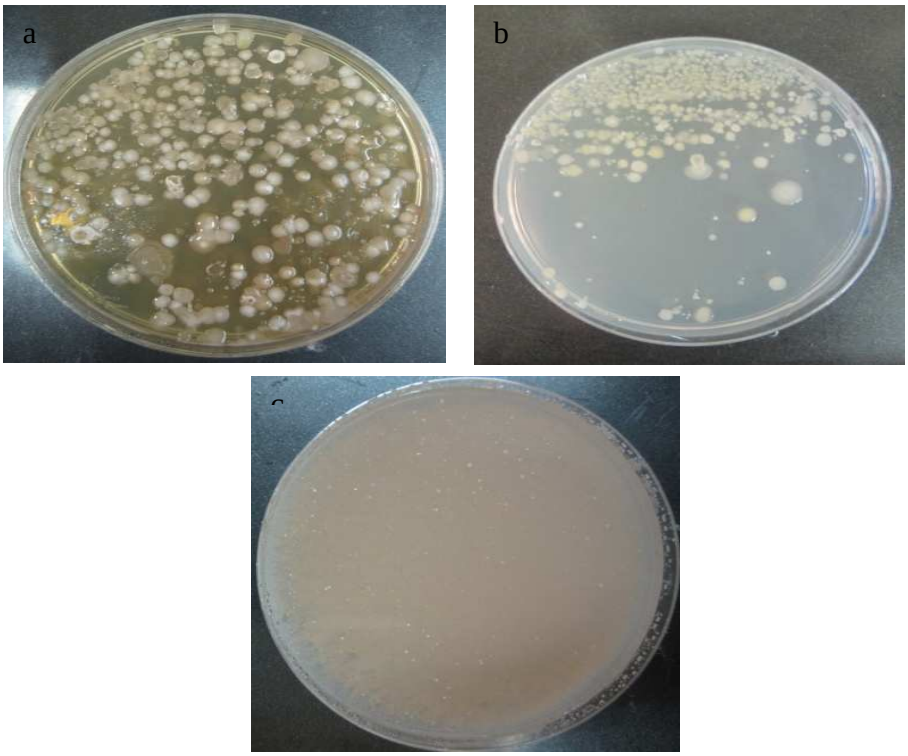

Figure1: Different media for isolating rhizobacteria, a) BHI agar medium, b) Nutrient agar medium, c) Soil extract Agar medium 10%

BHI agar was evaluated as the best medium with isolating 17 bacterial strains from rhizosphere of *Mentha L*. The lowest growth was observed on soil extract agar medium 10% (Fig1).

BHI agar is an enriched non-selective medium for the isolation and cultivation of most aerobic, anaerobic bacteria and other fastidious microorganisms. The basic nutritive properties are brain heart infusion from solids as well as meat peptones, with the addition of yeast extract [19].

Most isolated colonies were observed circular or rhizoid form with entire or undulated edges, rough surface, raised elevation and rod shape gram positive by macroscopic and microscopic analysis. Biochemical test results were shown in Table1.

Many researches such as Lawley *et al*. [20], Miller *et al*. [21], Yasuda and Katoh [22], Hasebe *et al*. [23], Nahas *et al*. [24], Kanazawa *et al*. [25], and da Silva and E. Nahas [26] have been revealed that gram positive bacteria as common flora in soil. Microbial populations in soils may be influenced by several factors such as drought, downpour and usage of chemical or biological fertilizer and etc.

Table1: Biochemical test results of bacterial strains

Tests / Strains	Indole production	MR	VP	Citrate test	H_2S production	Urea test	Starch hydrolyzes	Motility test	Gelatin hydrolyzes	Arabinose fermentation	Xylose fermentation	Catalase test	Oxidase test	Nitrate test	Mannose fermentation	Penicinn susceptibility test	Casein hydrolyzes	TSI
1	+	+	-	-	-	-	-	+	+	+	-	+	-	+	+	R	+	A/A
2	+	+	-	-	-	-	-	+	-	-	-	+	-	-	-	I	+	A/A
3	+	+	-	-	-	-	-	-	+	+	-	+	-	+	+	R	+	A/A
4	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	R	+	A/A
5	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	R	+	A/A
6	+	+	-	-	-	-	-	-	-	+	+	+	-	-	+	I	+	A/A
7	+	+	-	-	-	-	-	-	-	-	+	+	-	+	+	R	-	A/A
8	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	R	+	A/A
9	+	+	-	-	-	-	-	-	-	+	+	+	-	-	-	R	+	A/A
10	+	+	-	-	-	-	-	-	-	+	-	+	-	+	+	R	+	A/A
11	+	+	-	-	-	-	-	-	-	+	-	+	-	-	-	I	-	A/A
12	+	+	-	-	-	-	-	-	-	+	-	+	-	+	+	R	+	A/A
13	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	I	+	A/A
14	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	I	-	A/A
15	+	+	-	-	-	-	-	-	-	-	-	+	-	+	+	R	+	A/A
16	+	+	-	-	-	-	-	-	-	+	-	+	-	+	-	R	+	A/A
17	+	+	-	-	-	-	-	-	-	-	-	+	-	-	+	R	+	A/A

11.7 and 52.9% of isolates had ability to reduce nitrite and nitrate in medium, respectively. 47% of isolates were evaluated as phosphate solubilizing bacteria (Table2).

Some bacterial species have ability to solubilize inorganic phosphate compounds, such as tricalcium phosphate, dicalcium phosphate, hydroxyapatite, and rock phosphate by production of organic acids such as acid phosphatases which play a major role in the mineralization of organic phosphorus in soil [27].

Pseudomonas spp., *Bacillus* spp. and *Rhizobium* spp. are the most powerful phosphate solubilizers by producing organic acids such as lactic, isovaleric, isobutyric, acetic, glycolic, oxalic, malonic, and succinic acid. Chelating substances and inorganic acids such as sulphideric, nitric, and carbonic acid are considered as other mechanisms for phosphate solubilization [28].

According to both ability of consume nitrate and solublize phosphate (35.29%), three strains (3, 6 and 10) were collected as biofertilizer (Table2).

Table2: Bacterial isolation according to consume of nitrogenous and phosphorus compounds

Test results Sample	Reduction		Consumption(220nm)		Phosphorus solubility
	Nitrite	Nitrate	Nitrite	Nitrate	
Control	-	-	1.922	1.910	-
1	-	+	1.952	1.819	-
2	+	+	1.872	1.864	+
3	-	+	1.980	1.836	+
4	-	-	1.989	1.928	-
5	-	-	1.957	1.989	-
6	-	+	1.910	1.780	+
7	-	+	1.920	1.891	+
8	-	-	1.933	1.966	+
9	-	-	1.998	1.920	-
10	-	+	1.915	1.850	+
11	-	-	1.933	1.918	+
12	-	+	1.980	1.790	-
13	-	-	1.947	1.915	-
14	+	-	1.810	1.957	-
15	-	+	1.950	1.875	-
16	-	+	19.15	1.856	+
17	-	-	1.950	1.923	-

Maximum and minimum leaves size of *Mentha L.* obtained from the pots which inoculated by strain 6 and 10 respectively. The most inter-node distance was observed in the pot which inoculated by combination of three strains (Table3 and Fig 2).

The importance of bio-fertilizers effects on component traits like plant height, spike length, grain weight, flag leaf area and grains number per spike was reported previously [29]. Bacterial populations in biological fertilizers with different abilities such as fixing atmospheric N, production growth regulators hormones such as auxin, production different amino acids, various kinds of antibiotics, hydrogen cyanide and siderophore, could help to the growth and development of roots, shoots and improve the yield and quality by protecting the roots against soil-borne diseases [30].

Table 3: Morphological changes in *Mentha L.* inoculated with three strains as biofertilizer

Morphological changes **Bacterial strains as biofertilizer**	**Average leaf size (cm)**	**Average internode distance (cm)**	**Average wet weight (mg)**	
			Leaf	**Stem**
3	**1.3**	**3.2**	**200**	**100**
6	**1.7**	**2.8**	**70**	**4**
10	**0.9**	**2.4**	**192**	**121**
3+6+10	**1.8**	**4.4**	**410**	**220**

Figure2: *Mentha L.* inoculated by biofertilizers after 20-day period. a) strain 3, b) strain 6, c) strain 10 and d) combination of three strains.

GC/Mass analyses of *Mentha. L.* major products (leaves and stems), fertilization by isolated strains were shown in Table 4-7.

Table4: GC/Mass analyses of ethanolic extracts (leaf and stem of *Mentha L.*) fertilized by strain 3.

Products / Biofertilizer		Chemical Name	Formula	Purity (%)	Amount (%)	RT	Molecular weight(g/mol)
Strain3	leaf	1-Propene, 3,3'-oxybis-	$C_6H_{10}O$	61	0.453	5.0438	98.1430
		Molybdenum,bis[(1,2,3,4,5,6-u)-methylbenzene]-	$C_{14}H_{16}Mo$	40	0.102	7.2745	280.2201
		2,3-Bis[(trimethylsilyl)oxy]estra-1,3,5(10)-trien-17-one o-methyloxime -	$C_{25}H_{41}NO_3Si_2$	67	0.130	11.0967	459.76894
		● 2-(4,6-Bis(5-chloro-2-thienyl)-3-cyano-6-methyl-5,6-dihydro-2(1H)-pyridinylidene)malononitrile	$C_{18}H_{10}Cl_2N_4S_2$	68	0.136	12.8099	415.972382
		Piperonal	$C_8H_6O_3$	6.2	0.00374	17.3839	150.13
		5α-Cholestan-3-one dimethyl hydrazone	$C_{29}H_{52}N_2$	19	0.49	21.3943	428.413055
		Sumatriptan	$C_{14}H_{21}N_3O_2S$	28	0.140	24.2057	295.402
	stem	Naphthalene, 1,1'-(1,10-decanediyl)bis-	$C_{30}H_{34}$	32	0.261	17.3923	394.5910
		5,8,11-Heptadecatriynoic acid methyl ester	$C_{18}H_{24}O_2$	23	0.262	17.6604	272.38196
		Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	63	0.281	18.7859	428.10
		Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	55	0.242	19.6662	428.10
		Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	86	0.784	20.5037	428.10
		1,2-Dihydroindeno[1,2,3-cd]pyrene	$C_{22}H_{14}$	19	0.241	20.5183	278.109558
		1,2-Dihydroindeno[1,2,3-cd]pyrene	$C_{22}H_{14}$	33	0.220	20.9841	278.109558
		Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	41	0.243	21.4189	428.10
		Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	35	0.209	21.4254	428.10
		2-Phenanthrenol, 1,2,3,4,4a,4b,5,6,8a,9,10,10a-dodecahydro-4a,7-dimethyl-8-[3-cyano-3-(trimethylsilyloxy)propyl]-, acetate	$C_{25}H_{41}NO_3Si$	30	0.288	21.8758	431.68344
		Naphthalene, 1,1'-(1,10-decanediyl)bis-	$C_{30}H_{34}$	32	0.261	17.3923	394.5910

Table5: GC/Mass analyses of ethanolic extracts (leaf and stem of *Mentha L.*) fertilized by strain 6

Products / Biofertilizer		Chemical Name	Formula	Purity (%)	Amount(%)	RT	Molecular weight(g/mol)
Strain6	leaf	(1*S*,2*S*)-(+)-*trans*-1,2-Cyclopentanediol	$C_5H_8(OH)_2$	58	0.279	5.0479	102.13
		(1*S*,2*S*)-(+)-*trans*-1,2-Cyclopentanediol	$C_5H_8(OH)_2$	57	0.153	5.0577	102.13
		GLYCINE BENZYL ESTER	$C_9H_{11}N_1O_2$	31	0.129	5.9397	201.65
		Glycidamide,3-phenyl-, trans-	$C_9H_9NO_2$	39	0.152	6.2721	203.23712
		1-Hexene, 3,4,5-trimethyl-	C9H18	24	0.0185	7.9544	126.24192
		Nadolol di-methylboronic acid	$C_{19}H_{29}B_2NO_4$	6.1	0.00784	10.3316	357.06
		Bicyclo[2.2.2]oct-5-en-2-one, 7-syn-hydroxy-	$C_8H_{10}O_2$	61	0.148	10.7677	138.068085
		1-Propanamine,3-dibenzo[b,e]thiepin-11(6H)-ylidene-N,N-dimethyl-	$C_{19}H_{21}NS$	10	0.00905	13.5137	295.47
		Phenol,4-[2-(dimethylamino)ethyl]-	$C_{10}H_{15}NO$	76	0.355	16.2991	201.69
		Disiloxane, hexamethyl-	$C_6H_{18}OSi_2$	3.8	0.00423	17.3892	162.3775
		Ethinamate	$C_9H_{13}NO_2$	36	0.0761	18.4339	167.205
		7-(3-chloro-2-hydroxypropyl)guanine	$C_8H_{10}ClN_5O_2$	29	0.194	21.3430	243.052307
		Decaborane, chloro-	$B_{10}ClH_{13}$	47	0.318	21.3550	156.666
		1H-Indene, 2,3-dihydro-1,4,7-trimethyl-	$C_{12}H_{16}$	40	0.307	21.3663	160.2554
	stem	2,5-DIMETHYL-1-HEXENE	C_8H_{16}	59	0.325	5.2016	112.21
		3-Ethyl-5-(2-ethylbutyl)octadecane -	$C_{26}H_{54}$	34	0.185	5.2553	366.70696
		2,2-Dimethyl-3-hydroxypropionaldehyde	$C_5H_{10}O_2$	31	0.253	5.4999	102.1317
		1,3-Cyclopentadiene, 5-(1-methylethylidene)-	C_8H_{10}	30	0.0890	6.2926	106.1650
		3-ethyl-3-methyldiaziridine	$C_4H_{10}N_2$	24	0.100	6.2976	86.084396
		7-Methyl-7H-dibenzo[b,g]carbazole	$C_{21}H_{15}N$	42	0.155	7.2828	281.120453
		3,5,5-TRIMETHYL-1-HEXENE	C_9H_{18}	21	0.0104	7.9737	126.24
		3-Benzylsulfanyl-3-fluoro-2-trifluoromethyl-acrylic acid methyl ester	$C_{12}H_{10}F_4O_2S$	44	0.0879	20.2398	294.033752
		N,N'-Bis[2-(1,3-benzothiazol-2-yl)propan-2-yl]terephthalamide	$C_{28}H_{26}N_4O_2S_2$	14	0.0898	22.2559	514.149719
		2,3,5,6,7,8,9,10-Octahydro-1-phenyl-5-(p-bromophenylimino)(1H)cyclohepta[e][1,4]thiazepine	$C_{22}H_{23}BrN_2S$	63	0.1345	23.7863	427.40042
		Azafrin	$C_{27}H_{38}O_4$	42	0.08985	24.4641	426.59
		Piperoxan	$C_{14}H_{19}NO_2$	0.27	0.000550	27.3326	233.31

Table6: GC/Mass analyses of ethanolic extracts (leaf and stem of *Mentha.L*) fertilized by strain 10

Products / Biofertilizer		Chemical Name	Formula	Purity (%)	Amount (%)	RT	Molecular weight(g/mol)
Strain10	leaf	Heptane,1-nitro-	$C_7H_{15}NO_2$	63	0.246	5.0743	100.2019
		3-Methylene-1-oxaspiro[3.5]nona-5,8-dien-7-one	$C_9H_8O_2$	41	0.121	6.2982	148.15862
		4-(Anisylideneamino)-cinnamic acid	$C_{17}H_{15}NO_3$	50	0.0873	7.2830	281.3059
		Benzeneacetic acid, α,3,4-tris[(trimethylsilyl)oxy]-, methyl ester	$C_{18}H_{34}O_5Si_3$	44	0.144	9.2074	414.171417
		Borazine, 2-methyl-	$CH_8B_3N_3$	47	0.0816	10.7856	80.50
		3,9.alfa.;14,15-Diepoxypregn-16-en-20-one, 3,11.alfa.,18-triacetoxy-Mo	$C_{27}H_{34}O_9$	56	0.067	11.1109	502.55346
		10-(Methoxycarbonyl)-N-acetylcolchinol	$C_{22}H_{25}NO_7$	77	0.199	12.8155	415.4364
		Molybdenum, dicarbonylbis(.eta.-4-2-methylenecycloheptanone)-	$C_{18}H_{26}MoO_{4\text{-}6}$	57	0.0979	14.3322	402.33664
		Piperonal	C_8H_6O3	3.5	0.00484	16.6369	150.13
		5,6-Dicarba-nido-decaborane(12)	$C_2H_{12}B_8$	31	0.149	21.3824	142.11
	stem	2-Propenoic acid,2-methyl-, undecyl ester	$C_{15}H_{28}O_2$	55	0.214	5.0476	240.38
		Tricyclo[2.2.1.0(2,6)]heptan-3-ol	$C_7H_{10}O$	26	0.0880	6.2926	110.073166
		Molybdenum,bis[(1,2,3,4,5,6-u)-methylbenzene]-	$C_{14}H_{16}Mo$	42	0.0866	7.2815	280.2201
		3,4,5-Trimethyl-1-hexene	C_9H_{18}	18	0.00801	7.9688	126.2392
		Estra-1,3,5(10)-trien-17-one, 2,3-bis[(trimethylsilyl)oxy]-	$C_{25}H_{41}NO_3Si_2$	42	0.0825	11.0943	459.76894
		Piperonal	$C_8H_6O_3$	11	0.00904	17.3855	150.13
		Decaborane, ethyl-	$C_2H_{18}B_{10}$	24	0.110	21.3795	150.30
		Prost-13-en-1-oic acid,9,11,15-trihydroxy-6-oxo-, (9α,11α,13E,15S)-	$C_{20}H_{34}O_6$	41	0.107	21.9214	370.48
		2,3,3,3-tetrafluoro-1-(trifluoromethyl)-1-propenyl]benzene	$C_{10}H_5F_7$	28	0.0822	24.0242	258.139
		4,5,6,7-Tetrachloro-2-(2,4,5-trimethyl-3-thienyl)-1,3-benzodioxole	$C_{14}H_{10}C_{l4}O_2S$	15	0.0854	24.2560	381.915558
		Azafrin	$C_{27}H_{38}O_4$	48	0.100	28.4809	426.59
		11-HENEICOSANONE	$C_{21}H_{42}O$	28	0.109	29.9904	310.56

Table7: GC/Mass analyses of ethanolic extracts (leaf and stem of *Mentha.L*) fertilized by strains 3+6+10

Products / Biofertilizer		Chemical Name	Formula	Purity (%)	Amount (%)	RT	Molecular weight(g/mol)
Strains 3+6+10	leaf	2-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	55	0.165	5.0305	282.180664
		(E)-hept-2-en-1-ol	$C_7H_{14}O$	58	0.572	5.0404	114.18778000
		2-Trifluoroacetoxydodecane	$C_{14}H_{25}F_3O_2$	66	0.210	5.0612	282.34231
		8-Methylenebicyclo[4.2.0]oct-4-en-3-one	$C_9H_{10}O$	53	0.267	6.3025	134.1751
		1-[2,4-Bis(trimethylsiloxy)phenyl]-2-[(4-trimethylsiloxy)phenyl]propan-1-one	$C_{24}H_{38}O_4Si_3$	42	0.149	7.2797	474.81262
		3,4,5-Trimethyl-1-hexene	C_9H_{18}	15	0.0116	7.9703	126.24
		Terbutaline, N-trifluoroacetyl-o,o,o-tris(trimethylsilyl)deriv.	$C_{23}H_{42}F_3NO_4Si_3$	44	0.220	9.1975	537.83559
		2-Propanone, 1,3-diphenyl-	$C_{15}H_{14}O$	14	0.0112	14.9508	210.2711
		2-Octynoic acid, methyl ester	$C_9H_{14}O_2$	74	0.250	16.3159	154.2063
		Phosphonoselenoicdifluoride	HF_2PSe	54	0.142	17.3802	149.894913
		Decaborane, ethyl-	$C_2H_{18}B_{10}$	49	0.442	21.3704	150.30
	stem	Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	63	0.281	18.7859	428.10
		Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	55	0.242	19.6662	428.10
		Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	86	0.784	20.5037	428.10
		7-Methyl-7H-dibenzo[b,g]carbazole	$C_{21}H_{15}N$	42	0.155	7.2828	281.120453
		3,5,5-TRIMETHYL-1-HEXENE	C_9H_{18}	21	0.0104	7.9737	126.24
		3-Benzylsulfanyl-3-fluoro-2-trifluoromethyl-acrylic acid methyl ester	$C_{12}H_{10}F_4O_2S$	44	0.0879	20.2398	294.033752
		N,N'-Bis[2-(1,3-benzothiazol-2-yl)propan-2-yl]terephthalamide	$C_{28}H_{26}N_4O_2S_2$	14	0.0898	22.2559	514.149719
		2,3,5,6,7,8,9,10-Octahydro-1-phenyl-5-(p-bromophenylimino)(1H)cyclohepta[e][1,4]thiazepine	$C_{22}H_{23}BrN_2S$	63	0.1345	23.7863	427.40042
		Naphthalene, 1,1'-(1,10-decanediyl)bis-	$C_{30}H_{34}$	32	0.261	17.3923	394.5910
		5,8,11-Heptadecatriynoic acid methyl ester	$C_{18}H_{24}O_2$	23	0.262	17.6604	272.38196
		Molybdenum, tetrakis[μ-(acetato-O:O')]di-, (Mo-Mo)	$C_8H_{12}Mo_2O_8$	63	0.281	18.7859	428.10

Octynoic acid and Trifluoroacetoxydodecane, were determined in the mint leaves in control sample as main active compounds by GC/MS analysis. The results of current research showed biofertilizers could change in the main plant compounds.

Propene and Benzofuro benzopyran, were determined in the mint leaves and Molybdenum and Phenanthrenol in stems extraction inoculated with strain 3 as the main active compounds by GC/MS analysis. According to studies Propene has a major role in preventing ageing and reduces the plant's essential oil is peppermint [31].

Also molybdenum acts as a cofactor of some enzymes in the body and lack of it, causes serious problems in body functions. Use of biofertilizer (strain 3) could increase this compound rather than control.

Pentanoic acid and Decaborane chloro, were determined in the mint leaves and Dimethyl-3hydroxypropionaldehyde in stems extraction inoculated with strain 6 as the main active compounds by GC/MS analysis.

Pentanoic is monounsaturated essential fatty acids with a significant role in body health. This fatty acid should be supplied through food or food supplements [32]. So use of some biofertilizers such as strain 6 can increased this supplement production in plant.

Heptan adhesive is used as a solvent in the extraction of natural oils and oil is used to index the material composition of the leaf samples 10 and combination of three strains.

Propenoic acid and Decaborane ethyl, were determined in the mint stems inoculated with strain 10 as the main active compounds by GC/MS analysis.
Propenoic acid has antibacterial and anti-fungal property effects. It uses in animal feed for controling of *Salmonella* spp. outbreaks in cattle in the warm seasons [33].

The results showed that biofertilizers could effect on compounds diversity in plant extractions. The highest compounds number was observed in leaves sample when strain 3 used as biofertilizer with 21 compounds compared control sample with 12 compounds. Whereas 15, 11 and 12 kind of different compounds were obtained after using strain 6, 10 and combination of them as biofertilizers, respectively.

Biochemical and 16S rRNA sequencing analysis of the selected strains were confirmed *Bacillus subtilis*, *Bacillus endophyticus* and *Bacillus thuringiensis* with genetic affinity 96.4, 98 and 95% respectively.

CONCLUSION

In conclusion, the results showed that rhizobacteria strains such as *Bacillus* genera can be candidate as native biofertilizers. These fertilizers are eco-friendly and can be used for certain proposes with increase or decrease compounds such as aroma, antimicrobial, enzymes cofactors or plant morphological changes.

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