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Influence of Biofertilizers on the Essential Oil Content and Constituents of *Dracocephalum moldavica* L.

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Abstract: In this study the effect of three levels of vermicompost (0, 15 or 30 % V/Pot), two levels of biophosphate (treated and untreated) and two levels of *azotobacter* (treated and untreated) on content and constituents of essential oil of *Dracocephalum moldavica* was investigated. The Results showed that the essential oil content of dragonhead (*Dracocephalum moldavica*) and its constituents were significantly affected by biofertilizer treatments. The highest essential oil content (0.74 %) obtained by the treatment of vermicompost (30 % V/Pot). Fifteen components identified in the oil of plants which fertilized by biofertilizers. The highest geranyl acetate content (61.1 %) of essential oil were obtained by the treatment of vermicompost (30 % v/Pot), while the highest geraniol content in essential oil (24.2 %) obtained by the treatment of vermicompost (15 % V/Pot) along with untreated biophosphate and highest geranial content of essenitial oil (18.2 %) obtained by the treatment of vermicompost (15 % V/Pot) along with untreated azotobacter. Vermicompost had a promoting influence on most of vegetative growth parameters and it has been led to make accumulation of essential oil, chemical constituents including total carbohydrate and photosynthetic pigments content.

Key words: *Azotobacter*, biophosphate, *Dracocephalum moldavica*, essential oil content, essential oil constituents, vermicompost.

Introduction

Moldavian balm (*Dracocephalum moldavica* L.) is a hardy annual plant, with aromatic, balm-scented, green foliage and belongs to family *Lamiaceae* [25]. The oil content and its composition showed great variation due to plant origin. In Rumania, the percentage of essential oil ranged from 0.2-0.62 [26]. However, in Hungary, Halasz-Zelnik, *et.al.*,[13] and Hornok, *et.al.*,[14] reported that the essential oil at flowering stage reached 0.741 and citral was the major component of the oil (30-45 %). In Finland, Dastmalchi, *et.al.*,[6] stated that the maximum percentage of oil was 0.62 % during the flowering stage and the oil contained 90 % of oxygenated acyclic monoterpenes, i.e., geraniol, geranial, neral, nerol and geranyl acetate. In Egypt, El-Gengaihi, *et.al.*,[9] found that the oil was composed of acyclic oxygenated monoterpenes which reached to 93 % of the oil. Moldavian balm is widely used in folk medicine as a painkiller and for the treatment of kidney problems [15,25]. Extracts of the plant are used against toothache and colds as a poultice against rheumatism [6,26]; also, this extract acts as stimulated evolution in female rats

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and rabbits as it is used as antitumor [4].

Biofertilizers are important not only for the reduction of quality of chemical fertilizers but also for getting better yield in sustainable agriculture. Application of biofertilizers and no use of chemical fertilizers are rapidly gaining favor. Vermicompost is a product of bio-degradation and stabilization of organic materials by interaction between earthworms and microorganisms [5]; it is a finely-divided, peat-like material, with high porosity, aeration, drainage, water holding capacity and microbial activity, which make it an excellent soil conditioner [2,7].

Vermicompost contains plant-growth regulating materials, such as humic acids [3,23] and plant growth regulators like auxins, gibberellins and cytokinins, which are responsible for increased plant growth and yield of many crops [3]. Phosphate Solubilizing Bacteria (PSB) are being used as biofertilizer since 1950s [20]. Release of phosphorus by PSB from insoluble and fixed/adsorbed forms is an important aspect regarding phosphorus availability in soils. Microbial biomass assimilates soluble P and prevents it from adsorption or fixation [18].

Phosphate solubilization is the result of combined effect of pH decrease and organic acids production [10]. Phosphorus biofertilizers could help increase the availability of accumulated phosphate, efficiency of biological nitrogen fixation and increase the availability of Fe, Zn etc, through production of plant growth promoting substances [19]. Bacteria belonging to the genus *Azotobacter* are displaying a considerable nitrogen-fixing potential. They are abundant in the soils rich with organic matter and are able to synthesize a number of compounds stimulating plant growth (vitamins, heteroauxins, and gibberellins) and inhibiting phytopathogenic fungi [16].

The main aim of this study is to study the effect of application of different levels of vermicompost, biophosphate and/or *azotobacter* on the essential oil content and composition of *Dracocephalum moldavica* plant.

Material and methods:

Dracocephalum moldavica is a native plant of Iran. The seeds of *D. moldavica* were provided from Zardband Pharmaceutical Company in Tehran, Iran. This experiment was done during growing season of 2009 at Tarbiat Modarres University, College of Agriculture, at Paykan shahr, situated in suburb of Tehran, Iran. The seeds were sown on the first week of April in 2009; the factors were vermicompost (0, 15 or 30 % V/Pot), Biophosphate (treated and untreated by *Pseudomonas striata*) and/or *Azotobacter* (treated and untreated by *Azotobacter chroococcum*).

Vermicompost was applied during soil preparation for sowing; *Azobacter* and/or biophosphate were applied by seed treatment directly before sowing. The experiment was factorial on basis of randomized complete blocks design with twelve treatments and four replicates.

Treatments used in the field experiments were identified as follows: T0 (control), V30 (30 % V/Pot Vermicompost), V15 (15 % V/Pot Vermicompost), A (treated by *Azotobacter*), B (treated by Biophosphate), AB (treated by *Azotobacter* + biophosphate), AV15 (treated by *Azotobacter* + 15 % V/Pot vermicompost), AV30 (Treated by *Azotobacter* + 30 % V/Pot vermicompost), BV15 (treated by Biophosphate + 15 % V/Pot vermicompost), BV30 (treated by Biophosphate + 30 % V/Pot vermicompost), ABV15 (treated by *Azotobacter* + biophosphate + 15 % V/Pot vermicompost), and ABV30 (treated by *Azotobacter* + biophosphate + 30 % V/Pot vermicompost).

The black plastic plant pots with 32 cm depth were kept in open room conditions and prepared for sowing Moldavian balm seeds. Seedlings were thinned to four plants per pot 10 days after emergence. Irrigation was regularly provided during the vegetative period and all agronomic management practices were performed as needed. Shoot biomass was harvested at full flowering stage. The essential oil from shade dried biomass was isolated. Chemical properties of soil and vermicompost used in this study are presented in table 1.

Table 1. Chemical properties of soil and vermicompost

Sample	K(ppm)	P(ppm)	N(%)	EC (ds/m)	pH
Soil	0.064	0.0196	0.03	1.97	7.6
Vermicompost	1.75	0.53	3.29	2	6.8

Isolation of the essential oil

Shade dried aerial parts of *D. moldavica* (75 g three times) were subjected to hydro distillation for 3h using a Clevenger-type apparatus to produce oil according to the method recommended by the European pharmacopoeia [26]. The oil samples were dried over anhydrous sodium sulphate and stored in sealed vials at low temperature until analysis.

Gas chromatography (GC) analysis

GC Analysis was performed using a Shimadzu GC-9A gas chromatograph equipped with a Ph-5 fused silica column (10 m × 0.1mm, film thickness 0.4 µm). Oven temperature was held at 80°C/min for 3 minuet and then programmed to 280°C at a rate of 80°C/min. injector and detector (FID) temperature were 280°C; helium was used as carrier gas with a linear velocity of 0.5 ml/ min. percentage were calculated by electronic integration of FDI peak areas without the use of response factor correction. 0.1 µl was injected into the oven.

Gas chromatography/ mass spectrometry analysis

GC-MS analyses were carried out on a Varian 3400 GC-MS system equipped with a DB-5 fused silica column (30m × 0.25 mm i.d); Oven temperature was 40°C to 240°C at a rate of 4°C, transfer line temperature 260°C, carrier gas helium with a linear velocity of 31.5 cm/s, split ratio 1/60, Ionization energy 70 eV; scan time 1s; mass range 40-300 amu

The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices with those of authentic compounds or with data published in the literature. Mass spectra from the literature were also compared.

Results

Essential oil content

Results showed that vermicompost and biophosphate has a significant effect on essential oil content (table 2). The highest amount of essential oil yield (0.7425 %) was obtained by a V30 (table 4). figure 1 show results of comparison between control and other treatments.

Table 2. Analysis of variance for essential oil content versus treatments

Source	df	MS
Azotobacter	1	0.000003^{ns}
Biophosphate	1	0.026136^{**}
Vermicompost	2	0.069100^{**}
Azotobacter × Biophosphate	1	0.000336^{ns}
Azotobacter × Vermicompost	2	0.004811^{ns}
Biophosphate × Vermicompost	2	0.000144^{ns}
Azotobacter × Biophosphate× Vermicompost	2	0.001011^{ns}
Error	24	0.003044
Total	35	

(**Significant at α=0.01, ^{ns} non significant)

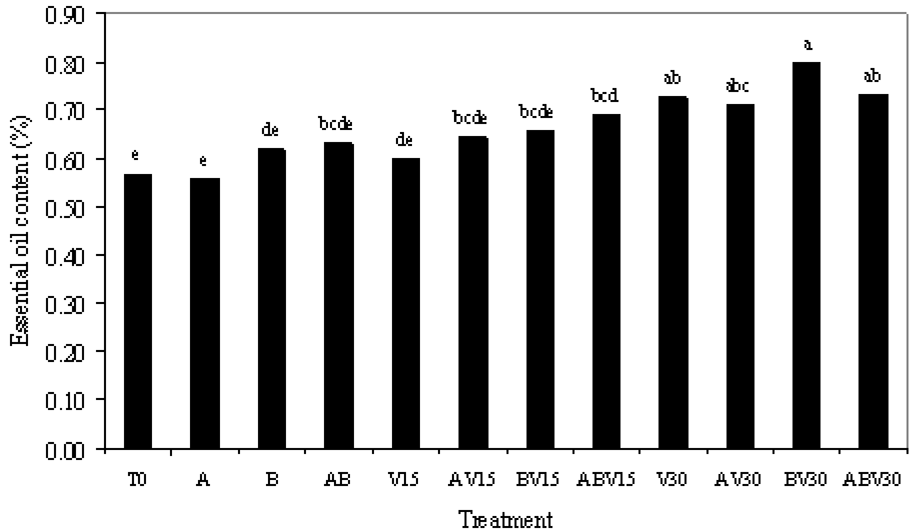

Figure 1. Influence of treatments on the essential oil content of *D. moldavica*
*Different letters on the top of each column indicate significant difference at P ≤ 0.05

Essential oil constituents

The effects of biofertilizer treatments on essential oil composition are presented in Table 3. Results showed that vermicompost, biophosphate (P≤0.01) and vermicompost × azotobacter (P≤0.05) has a significant effect on essential oil constituents. Fifteen compounds were identified in the essential oils of *D. muldavica*. As a result of GC and GC-MS analyses, *D. muldavica* contained sabinene, β-pinene, (Z)-β-ocimene, (E)-β-ocimene, linalool, *cis*-limonene ovide, *trans*-limonene ovide, *cis-chrysanthenol*, nerol, neral, geraniol, geranial, neryl acetate, geranyl acetate and β-bisabolene (Table 5). Results showed that the highest geranyl acetate content in essential oil (61.1 %) were obtained with V30. the highest geraniol content in essential oil (24.2 %) was obtained with V15× B0 and highest geranial content in essential oil (18.2%) was obtained with V15×A0 (table 4). Figure 2 show results of comparison between control and other treatments.

Table 3. Analysis of variance for essential oil constituents versus treatments

Source	**Geraniol**		**Geranial**		**Geranyl acetate**	
	MS	**df**	**MS**	**df**	**MS**	**df**
Azotobacter	d	-	2.0651*	1	4.875^{ns}	1
Biophosphate	23.849**	1	6.1796**	1	43.407**	1
Vermicompost	4.030^{ns}	2	9.7306**	2	24.197**	2
Azotobacter×Biophosphate	d	-	d	-	12.474*	1
Azotobacter×Vermicompost	d	-	1.8790*	2	d	-
Biophosphate×Vermicompost	8.122*	2	d	-	d	-
Biophosphate×Vermicompost *Azotobacter*×	d	-	d	-	d	-
Error	1.550	6	0.1382	5	2.028	6
Total		11		11		11

d: method of Danial 1959
(**Significant at α=0.01, ^{ns} non significant)

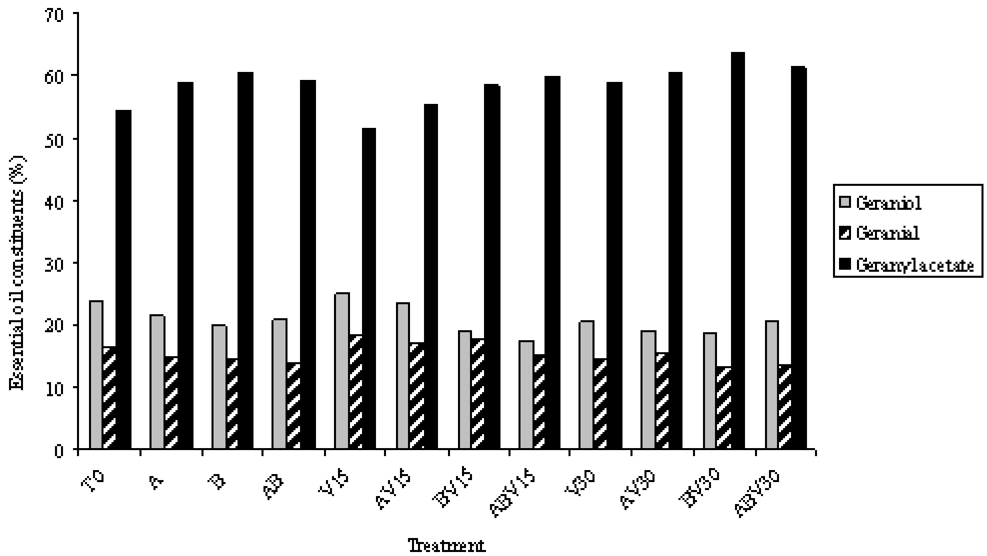

Figure 2. Influence of treatments on the essential oil constituents of *D. moldavica*

Table 4. Comparison of significant factors on essential oil content and constituents of *Dracocephalum moldavica*

Treatments	Essential oil content (%)	Geraniol (%)	Geranial (%)	Geranyl acetate (%)
A0	-	-	15.81 a	-
A1	-	-	14.98 b	-
B0	0.634 b	22.27 a	16.12 a	56.61 b
B1	0.688 a	19.64 b	14.68 b	60.41 a
V0	0.592 b	-	14.90 b	58.24 b
V15	0.647 b	-	17.15 a	56.20 b
V30	0.742 a	-	14.15 b	61.10 a
A0B0	-	-	-	54.95 b
A0B1	-	-	-	60.80 a
A1B0	-	-	-	58.27 a
A1B1	-	-	-	60.03 a
A0V0	-	-	15.47 b	-
A0V15	-	-	18.16 a	-
A0V30	-	-	13.82 b	-
A1V0	-	-	14.33 b	-
A1V15	-	-	16.13 b	-
A1V30	-	-	14.48 b	-
B0V0	-	22.75 b	-	-
B0V15	-	24.24 a	-	-
B0V30	-	19.83 b	-	-
B1V0	-	20.40 b	-	-
B1V15	-	18.36 b	-	-
B1V30	-	19.60 b	-	-

*Different letters on the top of each column indicate significant difference at $P \leq 0.05$

Table 5. Essential oil constituents (%) of *Dracocephalum moldavica* oil under different biofertilizers treatments

Constituents[a]	RI	Treatments											
		A	B	V15	V30	AB	AV15	AV30	BV15	BV30	ABV15	ABV30	T0
sabinene	977	0.0799	0.072	0.1609	0.0857	0.0748	0.0806	0.126	0.0549	0.0637	0.0931	0.0769	0.0763
β-pinene	985	0.3013	0.1278	0.0927	0.3339	0.1754	0.1423	0.1441	0.1808	0.1557	1.264	0.1457	0.2356
(Z)-β-ocimene	1041	0.1194	0	0.0684	0.1694	0	0.1288	0.0665	0.1049	0.0636	0.3903	0.0731	0.1199
(E)-β-ocimene	1056	0.2915	0.1335	0.183	0.4055	0.2056	0.1878	0.2302	0.1458	0.1776	0.7321	0.2232	0.3131
linalool	1109	0.6175	0.5813	0.67	0.4938	0.7231	0.5254	0.501	0.3136	0.4179	0.5041	0.464	0.6989
cis-limonene ovide	1154	0	0	0.1138	0	0.108	0.2455	0.1146	0	0	0.3182	0.0946	0.1176
trans-limonene ovide	1167	0.2137	0.1835	0.3576	0.3105	0.2411	0	0.4018	0.2494	0.3083	0.4508	0.2928	0.3274
cis-chrysanthenol	1180	0.2836	0.3212	0.6351	0.4369	0.3383	0.294	0.6564	0.3841	0.5055	0.9313	0.5004	0.5817
nerol	1228	0	0	0	0.5064	0	0	0	0	0	0.1718	0	0
neral	1245	0	0	0.132	0	0.0901	0	0	0	0	0	0.0982	0.1263
geraniol	1267	21.5795	19.9191	25.1701	20.6278	20.8829	23.308	19.0391	19.2022	18.6827	17.5265	20.5184	23.9244
geranial	1294	14.8376	14.4971	18.5507	14.37	13.8319	17.0539	15.4503	17.767	13.2611	15.2148	13.5144	16.4351
neryl acetate	1362	1.6506	2.4023	1.5938	2.4221	2.5376	2.2316	1.9455	2.3315	2.251	2.1509	2.2811	1.9744
geranyl acetate	1386	58.9958	60.4796	51.4332	58.9976	59.0386	55.1748	60.6318	58.3846	63.5253	59.8187	61.2392	54.4298
β-bisabolene	1500	0.634	0.8807	0.4726	0.1973	1.2122	0.1569	0.1301	0.5869	0.2182	0.3386	0.1783	0.3688

[a] Constituents Listed in Order of GC elution on DB-S column

Discussion

Results show that the vermicompost and biophosphate fertilizer increase the essential oil yield and constituents of *D. moldavica*. The Vermicompost treatments produced rather high and constant essential oil content. Applying vermicompost at 30 % pot volume caused an increase in essential oil content. This result may be due to the effect of vermicompost on accelerating metabolism reactions as well as stimulating enzymes. The increase in the essential oil yield might be due to either increase in vegetative growth or changes in leaf oil gland population and monoterpenes biosynthesis. Such findings were retrieved by many investigators such as Anwar, *et.al.*,[1] on French basil , El-Desuki, *et.al.*,[8] on sweet fennel, Hendawy,[12] on *Plantago arenaria*, Khalil, *et.al.*, [17] on *Tagetes erecta*, Liuc and Pank, [21] on roman chamomile, as well as Mona, *et.al*., [24] on fennel.

According to the obtained data, the Moldavian balm essential oil content is increased by application of biophosphate fertilizer. This behavior might be seen due to effects of biophosphate fertilizer on availability of phosphate and other beneficial microorganisms in the soil, which leads to better availability and uptake of macro and micro elements by plant [10]. Mamta, *et.al*.,[22] reported that treatment of Stevia with phosphate solubilizing bacteria resulted in increased stevioside and rebaudioside-A.

Concerning on obtained oil constituents results, reveals that, geranyl acetate is the major compound in treatments. Generally, it can be observed that, there is a positive correlation between total hydrocarbon compounds percentage and biofertilizers [27]. The inconsistent trend for the effect of various vermicompost levels on essential oil constituents of marjoram[5] plants and French basil is also reported [1,11]. As the results, it is concluded that, under Iran environmental condition, the application of vermicompost at level of 30 % pot volume with the biophosphate fertilizer to *D. moldavica* plants will lead to improving the productivity of this plants, due to its good growth, highest chemical constituents and more essential oil content.

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