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Original Article

Effects of Arbuscular Mycorrhizal (AM) Fungi on Essential Oil Content and Nutrients Uptake in Basil under Drought Stress

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Abstract

Study the effects of inoculation with two arbuscular mycorrhizal (AM) fungi, Gm (*Glomus mosseae* T.H. Nicolson & Gerd.)Gerd & Trappe) and Gi (*Glomus intraradices* N.C. Schenck & G.S. Sm.) on the herb yield, essential oil (EO) content and nutrient acquisition of basil (*Ocimum basilicum* L.) under drought stress conditions,The experiment conducted with 9 treatments and 4 replications. Drought stress treatments were applied by increasing the irrigation intervals from 4 days to 8 and 12 days. The root colonization, dry matter yield, oil content, oil yield and nutrients uptake decreased as the irrigation intervals increased. The AM fungi inoculation significantly increased the dry matter yield, oil content, oil yield and uptake of N, K, Zn, Fe and Cu as compared to Nm (non-mycorrhizal) plants in both well-watered and drought stressed condition. Analysis of essential oil by GC and GC/MS showed that Linalool, (E)-β-ocimene, eugenol and (Z, E)-farnesol, main components of oil, had no significant variation by drought stress or AM fungi inoculation. The effect of AM fungi inoculation on herb yield, oil content, oil yield and nutrient acquisition was more significant with *G. mosseae* than *G. intraradices*. Results suggest that inoculation of AM fungi could be a feasible procedure to increase growth, yield and essential oil production under water deficit conditions.

Key words: Arbuscular mycorrhiza, *Ocimum basilicum*, Root colonization, Essential oil

Introduction

Water stress is one of the most important environmental for growth and development in Mediterranean [1]. Arbuscular mycorrhiza symbiosis can protect host plants against its detrimental effects [2,3]. Several studies on the topic have demonstrated that contribution of AM symbiosis to plant drought tolerance results from a combination of physical, nutritional, physiological and cellular effects [3]. Often Mycorrhizal extension of the plant root surface facilitates potential uptake and translocation of P, N, K, Ca, S, Cu, Mo, and Zn [4-13].The AM symbiosis influences several aspects of plant physiology, such as plant rooting, closing of the nutrient cycles, nutrient acquisition, and plant protection [14]. Mycorrhizal improvement of drought tolerance occurs via drought avoidance, It can be a function of the often observed improved acquisition of phosphorus, nitrogen and other growth promoting nutrients by AMF plants [2]. The hyphae of (AM) fungi penetrate roots and grow extensively between and within living cortical cells, forming a very large and dynamic interface between symbionts [2]. Inoculation of plant roots with arbuscular mycorrhiza, *Glomus mosseae* or *Glomus etunicatum* W.N Becker & Gerd. improved growth, yield and nutrient uptake in wheat and in this experiment demonstrated the potential of mycorrhiza inoculation to reduce the effects of

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drought stress on wheat grown under field conditions [15].

The extra radical hyphae proliferate in the soil and provide the surface area for fungal uptake of phosphate [16] Copper [17] Zinc [18] or nitrogen [19]. During the establishment of the arbuscular mycorrhiza (AM) symbiosis, a range of chemical and biological parameters is affected in plants, including the pattern of secondary plant compounds [20]. However, little is known about their potential to enhance the productivity of aromatic plants. There have only been a few attempts to study the impact of VAM inoculation on the quantitative yield of essential oil in aromatic plants [21, 22]. Arbuscular mycorrhiza fungi are obligate symbiotic soil fungi that colonies the roots of the majority of plants and help to improve the performance of the plants in semi-arid conditions AM has importance due to its great capability to increase plant growth and yield under certain conditions. The major reason for this increase in the ability of plants in association with AM to uptake some nutrients such as phosphorus [23]. Basil (*Osimum basilicum*) is one of the important plants belonging to the Lamiaceae family includes at least 60 species and numerous varieties [10]. Tomato seedlings inoculated with *G. intraradices* had significantly higher uptake of N and P in both root and shoots regardless of intensities of drought stress [24].

It represents an important source of essential oil used in food, perfumery and cosmetics industries. Therefore an investigation was carried out to study the effects of inoculation of two species of VAM (Vesicular arbuscular mycorrhiza) fungi, *G. mosseae* and *G. intraradices* on some characteristic of basil plant under drought condition.

Material and Methods

This study was performed on a loamy sandy soil, collected from outside the township of Salmas (38º 11'N, 44º 46'E), West Azerbaijan province located in North-West of Iran. A greenhouse experiment was conducted during spring in the Iran at the department of horticulture, Urmia University. The experiment was randomized in factorial complete blocks with three drought stress level 4 (well water), 8 (mild water stress) and 12 (several water stress) days and three AM inoculums treatments (inoculation with *G. mosseae* (Gm), *G. intraradices* (Gi) and Control plants or non-mycorrhizal (NM),with four replications. Basil plants were grown in plastic pots containing a mixture of autoclaved (about 1.5 h in 121 temperature) loamy sand soil, AM inoculums, was obtained from institute of plant and soil (Iran) before sowing, a 40g of soil-based VAM fungal inoculums, consisting of root fragments and spores mixed with soil, was placed in a below the seeds. Some physio-chemical properties of soil used in this experiment are illustrated in table 1. Plants were irrigated according to need until the start of the drought treatment. Drought treatment was started 50 days after sowing. After three month growth, the plants were harvested. Plant shoots were washed thoroughly, dried at 70^{oc}, weighed and saved for mineral analysis. Nutrient concentration (N, K, Fe, Zn, Cu and Mn) were determined. Data were statistically analysis of variance with MSTATC program. Probabilities of significance were used to test for significance among treatments and interactions, and Dancans ($p<0.05$) were used to compare means.

Isolation and analysis of the essential oils

After three month growth (at full flowering stage), essential oil content was evaluated in aerial parts of host plants. For this purpose, 20 g were hydro-distilled in a Clevenger-type apparatus for 2 h and then percentage and yield of essential oils were calculated. The essential oils were dried over anhydrous sodium sulfate, stored in a dark glass vials and kept at 4 °C [25]. GC analysis was performed using an Ultra Fast Chromatograph (Thermo-UFM) equipped with a Ph-5 column (10 m × 0.1 mm, film thickness 0.4 _m). Oven temperature was kept at 60 °C for 3 min and then programmed to 285 °C at rate of 80 °C/min. Injector and detector (Fid) temperature were 280 °C and Helium (with 99.999% purity) was used as carrier gas with a linear velocity of 32 cm/s. Data were calculated by electronic integration of FID peak area without using of response correction factor. GC/MS analysis was also carried out on a Varian 3400GC/MS system equipped with a DB-1 fused silica column (60×0.25 mm, film thickness 0.25_m). Oven temperature program was 50 - 280 °C at a rate of 4 °C/ min, transfer line temperature 290 °C, carrier gas was Helium (with 99.999% purity) with a linear velocity of 31.5 cm/s, split ratio 1/60, Ionization energy 70 eV. 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 either with those of authentic compounds or with data published in the literatures [26, 27].

Table 1 The results of soil analysis

pH	EC	$CaCo_3$	O.c	Clay	Silt	Sand	Soil texture	N	K	Fe mg/kg	Zn
							Loam sandy				
	dS/m	(%)						(%)			
7.5	0.45	14.4	0.2	12	13	75		5	123	1.8	8

O.c: Organic carbon

Table 2 Effects of (*AM*) fungi and water-stress treatments on nutrient, dry yield, essential oil yield, Essential oil percent

Value sources	df	Means square								
		essential oil yield	essential oil percent	N	K	Dry yield	Fe	Z n	Cu	Mn
Replication	3	0.00 ^{ns}	0.01^{ns}	0.040^{ns}	0.16^{ns}	0.20^{ns}	885.28^{ns}	19.96^{ns}	29.85 ^{ns}	532.32^{ns}
Mycorrhiza	2	0.01**	0.33 **	0.38 **	13.64**	171.6**	9514.69**	245.00 **	133.58**	10554.25**
Irrigation	2	0.007 **	0.35**	0.23**	1.83 **	97.13**	4249.36 **	597.25**	340.58**	32496.75**
Mycorrhiza × Irrigation	4	0.001**	0.077**	0.08**	0.17 ^{ns}	8.21^{ns}	4669.94**	100.75 **	259.16**	12366.20**
Error	24	0.00	0.01	0.02	0.20	3.58	1090.99	20.06	14.89	648.19
Cv (%)		11.65%	13.66%	10.94%	13.63%	16.71%	9.17%	13.11%	11.03%	7.58%

ANOVA, NS = non significant, * = significant at 5% level, ** = significant at 1% level

Table 3 Effect of interaction between mycorrhiza and Irrigation on essential oil, and nutrient counteraction

Parameters Treatments	N (%)	Fe mg/kg	Mn mg/kg	Zn mg/kg	Cu mg/kg	Essential oil percent (%)	Essential oil yield (ml pot-1)
Control ×well water(ww)	1.118 cd	325.3 c	529.3 a	30.5 c	40.75 b	0.6200 c	0.01850 e
G. intraradices× (ww)	1.51 ab	412.5 b	482 b	31c	46.75 a	0.6425 c	0.02725 e
G. mossea × (ww)	1.695 a	479 a	541 a	65.25 a	30.75 c	0.6750 c	0.03425 de
Control ×mild (ws)	1.075 d	310.5 c	367.5 c	22.5 d	31c	0.6775 c	0.02475 e
G. intraradices× mild (ws)	1.462 b	385.3 b	252d	25 cd	38 b	0.8100 bc	0.05125 bc
*G. mossea*e× mild (ws)	1.31b c	426.3 b	260 d	45.5 b	40.5b	0.9425 b	0.1072 a
Control × several (ws)	1.092 cd	158.8 d	153 e	22.5 d	21.75d	0.9425 b	0.04275 cd
G.intraradices×several (ws)	1.1 cd	326 c	171 e	24 cd	27cd	1.160 a	0.06425 b
G.mosseae×several (ws)	1.3 bcd	400.3 b	266 d	41.25 b	38.5b	1.235 a	0.1112 a

*means in each column followed by the same letter are not significantly different at $p < 0.05$ according to Duncan's multiple range tests.

Results

Results showed that drought stress had significantly effects on, shoot dry yield, essential oil yield, essential oil percent, and nutrient concentration (N, K, Fe, Mn, Zn and Cu) in (P<0.01 or 0.05) (Table 2). Leaf nutrient concentration, essential oil yield and shoot dry yield declined under water stress condition in AM and non-mycorrhizal plants. Highest upon characteristics were achieved under non-stressed condition and in mycorrhizal plant inoculation. Also the results showed that mycorrhizal inoculation had significantly effects on shoot dry yield, essential oil yield, essential oil percent and nutrient concentrations (N, K, Fe, Mn, Zn and Cu) in P<0.01(Table 2). shoot dry yield, essential oil yield, essential oil percent and nutrient concentration (N, K, Fe, Mn, Zn and Cu) were significantly higher in most case in AM especially *G. mosseae* and well watered plants (Table3). Interaction between drought stress and mycorrhizal colonization was significant (P<0.05) for N, Fe, Mn, Zn, Cu, essential oil yield and essential oil percent. In addition were not significant for K content and shoot dry yield (Table 2). mycorrhizal structure was not found in non-mycorrhizal plants. After root staining, different fungal structures could be observed in host plant roots including extraradical hyphae, vesicles as well as Arbuscules (Fig. 1). Increased growth and development in AM plants compared to non-mycorrhizal ones, was reported for many different species [28]. There are significant differences in essential oil percent as well as essential oil yield between inoculated and

non-inoculated plants and among different fungal species (Table 3). Plants inoculated with Gm showed significant increase in essential oil percent and yield in comparison with other treatments. There was difference in the composition of essential oils in leaves among treatments (Table 4). Chemical analysis of essential oil showed that in *O.basilicum*, Linalool and Eugenol was the most abundant oil, followed by -(E)-ß-ocimen, (E), Z-farnesol, α-humulen1 and all the other oils in decreasing order. Concentration of Linalool increased by 10 and 8% on inoculation with G. mosseae and *G. intraradices* respectively as compared to control in non-stressed condition. Also In the mild stress condition (8 days) concentration of Linalool increased by 13 and 15% on inoculation with *G. mosseae* and *G. intraradices* respectively as compared to control (Table 4). The most effective treatment, with respect to improving the growth of basil, was the inoculation with a *G. mosseae*.

Discussion

The inoculation with AM fungi in this study was the most effective treatment for improving the growth of plants under both well-watered and water-stress condition, compared to control plants. In this sense, the mycorrhiza effect could be interpreted as an indirect response to improved nutrient status, particular of phosphorus presented in pervious paper.

Table 4 Relative abundance of the main compounds in leaf essential oils of *Ocimum basilicum* L. inoculated with different AM fungi

		Treatments								
Compound	RI	T1	T2	T3	T4	T5	T6	T7	T8	T9
ß-pinene	979	1.12	0.82	0.79	1.09	0.97	1.06	1.42	0.72	1.45
Miresen	990	0.32	0.19	0.19	0.19	-	0.19	0.34	-	-
1,8 -cineol	1035	0.99	0.69	0. 97	0.96	0.77	1.12	0.97	0.51	1.04
-(E)-ßocimen	1050	3.33	3.11	3.12	3.47	3.33	3.04	5.41	4.24	5.52
Lnalol	1101	65.92	73.71	75.50	61.93	75.65	73.29	71.11	63	70.58
Camphor	1142	0.04	0.06	0.18	-	0.08	0.17	-	-	0.90
Terpinen-4-ol	1177	0.73	0.50	0.57	0.93	0.55	0.74	0.68	0.73	0.80
α-terpineol	1192	0.15	0.11	0.04	0.13	0.09	0.10	0.18	-	0.14
γ-terpineol1	1199	0.18	0.14	0.26	0.22	0.16	0.29	0.27	-	-
Meth chavico	1200	1.91	0.71	1.23	1.56	0.68	1.42	1.71	0.73	1.06
Geraniol	1253	0.77	1.17	1.48	0.07	1.52	1.11	0.49	-	0.36
Bornyl acetate	1292	0.39	0.25	0.35	1.25	0.48	0.56	0.49	0.49	0.64
Eugenol	1362	5.88	4.65	3.32	3.47	3.14	2.55	2.92	2.82	3.28
Metyl eugenol	1408	0.92	0.11	0.05	1.10	0.07	0.10	0.62	0.61	0.23
E-caruphyllene	1420	0.13	0.14	0.16	0.13	0.12	0.19	0.14	-	0.18
α-trans-bergamotene	1432	1.18	1.36	1.5	1.68	1.37	1.86	1.11	1.09	1.67
α-humulen1	1458	0.02	2.46	1.90	3.80	2.69	3.24	3.71	3.41	3.72
Germacrene D	1485	0.88	0.67	0.74	0.66	0.63	0.84	0.60	0.54	0.82
Bicyclogermacrene	1500	0.08	0.06	0.06	0.15	0.06	0.09	0.07	-	-
α-bisabolene	1510	0.36	0.28	0.18	0.29	0.19	0.24	0.27	-	0.31
GermacreneA	1513	0.36	0.34	0.15	0.21	0.19	0.22	0.23	-	0.24
γ-cadinene	1517	0.45	0.75	0.05	0.15	0.10	0.12	0.16	-	0.14
trans-calamenene	1531	0.92	1.12	0.56	0.54	0.39	0.54	0.40	0.42	0.56
Spathulenol	1576	1.33	0.13	1	1.31	1.10	1.24	1.25	1.34	1.46
α-cadino	1654	0.15	0.15	0.13	0.12	0.12	0.14	0.12	-	0.17
-(E), Z-farnesol	1701	3.01	3.01	2.87	3.28	2.81	3.02	2.98	3.71	3.48

T1, T2, T3, T4, T5, T6, T7, T8, T9: well water (ww) and non-mycorrhizal (NM), ww and G. intraradices, ww and G. mosseae, mild (ws) and NM, mild (ws) and G. intraradices, mild (ws) and G. mosseae, several (ws) and NM, several (ws) and G. intraradices, several (ws) and G.mosseae respectively. RI: Retention

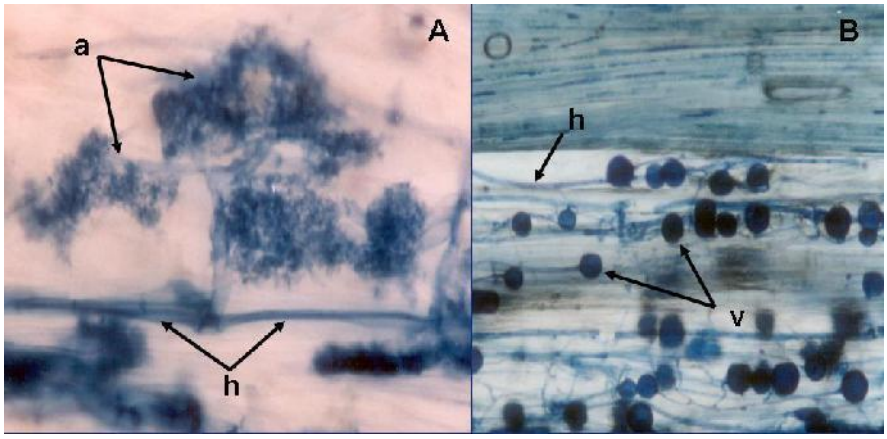

Fig. 1 Arbuscular mycorrhizal fungal structures in well watered plant roots. A. arbuscules (a) and fungal hyphae (h) in root cortex region of *O. basilicum* (100 ×). B. vesicles (v) and fungal hyphae in root cortex region of *O. basilicum* (40 ×)

Thus, inoculation of basil plant not only to enhance growth but also to improve the shoot dry yield, percent of essential oil and concentration of composition of essential oil. Essential oil (EO) is terpenoids based on C_5 subunits (isoprenoid). The biologically active isoprene derivatives are isopentenyl pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP). Biologically active isoprenoid requires acetyl-CoA, ATP and NADPH for synthesis. Hence, the biosynthesis of essential oil is dependent on inorganic phosphorus content in the plant [29]. The most well known benefit of AM to the host plant is increased absorption of mineral nutrients especially phosphorus [11]. Our result showed increased concentration of phosphorus in plants inoculated with mycorrhiza fungi compared with control plants. The increased yield in total essential oil in fungi-treated *O. basilicum* plants could of peltate glands, the structures responsible for oil production [30]. Three studies carried out on *mentha arvensis* L. indicated a relation between the presences of AM fungi, increased a growth, essential oil accumulation [22, 31, 32]. Similar results were observed about *corianderum sativum* L. [33]. Kapoor *et al*; have been studied three different plant species (*Anethum graveolens* L., *Trachyspermum ammi* L.Sprague, and *Foeniculum vulgare* Mill) and two fungal species (*Glomus macrocarpum* Tul. & C. Tul and *Glomus fasciculatum* (Thaxt.) Gerd. & Trappe) showing that both fungi increased plant growth, phosphate content, and the concentration of essential oils in the fruits [34, 35]. After 63 days, *O. basilicum* colonized by *Gigaspora rosea* BEG 9.

presented a larger number of gland in comparison with the other mycorrhiza treatments, suggesting that colonization by this fungus can stimulate the production of peltate gland [36]. They reported this greater number of glands may be related to alterations in the hormonal profile of the plants because increased levels of auxins, cytokinins, and gibberellins were recorded in AM plants [37-39]. The positive effects of AM on upon parameters were reduced by drought stress. The increasing of mineral nutrition might mainly be ascribed to a greater absorptive surface inside the plant, due to the effective absorptive area of roots by formation of an extra radical hypha network that enhances efficiency in absorption of nutrients [40, 41]. Mycorrhiza colonization in the ww plants resulted in the highest nutrient concentration in most cases. Leaf concentration of all macro-and micro-nutrients tested were reduced in the ws treatment compared to the ww. Nutrient concentration in the ws+M treatment were comparable to those in ww-M (except for Fe and Mn) interaction between irrigation and mycorrhiza colonization was significant ($P<0.05$) for K, Ca, Fe, Mn, Zn and not significant for N, P and Mg (Yield was positively correlated with all macro-and micro-nutrient in leaves [42]. It has been demonstrated that AMF increased p plant acquisition in several ways: direct acquisition through the hyphae, transference to the root, and by inducing changes in efficiency of p uptake through the plant root [43]. It has been accepted that mycorrhiza improve phosphorus acquisition by plant. Nitrate transport from roots to shoots is under the control of hormonal balance, and is promoted by high levels of cytokinins, which have been reported to increase under AM colonization [44]. Increased nitrate reductase activity only in root and shoots of *Juniperus oxycedrus* plants inoculated with the mixture of three exotic AM fungi, leading to much higher shoot N content than in plants neither inoculated plants under water stress condition [45]. In general, the inoculation of soil with *G. intraradices* had stronger effects on the physiology of the AM root plants in particular in glutamine synthetase, arginase and urease are two key enzymes in the transference of nitrogen from mycelium into plant root in Am symbiosis [46,47].

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