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ORIGINAL ARTICLE

Mycorrhiza effect on Yield and Component yield of *Nepeta pogonosperma* under Water Deficit Conditions

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ABSTRACT
*Nepeta pogonosperma was identified as a new species in 1984 that its essential oil have Anti-inflammatory effect. in order to investigation the effect of mycorrhiza on yield and component yield, essential oil percentage and essence yield of Nepeta pogonosperma, this experiment was conducted at Alborz research station, Research Institute of Forests and Rangelands, Karaj, Iran. The experiment was conducted in split plot in the form of a randomized complete block design with three replications. The main factor was water deficit in 3 levels (80-100% FC, 40-60% FC, 20-40%FC), sub factor was mycorrhiza in four levels that were non-use of mycorrhiza, inoculated with Glomus intraradices, inoculated with Glomus mosseae and inoculated with Glomus intraradices + Glomus mosseae. Analysis of variance showed that the effect of water deficit was significant on leaf yield, stem yield, flower yield, shoot yield, essential oil percentage and yield at P≤0.01. The effect of inoculation with mycorrhiza was significant on leaf yield, stem yield, flower yield and shoot yield at P≤0.01 and on essential oil percentage at P≤0.05. Mean comparison of interaction with two factors showed that the maximum stem yield (623.4 kg/ha), flower yield (233.7 kg/ha) and shoot yield (1558.5 kg/ha) were related to 80-100% FC * inoculated with Glomus intraradices + Glomus mosseae. The inoculated plants with symbiotic Mycorrhiza can achieved Optimal yield, also, symbiosis helps plants under stress conditions that have a good yield.*
Keywords: essential oil, symbiosis, Nepeta pogonosperma, medicinal plant, Iran

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INTRODUCTION

Plants are frequently subjected to different abiotic environmental stresses that determine geographic distribution and adversely affect growth, development, and agronomy yield. Drought is one of the major constraints on plant productivity in worldwide and is expected to increase with climatic changes [1, 2]. It is known as a major abiotic factor that limits plant's growth and production. Although the general effects of drought on plant growth are fairly well known, the primary effects of water deficit at the biochemical and molecular levels are not well understood [3]. Lebaschy and Sharifi Ashoorabadi [4] concluded that higher drought stress levels reduced shoot weight in some medicinal plants such as *Salvia officinalis* and *Achillea millefolium*. Ardakani *et al*. [5] reported that drought stress affected shoot yield, essential oil percentage and yield, leaf yield and stem yield in balm (*Melissa officinalis*). Water deficit (commonly known as drought) can be defined as the absence of adequate moisture necessary for normal plant grow and to complete the life cycle [6].

The symbiotic relationship between arbuscular mycorrhizal (AM) fungi and the roots of plants is widespread in nature, and several ecophysiological studies have demonstrated that AM symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance, as

demonstrated in a number of host plant and fungal species [7]. The alleviating effect of AM symbiosis in response to drought generally relies on the positive effects of AM fungi on the uptake and transport of water and on an improved uptake of nutrients, especially of available soil phosphorus (P) and other immobile mineral nutrients, resulting in the hydration of plant tissues, a sustainable physiology and a clear promotion of growth [8].
Arbuscular mycorrhizal symbiosis is formed by approximately 80% of the vascular plant species in all terrestrial biomes [9]. Arbuscular mycorrhizal fungi (AMF) are of great ecological importance, since arbuscular mycorrhizae is the most widespread plant symbiosis that often improves plant productivity [10]. The main advantage of mycorrhiza to the host plants is the extension of the penetration zone of the root fungus system. The interconnected networks of external hyphae act as an additional catchment and absorbing surface in the soil [11]. The increased efficiency of mycorrhizal roots versus non mycorrhizal roots is caused by the active uptake and transport of nutrients especially immobile minerals like P, Zn and Cu [12].
There are few reports on AM studies of family Lamiaceae including *Ajuga pyramidalis*[13], *Betonica officinale* [14], *Clinopodium gracile* [15], *Lavendula angustifolia* [16], *Thymus polytrichus* [17], *Ocimum basilicum* [18], *Salvia azurea* [19] and *Mentha species* [20, 21]. Mycorrhizal inoculation not only promoted the growth of medicinal plants but also improved the productivity and quantity of chemicals. Hence, there is an upcoming demand for research in improving the quality and quantity of drugs produced from native medicinal plants in relatively less time with application of AM fungi [22]. Many species belonging to the Lamiaceae, including sweet basil, form arbuscular mycorrhizas. In addition to increasing uptake of poorly available nutrients such as phosphorus and nitrogen [23] or conferring protection against pathogens [24]. Relatively little is known about the effects of AM colonization on the accumulation of active phytochemicals in shoots of medicinal plants, which are often the harvest products. However, it was reported that *Glomus mosseae* directly increases the essential oil content in shoots of *Origanum sp*. [25] as well as sweet basil [26].
Nepeta contains about 300 species, which are distributed in central and southern Europe, East, central and southern Asia, among them Iran is one of the centers of origin of this genus with 75 species and approximately 53% endemics which have been used as herbal remedies. Nepeta pogonosperma Jamzad & Assadi was identified as a new species in 1984 [27]. Anti-inflammatory effects of compounds of its essential oil have also been investigated in other studies [28, 29].
The aim of this work was to evaluate the influence of water stress and inoculation with *Glomus mosseae* and *Glomus intraradices* on yield and essential oil of *Nepeta pogonosperma*.

MATERIALS AND METHODS
In order to investigation the effect of water deficit and mycorrhiza, This project was performed in Research Institute of Forest and Rangelands, Karaj, Iran (Latitude: 35° 38´ N; Longitude: 51°E; Altitude: 1321 meter (m)) in 2015. The soil of the experimental region was loamy with pH 7.48 (Table 1).
The experiment was conducted in split plot in the form of a randomized complete block design with three replications. The main factor was water deficit in 3 levels (80-100% FC, 40-60% FC, 20-40%FC), sub factor was mycorrhiza in 4 levels that were non-use of mycorrhiza, inoculated with *Glomus intraradices,* inoculated with *Glomus mosseae* and inoculated with *Glomus intraradices* + *Glomus mosseae*.
The way to use drought stress treatments was weighing method with the help of TDR. In the field conditions for about 3 weeks after transplanting the seedling when seedlings were quite stable in terms of deployment on the ground, all plots were irrigated uniformly. 24 hours after irrigation, one sample of soil was taken from each plot at the root zone (from surface to a depth of 30 cm) and immediately weighed. To determine the dry weight of soil, samples were transferred to an oven at 105 °C for 24 hours [30]. Then the soil moisture percentage at field capacity condition was calculated using the Levitt formula [31]. The time of stress treatments use was from the start of stem elongation. Analyses of the statistics and data on the application components were done using SAS software and comparison of averages at 5% of probability level. Analyses of growth process data were done using Excel software.
Flowering shoots after harvested, transferred to the laboratory. Samples were dried at shade and room temperature, and then essential oil extraction was done by water distillation method and using a Clevenger for 2 h [32].

RESULTS AND DISCUSSION
Variance analysis indicated (Table 2) that water deficit significantly affected leaf yield, stem yield, flower yield, shoot yield, essential oil percent and yield ($\alpha \leq 0.01$).

There was significant difference between mycorrhiza inoculated treatments on leaf yield, stem yield, flower yield and shoot yield ($\alpha \leq 0.01$) and on essential oil percent ($\alpha \leq 0.05$). The effect of interaction between water deficit and mycorrhiza was different for stem yield, flower yield and shoot yield ($\alpha \leq 0.01$).
Means comparison showed that leaf yield (609.4kg/ha), stem yield (519.1 kg/ha), flower yield (186.9 kg/ha) and shoot yield (1315.5 kg/ha) was the highest in non stress treatment. The highest essential oil percentage (2.8%) was observed in severe water deficit. The maximum amount of essential oil yield was belonged to the medium stress with 14.3 kg/ha (Table 3).
According to the results of Table 4, Means comparison indicated that leaf yield had the highest amount in inoculation with *Glomus mosseae* + *Glomus intraradices* with 427.5 kg/ha. The inoculation with *Glomus mosseae* + *Glomus intraradices* had the maximum amount of stem yield (392.8 kg/ha), flower yield (141.7 kg/ha) and shoot yield (962 kg/ha). The highest percent of essential oil was found in non inoculation with 2.06%.
mean comparisons of interaction between water deficit and mycorrhiza showed that stem yield was the highest in non stress and inoculation with *Glomus mosseae* + *Glomus intraradices* with 623.4 kg/ha (Fig. 1).
The maximum amount of flower yield was belonged to the non stress * inoculation with *Glomus mosseae* +*Glomus intraradices* with 233.7 kg/ha (Fig. 2).
The highest shoot yield (1558.5 kg/ha) was observed in non stress * inoculation with *Glomus mosseae* + *Glomus intraradices* (Fig. 3).
In this study, reduction in plant growth and yield, with increasing levels of stress (Table 2) due to the fact that drought stress limits plant's growth and production [3] expected. All of the yield component such as stem, leaf, flower and shoot yield Were significantly reduced. This result, in Lebaschy and Sharifi Ashoorabadi [4] research that showed drought stress levels reduced shoot weight in Salvia officinalis and Achillea millefolium, Was also observed. Ardakani *et al*. [5] also reported that drought stress affected shoot yield, essential oil percentage and yield, leaf yield and stem yield in balm.
On the other, Arbuscular mycorrhizal symbiosis help to uptake and transport of water and on an improved uptake of nutrients, especially of available soil phosphorus [8] so that achieved the highest yield in treatment with inoculation with two species of mycorrhiza.
Looking at the results of interaction, when the water needed by plants is fully provided and Symbiosis with mycorrhiza also take place, Provides ideal conditions for plant, which will result in increased yield.
AM symbiosis is a key component in helping plants to cope with water stress and in increasing drought resistance [7]. It was found that mycorrhiza could help to improve yield in stress conditions. This result was also observed in other plants As in *Lavendula angustifolia* [16], *Thymus polytrichus* [17], *Ocimum basilicum* [18]. In total, symbiotic could dampen the damaging effect of stress and not let that happen yield loss.

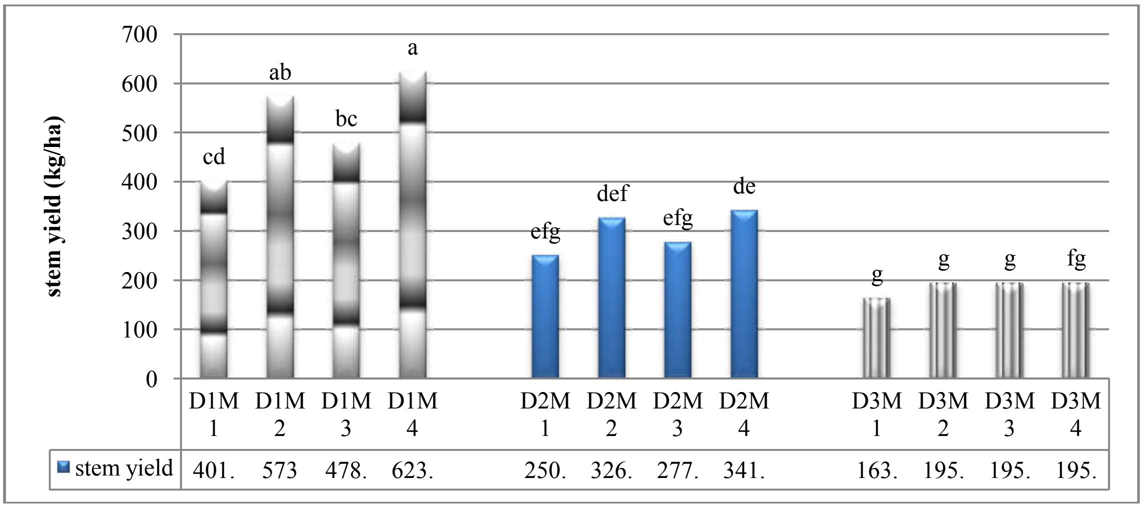

Figure 1: The interaction between water deficit and mycorrhiza on stem yield.
D, water deficit; D1, non stress; D2, medium stress; D3, sever stress
M, Mycorrhiza; M1, *Glomus intraradices*; M2, *Glomus mosseae*; M3, *G. intraradices+ G. Mossese*

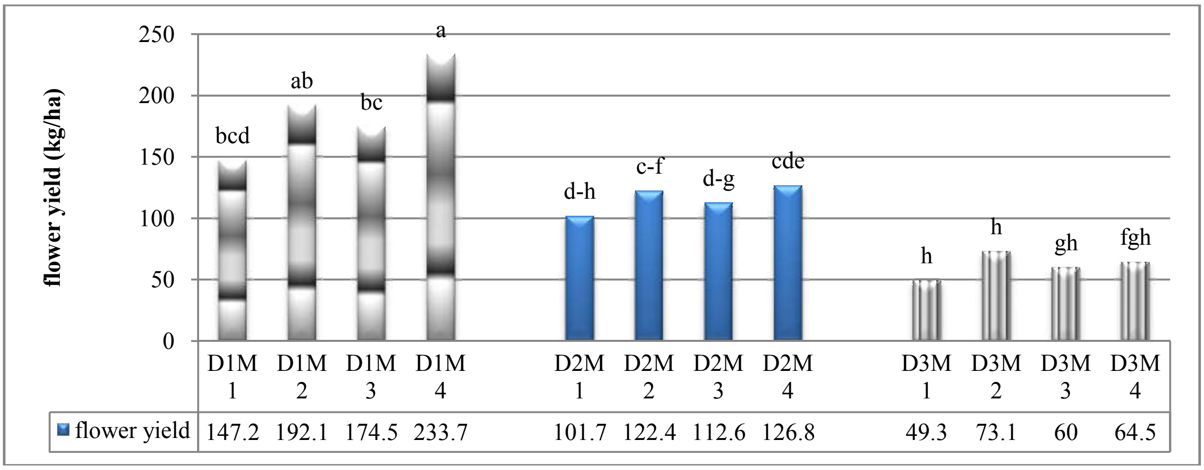

Figure 2: The interaction between water deficit and mycorrhiza on flower yield
D, water deficit; D1, non stress; D2, medium stress; D3, sever stress
M, Mycorrhiza; M1, *Glomus intraradices*; M2, *Glomus mosseae*; M3, *G. intraradices+ G. Mossese*

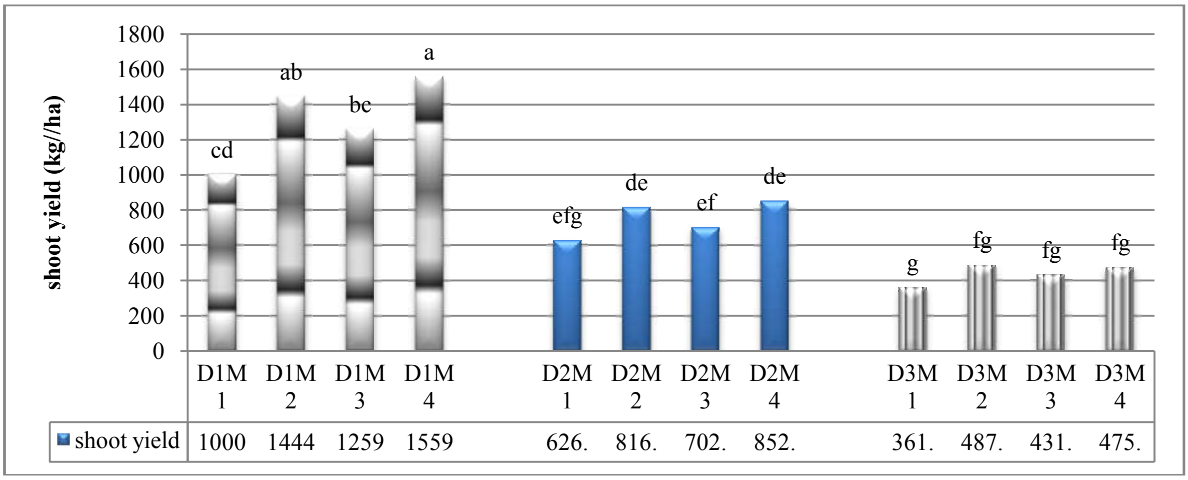

Figure 3: The interaction between water deficit and mycorrhiza on shoot yield
D, water deficit; D1, non stress; D2, medium stress; D3, sever stress
M, Mycorrhiza; M1, *Glomus intraradices*; M2, *Glomus mosseae*; M3, *G. intraradices+ G. Mossese*

Table 1: Physical and chemical properties of soil.

Clay %	Silt %	Sand %	K (ave.) ppm	P (ave.) ppm	Zn (ave.) ppm	Fe (ave.) ppm	O.C %	O.M %	Nt %	pH of paste	EC	S.P	S.M %
35.71	38.78	25.51	380	8.16	0.37	3.18	1.33	2.29	0.09	7.48	1.02	35.29	2.04

Table 2: Variance analysis of water deficit and Mycorrhiza on *Nepeta pogonosperma*.

SOV	df	Leaf yield	Stem yield	Flower yield	Shoot yield	Essential oil percentage	Essential Oil yield
Block	2	43584**	67919**	7942**	310597**	0.8**	8.7ns
Water deficit (A)	2	555256**	334139**	47271**	2370081**	13.3**	70.5**
Error a	4	18670	19486	4783	112345	0.11	13.2
Mycorrhiza (B)	3	36041**	25587**	2968**	161187**	0.24*	9.2ns
A*B	6	6228ns	5169**	826**	29457**	0.07ns	1.1ns
Error	18	3096	582	142	6278	0.05	4.6
C.V (%)		14.7	7	9.8	9.5	12.9	17.9

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 3: The effect of water deficit on *Nepeta pogonosperma*.

Water deficit stress	Leaf yield (kg/ha)	Stem yield (kg/ha)	Flower yield (kg/ha)	Shoot yield (kg/ha)	Essential oil (%)	Essential oil yield (kg/ha)
Non stress	609.4a	519.1a	186.9a	1315.5a	0.74c	9.5c
Medium stress	334.6b	299.04b	115.9b	749.5b	1.9b	14.3a
Severe stress	185.4c	191.8c	61.7c	438.9c	2.8a	12.1b

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 4: The effect of mycorrhiza on *Nepeta pogonosperma*.

Inoculation with mycorrhiza	Leaf yield (kg/ha)	Stem yield (kg/ha)	Flower yield (kg/ha)	Shoot yield (kg/ha)	Essential oil (%)	Essential oil yield (kg/ha)
No inoculation	291.5b	271.8c	99.4b	662.8b	2.06a	10.9a
Glomus intraradices	421.9a	364.9ab	129.2ab	916.05a	1.8ab	13.2a
Glomus mosseae	365.04ab	317.1bc	115.7ab	797.9ab	1.8b	11.4a
G. mosseae + G. intraradices	427.5a	392.8a	141.7a	962a	1.6b	12.3a

Means in a column followed by the same letter are not significantly different (P≤0.05).

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