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Sustainable production of fennel (*Foeniculum vulgare* Mill.) by seed inoculation with mycorrhizae strains under drought stress conditions

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Abstract

In order to study effect of seed inoculation with Mycorrhiza strains on yield of fennel under drought stress conditions a factorial field experiment was carried out at the Research Station of Islamic Azad University, Tabriz Branch, Iran, during 2011-2012 on Fennel (*Foeniculum vulgare* Mill.) *cv. Malayer*. Studied factors were irrigation intervals (70mm, 100mm and 130mm evaporation from pan class A) and Mycorrhizae strains (*Glomus interadics* and *G. mosseae*). There was a field with non-inoculation with Mycorrhizae as control plot. Mean comparisons revealed that the lowest green cover percentage of fennel (0.13%) could be obtained from non-inoculated seeds, but the highest one from those seeds inoculated with *G. mosseae*. Appearance of leaf senescence symptoms in fennel plants happened earlier when seeds were sown without inoculation. But appearance of these symptoms delayed nearly 5 days. Lower number of secondary branches (8.2 branches) observed in normally irrigated treatment (70mm evaporation from pan). Plants under sever water deficit produced higher seed yield than 100mm evaporation level. Seeds inoculated with *G. interadics* strain produced higher seed yield (75.25 g m^{-2}) than non-inoculated seeds (55.04 g m^{-2}). Essential oil percentage of fennel seeds only affected by mycorrhizal inoculation treatment, and those seeds inoculated with *G. interadics* was in a better condition. The stepwise regression analysis verified that the green cover percentage and secondary branches had a marked increasing effect (R^2=0.71) on the seed yield of fennel. The present study concluded that seed inoculation with mycorrhizae strains can play a major role in improvement of seed yield in fennel under water deficit conditions.

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Introduction

Fennel (*Foeniculum vulgare* Mill.) is one of the medicinal plants of the Apiaceae family; due to its estrogenic activities and usage as a carminative and antimicrobial; it has also been used to increase the production of milk in humans and animals (Mahfouz and Sharaf-Eldin, 2007). In Iran, fennel is grown in semi-arid regions as a cold season crop (Omidbeigi, 2007). It is considered to be one of the most important medicinal plants, after saffron (*Crocus sativus* L.) and cumin (*Cuminum cyminum* L.) in Iran.

One of the major crop productivity constraints in the world is the unavailability of crop nutrients in both adequate amount and proper form to crop plants (Hussain *et al.* 2006). The roles of macro-nutrients in crop nutrition are undisputable and thus they are quite important for achieving higher yields (Arif *et al.* 2006). However, they are limiting factors in most of the soils and thus must be supplemented through proper crop nutrients management (Hossain *et al.* 2006). There are number of microbial inoculants like mycorrhizae used as biofertilizers which have been given much attention as they are responsible to plant growth and yield of medicinal plants under field inoculation (Badran and Safwat, 2002).

Phosphorus is one the most essential elements for plant growth after nitrogen. However, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils (Mehrvarz *et al.*, 2008). A mycorrhiza is a symbiotic (generally mutualistic, but occasionally weakly pathogenic) association between a fungus and the roots of a vascular plant. In a mycorrhizal association, the fungus colonizes the host plant's roots, either intra-cellularly as in arbuscular mycorrhizal fungi (AMF), or extra-cellular as in ecto-mycorrhizal fungi (Kirk *et al.*, 2001).

Sharma (2002) reported that one of the advantages of feeding the plants with phosphorus is to create deeper and more abundant roots. Arpana *et al.* (2002) reported that a great proportion of phosphorus in chemical fertilizer becomes unavailable to the plants after its application in the soil. They referred this to formation of strong bonds between phosphorous with calcium and magnesium in alkaline pH and the same bonds with iron and aluminum in acidic soils. The mobility of this element is very slow in the soil and cannot respond to its rapid uptake by plants (Jahan *et al.*, 2007).

Drought stress after pathogens is the main factor in yield decrease among environmental stresses (Sabagpour, 2006). Studies have shown that water deficit caused to decrease in growth, leaf area, stem height, chlorophyll content and root growth decrease (Levitt, 1980). In a field experiment on rapeseed, Dehshiri *et al.* (2006) showed that treatments as irrigation after 50, 80 and 110 mm evaporation from pan did not have effect on seed yield, seed number per pod and oil yield. Nesmith and Ritche (1992) showed that drought stress occurrence in seed filling period causes to dry matter accumulation decrease in seed as a result of effective seed growth period shortening.

Phosphorous is required in large quantities for plants to grow, and is mainly provided in the form of synthetic chemical fertilizers. Such products pose a health hazard and microbial population problem in soil, besides making the production cost high (Badran and Safwat, 2002). The present study was conducted for evaluation the effect of seed inoculation with mycorrhizae strains on yield of fennel under drought stress conditions.

Materials and methods

A factorial field experiment was carried out at the Research Station of Islamic Azad University, Tabriz Branch, north-west of Iran, during 2011-2012 on Fennel (*Foeniculum vulgare* Mill.) *cv. Malayer*. The climate of research site is semi-arid cold with an average annual precipitation of 270 mm. The soil was sandy-loam with EC of 0.72 dS m^{-1}; pH of 7.9; total nitrogen of 0.09%; phosphorous and potassium contents of 70 mg kg^{-1} and 375 mg kg^{-1}; and Fe and B contents of 6 mg kg^{-1} and 1 mg kg^{-1}. The experimental field had been in a corn-potato rotation cycle for the last two years. The field was ploughed twice (October 2011 and March 2012) and manured with 10 t $ha^{-1}$and fertilizer applied in spring and before sowing, based on soil analysis, was 200 kg ha^{-1} of urea. The field then harrowed to prepare the final seed bed. Plots were arranged in a randomized complete block design with three replications. Plots were 4×3 m in size with four rows of planting.

Studied factors were irrigation intervals (70mm, 100mm and 130mm evaporation from pan class A) and Mycorrhizae strains (*Glomus interadics* and *G. mosseae*). There was a field with non-inoculation with Mycorrhizae as control plot.

Statistical analysis

All data were statistically analyzed based on RCBD using MSTAT-C software. The means of the treatments were compared using the least significant difference test at * $P < 0.05$. The stepwise regression analysis was also carried out for the data obtained to test the significance of the independent variables affecting the seed yield as a dependent variable.

Results and discussion

Based on analysis of variance (Table 1), effects of irrigation intervals on leaf senescence, number of secondary branches, seed yield and essential oil yield were significant. Also, effects of mycorrhizae strains on green cover percentage, chlorophyle content index, leaf senescence, number of secondary branches, seed yield, essential oil percentage and it,s yield were significant.

Table 1. Variance analysis of effects of irrigation intervals and Mycorrhizae strains on studied traits in Fennel *cv. Malayer*.

		Mean squares						
SV	df	Green cover percentage	Chlorophyle content index	Leaf senescence	Number of secondary branches	Seed yield	Essential oil (%)	Essential oil yield
Replicate	2	0.0144	0.0137	16.037	1.1481	168.8474	0.0370	2335.44
irrigation intervals (I)	2	0.0046	0.0359	4.5925	8.4814**	641.9548**	0.0370	7177.33*
Mycorrhizae (M)	2	0.2288**	0.4714**	205.8148**	59.7037**	2653.4265**	3.7037**	25790..33**
I × M	4	0.0020	0.0203	9.981	1.4814	162.2675	0.2037	1681.67
Error	16	0.0233	0.0374	14.5787	0.9398	80.4436	5.9259	1596.07
CV (%)	-	17.81	14.47	6.92	10.282	13.86	23.14	21.56

*, ** mean significant difference at 5% and 1% probability levels, respectively.

Green cover percentage

Mean comparisons revealed that the lowest green cover percentage of fennel (0.13%) could be obtained from non-inoculated seeds, but the highest one from those seeds inoculated with *G. mosseae* (Table 2). Based on correlation analysis there is a positive and significant relationship between green cover percentage and seed yield (r=0.70**). It seems that any increment in green cover could be increase yield of fennel under experiment condition.

Table 2. Mean comparisons of some of studied variables affected by mycorrhizal inoculation.

Treatments	Green cover (%)	Chlorophyle content index	Leaf senescence (days after emergence)	Number of secondary branches	Seed yield (g m^{-2})	Essential oil (%)	Essential oil yield (mL m^{-2})
G. interadics	32b	1.46a	58a	10.5a	75a	2.96a	219.8a
G. mosseae	48a	1.40a	57a	10.3a	73a	3.00a	209.4a
Control	15c	1.09b	49b	6.2b	44b	1.84b	143.1b

Means in each column with the same letter have not significant difference at 5% probability level.

Chlorophyle content index

Mycorrhizal inoculation influences chlorophyle content of fennel leaves (Table 2). Gupta *et al.* (2002) reported that mint (*Mentha piperita*) seeds inoculation with vesicular arbuscular mycorrhizae significantly improved leaves chlorophyle, biologic yield and root symbiosis percentage in crop plants compared to the control. Findings of Kapur (2002) on dill (*Anethum graveolens*) has also been supported the results of this study.

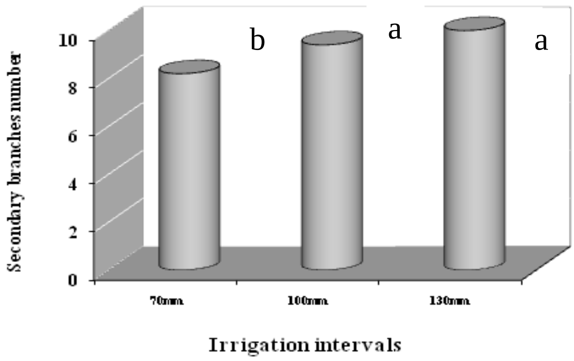

Fig. 1. Effect of irrigation intervals on number of secondary branches in fennel plant.

Leaf senescence

Appearance of leaf senescence symptoms in fennel plants happened earlier when seeds were sown without inoculation. But appearance of these symptoms delayed nearly 5 days (Table 2). Leaf senescence had positive and significant relationship with yield (r=0.58**). With considering of effect of Mycorrhizal inoculation on green cover percentage, the obtained results were not un-expected.

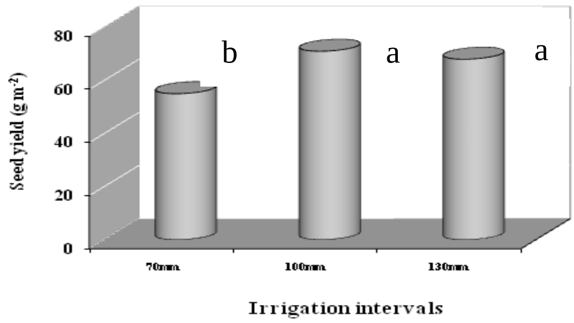

Fig. 2. Effect of irrigation intervals on seed yield of fennel plant.

Number of secondary branches

Lower number of secondary branches (8.2 branches) observed in normally irrigated treatment (70mm evaporation from pan). While, fennel plants under limited irrigation levels produced greater secondary branches (10 branches) than control (Fig. 1). Similarly, when seeds bio-primed with *G. interadics*, plants experienced a significant increase in secondary branches up to 11 branches (Table 2). Umbels in fennel improve on secondary branches, and it seems that any increase in the branches could be improving seed yield.

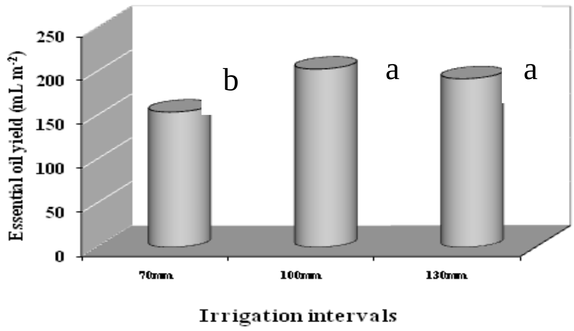

Fig. 3. Effect of irrigation intervals on essetial oil yield in fennel seeds.

Seed yield

Plants under sever water deficit (irrigated when done 130mm evaporation from pan) produced higher seed yield than 100mm evaporation level (Fig. 2). Gabler (2002) in an experiment conducted on coriander (*Coriandrum sativum*) reported that water stress caused to increasing of 1000 seeds weight and yield in crop plants, but essential oil percentage reduced. Seeds inoculated with *G. interadics* strain produced higher seed yield (75.25 g m^{-2}) than non-inoculated seeds (55.04 g m^{-2}) (Table 2). In non-inoculated seeds with increasing of phosphorous fertilization rate grain yield improved significantly, and yield produced in 100% recommended dose of phosphorous was nearly 6.43 t ha^{-1}. Whereas, in those seeds inoculated with Mycorrhizae strains and fertilized with 67% recommended dose of phosphorous, yield improved 31% (Poshtvareh and Mirshekari, 2011).

Essential oil

Essential oil percentage of fennel seeds only affected by mycorrhizal inoculation treatment, and those seeds inoculated with *G. interadics* was in a better condition (Table 2). In fennel studied cultivar *Malayer*, with a view to essential oil yield, there is no significant difference between 100mm and 130mm water deficit levels, and both of them produced greater yield than control (Fig. 3). Similarly, there is no significant difference between mycorrhizae strains too. The treated seeds produced nearly 110 mL m^{-2} greater essence compared to the control (Table 2).

Multiple regression analysis

To formulate the relationship between five independent growth variables measured in our experiment with a dependent variable, multiple regression analysis was carried out for the green cover percentage (X_1), chlorophyle content index (X_2), secondary branches (X_3) umbel number per plant (X_4), and essential oil percentage (X_5) as independent variables and seed yield as a dependent variable. The multiple regression equation is shown as follows:

Seed yield (g m^{-2}) = 0.333 + 39.45 (X_1) + 0.99 (X_2) + 4.29 (X_3) + 0.56 (X_4) + 0.59 (X_5).

Furthermore, the stepwise regression analysis was also carried out for the data obtained to test the significance of the independent variables affecting the seed yield as a dependent variable. The resulted stepwise regression equation is shown as follows:

Seed yield (g m^{-2}) = 0.521 + 38.43 (X_1) + + 7.77 (X_3).

The stepwise regression analysis verified that the green cover percentage and secondary branches had a marked increasing effect (R^2=0.71) on the seed yield of fennel.

Conclusion

Our study concluded that seed inoculation with mycorrhizae strains can play a major role in improvement of seed yield in fennel under water deficit conditions.

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