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# Effects of planting date and biofertilizer on seedling growth of *Thymus daenensis* Celak and *T. vulgaris* L. Cultivated in Borujerd, Iran

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**Abstract:**

Seedling establishment is a crucial stage in the restoration of degraded rangelands. The effects of sowing dates and bio-fertilizers on the growth and establishment of *Thymus daenensis Celak* (native) and *Thymus vulgaris L.* (introduced) were studied over two growth seasons at Islamic Azad University, Borujerd branch, Lorestan, Iran. The treatments consisted of four bio-fertilizers treatments i.e., mycorrhiza fungi, Nitrogen-Fixing free-living Bacteria (*NFB*), Phosphate Solubilizing Bacteria (*PSB*), and a control group, two species of *Thymus*sp. and two transplantation dates (November 2017 and March 2018) were also included. *PSB* and *NFB* (50 mm/lit of dissolved water) as well as mycorrhiza were applied twice: once during seed sowing in pots and again after transplanting the seedlings in the field. The results demonstrated that *T. vulgaris* exhibited superior performance (ranging from 31.8% to 51%) in various plant traits such as height, canopy cover, leaf area, dry leaf weight, dry shoot weight, aerial dry weight, root dry weight, shoot-to-root ratio, and root volume compared to *T. daenensis*. The transplantation date did not significantly affect seedling survival rate and shoot growth. However, seedlings transplanted in November had a higher root-to-shoot ratio (0.94), root dry weight (3.48 mm), root volume (3.56 m3), main root length, and mycorrhiza colonization (11.4%). In exceptionally wet year in 2018, *T. vulgaris* performed better than *T. daenensis.* However, we anticipate *T. daenensis* to outperform *T. vulgaris* during normal and dry years, particularly with the application of *PSB* biofertilizer in its root medium. We recommend simultaneous comparisons of plant growth and thymol concentration under various cultivation and/or biofertilizer treatments on *Thymus* species in future studies.

**Keywords:** Rangeland restorations; *Mycorrhiza*; Free living bacteria; Seedling establishment

## Introduction

Arid land restoration using medicinal plants offers dual benefits of soil conservation and income source for locals. Such projects are highly welcomed by landowners and local communities. The primary and critical stage in the restoration of degraded rangelands is the establishment of seedlings, which often fails due to harsh microclimatic conditions (Moradshahi et al., 2004; Sangtarash et al., 2009; Omidi et al., 2009). Although direct seeding is the main method of plant establishment, it is characterized by slow and steady seedling growth and establishment under field conditions. In natural rangelands, new seedlings must cope with drought, nutrients deficiencies, and competition with neighbouring plants, all of which can hinder plant establishment, growth, and adaptation (Evans et al., 2003; Habibi Savadkoohi et al., 2008). For medicinal plants in their natural habitats, in particular, seedling establishment and time to harvest can take 2-3 years, resulting in the increased management costs and reduced income for stakeholders. Therefore, the transfer of seedlings to the field should aim to minimize environmental stress while quickening the establishment process (Dharmalingam and Basu, 1993; Hosseinzade et al., 2008). In the harsh climate conditions of arid zones, cultivating seedlings in pots is considered a method to alleviate environmental stresses on young seedlings during the establishment period under field conditions (Jankju, 2016). Thus, this research focuses on testing the methods that can enhance seedling growth and survival for pot-cultivated plants under natural field conditions.

Large areas of rangelands across the worlds were deteriorated due to improper utilizations, overgrazing, and ploughing by farmers (Wanga et al., 2014; Tarhouni et al., 2017). The resulting soil erosion depletes soil nutrients and creates harsh microclimate conditions. Consequently, treatments that enhance soil fertility and improve soil moisture can promote seedling establishment under natural field conditions (Nassiri Mahallati et al., 2001). In recent decades, the use of biofertilizers has been introduced as a means to increase soil fertility and create favorable soil conditions (Yousefi et al., 2011) to improve seedling establishment and growth (Hamidi, 2006). Biofertilizers including beneficial bacteria and fungi enhance the chemical and biological properties of the soil. Therefore, managing microbial communities and their coexistence can play a crucial role in restoring damaged ecosystems (Renanta et al., 2010).

Soil microorganisms play important roles in the cycling and absorption of nutrients, as well as vegetation recovery (Smith et al., 2010). The application of *Mycorrhiza fungi*, specifically *Glomus mosseae*, has been shown to increase the plant height and yield of *Origanum sp*. (Kaosaad et al., 2006) and *Thymus vulgaris* (Dolatabadi et al., 2012). Previous research has demonstrated a greater impact of fungi on soil particle sustainability compared to bacteria and actinomycetes, which have relatively lower effects (Martin et al., 2012; Fokom et al., 2013).

In terms of nutrient absorption and access, *Mycorrhiza fungi* can provide a wide area for transferring nutrients, especially phosphorus, to the plant through numerous internal hyphal branching (Baum et al., 2015; Habibzade et al., 2015; Rahimzadeh and A., 2017). The increased growth and efficiency of water consumption can lead to changes in the chemical mixture of root secretions via nutrients absorption (Azul et al., 2010). Mycorrhiza may also enhance sustainability in ecosystem performance, as well as plant species yield and growth (Klironomus, 2003; Sambandan, 2014).

It has been reported that the application of *Azospirillum* bacteria increased seed germination, root growth, and the dry and wet weight of *Eragrostis* sp., *Bouteloua* sp., and *Raphanus sativus* (Hector et al., 2004) Several other studies have also indicated that inoculation with *Azospirillum* bacteria can increase plant biomass, nutrient absorption, nitrogen content in tissues, plant length, leaf size, and root length (Bashan et al., 2004) and can ultimately improve forage production, root growth, and plant biomass (Hector et al., 2004), as well as higher seed germination. Additionally, these bacteria may increase the solubility of materials and nutrients, help plants to defend against pathogens, pests, and environmental stresses, and enhance plant growth intensity (Chen, 2006; Nagananda et al., 2010).

Numerous studies have been conducted on the effects of *Mycorrhiza* and bacterial bio-fertilizer on increasing soil fertility and plant growth. However, the current literature mostly focuses on crops and fruits (Ozturk et al., 2003; Gholami et al., 2009). Few studies have been undertaken in natural rangelands, despite 90% of roots showing symbiosis with *Mycorrhiza* under natural field conditions (Brandreth, 2002). Moreover, while the individual impact of *Mycorrhiza* and bacterial fertilizers has frequently been studied, few studies have compared them simultaneously (Renanta et al., 2010).

The development of medicinal plants in ploughed rangelands and abandoned low-yield rainfed farmlands is important for soil and water conservation, as well as the well-being of the local inhabitants. Therefore, we compared the growth and survival of *T. daenensis*, an endemic species in the Zagros Mountains in western Iran (Shahnazi et al., 2007) with *T. vulgaris*, an introduced species that is widely found in agricultural and natural lands. The research aimed to investigate the efficacy of bio-fertilizers such as *Azotobacter, Phosphobacteria*, and *Mycorrhizae* fungi in improving the yield and production of the *Thymus* species. Additionally, we were interested in determining the optimal transplantation date for these species under field conditions. The research aims to answer the following questions: 1) Can the single or combined application of *Mycorrhiza, Azotobacter* and *Phosphobacteria* increase plant growth and establishment of *Thymus* species compared to the control? and 2) Which planting date for seedling transplantation (autumn or late winter) may significantly enhance the growth and establishment of *Thymus* species under field conditions?

## Materials and Methods

### Study area

This study was conducted in the rangelands of Borujerd, Iran. The study area is located at 33°55’ N latitude and 48°41’ E longitude, at an elevation of 1629 meters above msl. The biome is a semi-arid steppe, with a mean annual precipitation of 460.8 mm. The average, absolute minimum and maximum temperatures are 14.6 °C, 41.4 °C, and - 22 °C, respectively. The study area covered approximately two hectares and resembled a north- facing slope with an inclination rate of 30-40

### Seed cultivation and seedling transplant

Seeds of T. *daenensis* and *T. vulgaris* were sown in plastic pots (30 cm depth and 15 cm wide) in 15th of March 2018, in a glasshouse with no control on air humidity, temperature and light. Seedlings were transferred to the real environment conditions in early May and grown till the transplantation dates. Half of the seedlings (240) were transplanted in mid-autumn ($4^{th}$ of November 2018) and the remaining pots were transplanted at $15^{th}$ of March 2019. Seedlings were planted in the hand-made pits (50 cm wide and 30 cm depth), in 40 cm within and 100 cm distance between the rows. Seedlings were planted in 10 cm distance within each pit.

### Growth media

Soil for the pot was taken from the natural rangeland site where the experiment had been planned to be conducted. Soil parameters were analysed according to standard methods, and the results indicated a loamy-sand soil and low fertility in terms of nitrogen (0.06 mg/kg), and organic carbon (0.6%). It contained a reasonable amount of phosphorus (13.4 mg/kg) and high potassium (293 mg/kg) levels, which led to relatively high pH (7.8). EC was 0.4 dS/m and soil depth varied from 10 to 30 cm.

### Study design

The experiment was designed as a completely randomized block and treatments were applied as split plot with three replications. Biofertilizers were considered as the main plot, plant species as subplot and cultivation times as the sub-sub plot. Main treatments were seedling cultivation planting date (November 2018 or March 2019), four biofertilizers and two *Thymus species* i.e, T. *daenensis* and *T. vulgaris*. Biofertilizer treatments included application of *NFB, PSB*, *Mycorrhiza* and control (no biofertilizer). *PSB* contained *Pseudomonas sp.* and *Bacillus sp. NFB* contained *Bacillus sp., Azotobacter sp.* and *Azospirillum sp.* All the bacteria strains had been taken from the natural fields of Iran and processed by Khosheparvaran Biotechnology Company Mashhad, Iran. For both the phosphate soluble bacteria and free- living nitrogen fixing bacteria, we used 50 ml of biofertilizers and dissolved it within 2 L water. For *Mycorrhiza* treatment, we used 10 g of soil contained *Glomus mosseae* in each pot. All (*NFB*) and (*PSB*) and *Mycorrhiza* treatments were applied twice on *Thymus* seedlings; once at the time of seed cultivation within pots and at the second time was at seedling transplantation into the field.

### Growth measurements

Seedling growth and survival rate were measured at 12$^{th}$ of May 2019 for all seedlings transplanted in the autumn or spring. At this planting date, numbers of alive seedlings were measured together with plant height and canopy cover. Furthermore, three replicates of each treatment (total of 80 plant samples (were harvested and such traits as stem dry weight, leaf dry weight, root dry weight, shoot dry weight, leaf area, total root length, average root diameter, root volume, root area, main root length and *Mycorrhiza* colonization percentage were measured in the laboratory.
To measure canopy cover, three individual plants were randomly selected and their photos were taken from the 1m above. Canopy cover was calculated by image processing software, JMicroVision V1.27. Leaf area was measured by Leaf Area Meter (Delta-T England). Root volume was measured based on Archimedes'principle, i.e., water volume increased by floating roots. Root area, diameter and length were measured by Delta-T Scan scanner (CB50EJ). Finally, for measuring dry weights, plant samples were placed in an oven at 70 °C temperature for 48 h.
Colonization percentage was measured using Malibari et al. (1988) method. In which *Mycorrhiza* contamination percent was estimated for each piece of root separately and then, the total contamination percent was estimated. Vesicle's surface and hypha length were estimated by JMicroVision software.

### Data analysis

Data were analysed as Two-Way ANOVA and means were compared using Duncan's test at 95% probability level in SPSS statistical software.

## Results

The effects of biofertilizer, planting date, species types, and the interaction between them were compared to assess their impact on the survival rate, morphological growth (Table 1), and root traits (Table 2) of *Thymus* species. None of the main factors, namely transplanting date, biofertilizer, and

**Table 1.** Effects of biofertilizers, planting date, species, and their interaction effects on survival rate and growth traits of *Thymus* sp.

| SOV | DF | Survival rate | Canopy cover | Leaf area | Plant height | Leaf dry weight | Stem dry weight | Shoot dry weight |
|---|---|---|---|---|---|---|---|---|
| Block | 2 | 1654 ns | 2025 ns | 2754 ns | 99.6 ns | 0.235 ns | 0.058 ns | 1.48 ns |
| Biofertilizer (F) | 3 | 5.1ns | 151ns | 1034 ns | 18.06** | 0.046 ns | 0.010 ns | 0.079 ns |
| Error1 | 6 | 46.8 ns | 133 ns | 838 ns | 8.32 ns | 0.118 ns | 0.266 ns | 0.550 |
| Species (S) | 1 | 112 ns | 2386** | 25.7 ns | 66.1** | 0.573* | 0.321ns | 1.11* |
| F×S | 3 | 68.1ns | 243 ns | 281ns | 15.8** | 0.043 ns | 0.402 ns | 0.69 ns |
| Error2 | 8 | 37.5 ns | 146 ns | 453ns | 9.95 ns | 0.128 ns | 0.070 ns | 0.098 ns |
| Planting date (D) | 2 | 1852 ns | 2297* | 1296 ns | 1021ns | .152 ns | 1.314 ns | 2.27* |
| F × D | 6 | 5.57 ns | 250 ns | 2085** | 33.9** | 0.149 ns | 0.408 ns | 0.504 ns |
| S×D | 2 | 154 ns | 420 ns | 1293 ns | 6.42** | 0.076 ns | 0.207 ns | 0.708 ns |
| F×S×D | 6 | 48.6 ns | 512 ns | 510 ns | 25.1** | 0.141ns | 0.311ns | 0.675 ns |
| Error3 | 32 | 224 ns | 299 ns | 798 ns | 8.59 ns | 0.153 ns | 0.192 ns | 0.334 ns |
| CV% | | 7.31 | 17.6 | 34.9 | 15.71 | 17.5 | 25.1 | 12.6 |

*, ** and ns indicate a significant difference in P > 0.05, P > 0.01, and non-significant differences, respectively.

**Table 2.** Effects of biofertilizers, planting date, species, and their interaction effects on root traits of *Thymus* sp.

| SOV | DF | Root dry weight | Root volume | Root to shoot ratio | Root area | Root diameter | Root colonization with *Mycorrhiza* |
|---|---|---|---|---|---|---|---|
| Block | 2 | 0.019 ns | 1.40 ns | 0.236 ns | 118 ns | 0.101ns | 7.18 ns |
| Biofertilizer (F) | 3 | 2.57** | 0.502** | 0.329** | 86.7 ns | 0.055 ns | 12.3 ns |
| Error1 | 6 | 0.329 ns | 0.377 ns | 0.056 ns | 83.5 ns | 0.116 ns | 8.85 ns |
| Species (S) | 1 | 0.258 ns | 1.69 ns | 0.021ns | 91.8 ns | 0.015* | 0.546 ns |
| F×S | 3 | 0.216 ns | 0.730 ns | 0.032 ns | 139 ns | 0.052** | 8.11 ns |
| Error2 | 8 | 0.158 ns | 0.206 ns | 0.095 ns | 90.4 ns | 0.022 ns | 3.57 ns |
| Planting date (D) | 2 | 8.63** | 5.56* | 1.10** | 4025** | 1.16* | 113* |
| F × D | 6 | 1.36 ns | 0.762 ns | 0.218 ns | 93.7 ns | 0.026 ns | 2.19 ns |
| S×D | 2 | 0.197 ns | 0.344 ns | 0.056 ns | 42.9 ns | 0.024 ns | 1.90 ns |
| F×S×D | 6 | 0.410 ns | 0.294 ns | 0.090 ns | 176 ns | 0.059** | 2.94 ns |
| Error3 | 32 | 0.390 ns | 0.494 ns | 0.067 ns | 122 ns | 0.064 ns | 4.32 ns |
| CV% | | 2.34 | 16.7 | 21.1 | 40.7 | 18.45 | 21.4 |

*, ** and ns indicate a significant difference in $P > 0.05$, $P > 0.01$, and non-significant differences, respectively.

planting date had a significant effect on the survival rate and stem dry weight of *Thymus* species. However, the main effect of biofertilizer was found to be significant ($P<0.05$) for plant height, root dry weight, root volume, and root-to-shoot ratio. Similarly, the main effect of species was significant ($P<0.05$) for canopy cover, plant height, leaf dry weight, shoot dry weight, and root diameter. Furthermore, the main effect of planting date was significant ($P<0.05$) for canopy cover, shoot dry weight, root dry weight, root volume, root-to-shoot ratio, root area, root diameter, and root colonization with *mycorrhiza*.

The interaction effects between biofertilizer and species were found to be significant ($P<0.05$) for root diameter. Similarly, the interaction effects between biofertilizer and planting date were significant ($P<0.05$) for leaf area and plant height. Additionally, the interaction effects between species and planting date were significant ($P<0.05$) for plant height. Lastly, the interaction effects among biofertilizer, species, and planting date were significant ($P<0.05$) for plant height and root diameter (Tables 1 and 2).

### Canopy cover

*T. vulgaris* exhibited 18% larger canopy cover than that in *T. daenensis* (Figure 1 a). Additionally, for both species, seedling planted in March resulted in 12.5% higher canopy cover compared to those planted in November (Figure 1b).

### Leaf area

Both species displayed the most significant response to *NFB* treatments, with a greater effect observed in November compared to March (Figure 2). Specifically, the application of *NFB* treatment on *Thymus* seedlings in November resulted in a 77% increase in leaf area compared to those growing under *PSB*, *Mycorrhiza*, and control treatments.

### Plant height and root diameters

By the end of this study, *T. vulgaris* seedlings exhibited a 51% greater canopy height compared to *T. daenensis* seedlings. The application of phosphate-dissolving bacteria did not yield significant differences in the two planting dates of November and March for both species. While the use of Mycorrhiza fungus had no significant effect on *T. daenensis* across the two planting dates, it resulted in a 27.2% increase in plant height for *T. vulgaris* when comparing the March date with November (Table 3).

### Root diameters

For seedlings transplanted in March, NFB treatments resulted in a 26% higher root diameter for *T. daenensis* compared to *T. vulgaris*. The utilization of *Mycorrhiz*a fungi in both November and March planting dates did not yield a significant difference between the *Thymus* species. However, for seedlings transplanted in November, *T. daenensis* exhibited a 66.6% larger root diameter compared to *T. vulgaris*. Overall, the largest root diameters were observed in *T.*

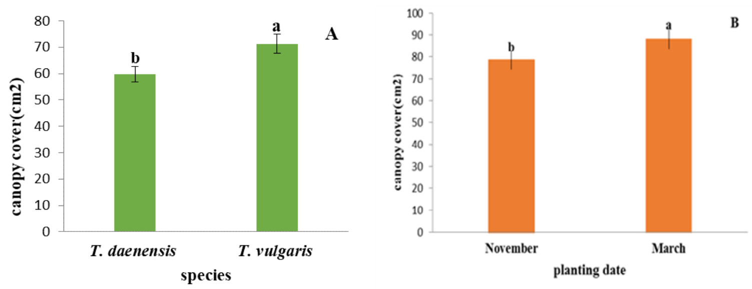


**Figure 1.** Effect of species (a) and planting date (b) on the canopy of *Thymus* species.

**Table 3.** Effects of biofertilizer treatments, plant species and date of cultivation on canopy height and root diameter of *Thymus* species.

| Treatments | Species | Planting date | Plant height (cm) | Root diameter (mm) |
|---|---|---|---|---|
| NFB | *T. daenensis* | November | 21.0 b | 0.90 abd |
| | | March | 20.8 b | 0.63 cd |
| | *T. vulgaris* | November | 31.8 a | 0.93 ab |
| | | March | 23.1 ab | 0.50 d |
| PSB | *T. daenensis* | November | 19.2 b | 1.00 a |
| | | March | 22.1 b | 0.52 d |
| | *T. vulgaris* | November | 20.6 b | 1.01a |
| | | March | 20.2 b | 0.59 d |
| *Mycorrhiza* | *T. daenensis* | November | 22.7 ab | 0.92 ab |
| | | March | 21.7 b | 0.53 d |
| | *T. vulgaris* | November | 20.0 b | 0.94 a |
| | | March | 27.6 ab | 0.53 d |
| Control | *T. daenensis* | November | 18.2 b | 1.08 a |
| | | March | 21.2 b | 0.61d |
| | *T. vulgaris* | November | 18.9 b | 0.65 bcd |
| | | March | 24.9 ab | 0.54 d |

Similar letters indicate no significant differences at the 5% probability level. Abbreviations NFB: Nitrogen fixing free - bacteria, PSB: Phosphorus-soluble bacteria.

*daenensis* planted in November under the control treatment, measuring 1.08 mm. On the other hand, the smallest root diameter of 0.50 mm was recorded for *T. vulgaris* planted in March with NFB treatment (Table 3).

### Root dry weight

Plants transplanted in November exhibited a 32% more root weight compared to those planted in March. *Thymus* seedlings that were inoculated with Mycorrhiza fungi demonstrated 49% and 22.2% greater root dry weight than those inoculated with bacteria and phosphate fertilizers, respectively. However, these differences were not statistically significant when compared to the control treatment (Table 4).

### Root colonization with Mycorrhiza fungi

The *thymus* seedlings planted in November had a root colonization that was 50% higher compared to those planted in March (Figure 3). The root surface increased by 65% and the root dry weight increased by 14.4% when the seedlings were transplanted in March compared to November. Additionally, the stem dry weight showed an increase of 25.8%. In terms of *Thymus vulgaris*, it produced 15.9% more leaf dry matter weight and 14.4% more shoot dry weight compared to *T. daenensis* (Table 4).

The application of NFB resulted in a reduction of root dry mass compared to shoot dry mass. The shoot-to-root ratio was 44.7% higher for seedlings transplanted in March than in November (Table 4). Furthermore, the mycorrhiza fungus increased the root volume of two species, resulting in a 32.3% and 19.9% higher root volume compared to the *NFB* and *PSB* treatments, respectively. Additionally, we observed that seedling transplantation in November rather than March led to a 23.6% higher RV for *Thymus* species (Table 4).

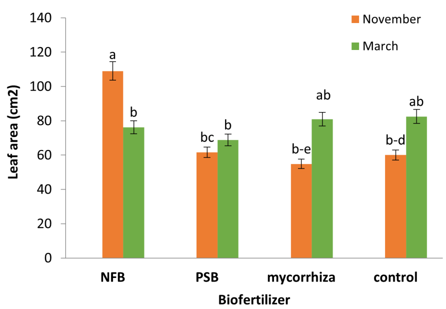


**Figure 2.** Effect of planting dates and biofertilizer interaction effect of on leaf area index of *Thymus* species Abbreviations; NFB: Nitrogen-fixing free bacteria, PSB: Phosphorus-soluble bacteria.

## Discussion

### Planting dates

We transplanted seedlings of two *Thymus* species on November 2018 and March 2019, when they had been grown in

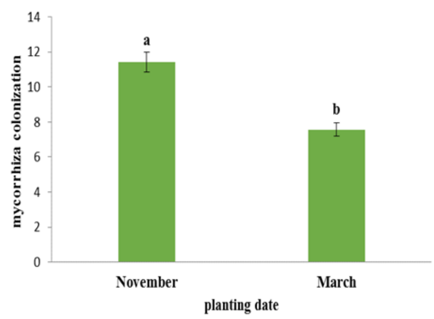


**Figure 3.** Effects of seedling planting date on *Mycorrhiza* colonization percentage.

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**Table 4.** The effect of biofertilizer treatments, species types and planting date on root morphological traits of *Thymus* species.

| Treatments Treatments | Root area ($mm^2$) | Leaf dry weight (g) | Stem dry weight (g) | Shoot dry weight (g) | Root dry weight (g) | Root to shoot ratio | Root volume ($cm^3$) |
|---|---|---|---|---|---|---|---|
| NFB | 32.5a | 1.89a | 1.43a | 3.32a | 2.36b | 1.42a | 2.69b |
| PSB | 30.6a | 1.76a | 1.41a | 3.17a | 2.88ab | 1.14ab | 2.97ab |
| *Mycorrhiza* | 24.9a | 1.80a | 1.46a | 3.26a | 3.52a | 0.98b | 3.56a |
| Control | 26.8a | 1.88a | 1.47a | 3.35a | 3.48a | 1.06b | 3.64a |
| | | | **Species** | | | | |
| *T. daenensis* | 30.5a | 1.70b | 1.36a | 3.06b | 2.95a | 1.11a | 3.03a |
| *T. vulgaris* | 26.9a | 1.97a | 1.52a | 3.50a | 3.17a | 1.19a | 3.40a |
| | | | **Planting date** | | | | |
| November | 21.7a | 1.78a | 1.28b | 3.06b | 3.48a | 0.94b | 3.56a |
| March | 35.8a | 1.88a | 1.61a | 3.50a | 2.63b | 1.36a | 2.88b |

Similar letters are not significantly different from each other at the 5% probability level.

pots for about 8 and 12 months, respectively. The date of planting in March was coincident with the early days of spring, when there were favourite conditions for the early transplanted seedlings in terms of temperature, precipitation and soil moisture storage. The mild environmental conditions in early days of plant establishment can stimulate the vegetative and reproductive growth stages and hereby increase their growth performances (Hosseinzade et al., 2008; Dharmalingam and Basu, 1993). Furthermore, the newly established plants would gain more opportunities for benefiting from the early season sporadic rainfall in the arid ecosystems (Jankju Borzelabad and Griffiths, 2006). We did not find clear effects of transplanting date on aboveground biomass during the first growing season; i.e., higher canopy diameters for the seedlings that had been transplanted in March were compensated by higher leaf area and those in November. However, for all root growth parameters, plant cultivation in November was more beneficial for both *T. daenensis and T. vulgaris*. Thymus seedlings that had been transplanted in November displayed thicker root diameters, greater root weight and root volume, and higher biomass allocation to roots than shoots (higher root: shoot ratio). Furthermore, planting *Thymus* seedlings in November provided more opportunity for *Mycorrhiza* inoculation as compared those of planted in March.

A proper date for plant transplantation into the field provides a set of environmental factors that are suitable for seedlings establishment and survival (Hoffmann and Kluge-Severin, 2010; Khajepour, 2001). In this experiment, the *Thymus* species favoured mild temperature, high light and soil moisture in the early days of spring, which could increase their canopy growth. Therefore, the reason for the larger canopy size of the *Thymus vulgaris* as compared with *T. daenensis* might be due to its higher capability for using the environmental resources. Since the study site is located in a semiarid area, higher plant investment on root traits would be more beneficial for its long-term survival (Salmani Biary et al., 2010). The ability of a plant to avoid drought is measured by the mechanism of change and distribution of the root system in deep soils, which increases water absorption efficiency (Songseri et al., 2008). Plant genotypes with larger root volume occupy more soil and hence absorb more water that eventually leads to higher drought resistance (Adda et al., 2005; Wanga et al., 2009). Earlier seedling plantation provide more opportunities for making adaptation to the field conditions. This was found as higher *Mycorrhiza* colonization for *Thymus* seedlings that were transplanted in November, as compared with those cultivated in March. *Mycorrhiza* symbiosis may help plants for higher uptake of nitrogen, phosphate, potassium, iron and other nutrients (Wu et al., 2005). They may also be effective in improving the physical, chemical and biological properties of soil (L. and Kuyper, 2006). Accordingly, we expect higher drought tolerance for seedling cultivated in November than March.

### Biofertilizer treatments

We applied two free-living bacteria fertilizers (*NFB* and *PSB*) and a *Mycorrhiza* inoculation treatment on *Thymus* species and compared them with control plants. Generally, the biofertilizers increased total biomass of *Thymus* species. However, their effects on the above versus belowground organs varied depending on the type of biofertilizers. Currently, it has been proven that these micro-organisms increase nutrients in the rhizosphere environment and also increase the absorption of nutrients; furthermore, they help to control plant pathogens and increase plant resistance to environmental stresses through several mechanisms (Chen, 2006; Nagananda et al., 2010). Therefore, for the species studied in this research, the positive interaction between *Mycorrhiza* symbiosis and higher growth of seedlings that were planted in November can ultimately improve plant growth and subsequently increase the establishment of *Thymus* species in autumn than winter.

### *T.daenensis vs. T. vulgaris* performances

We compared the performances of a native and introduced *Thymus* species. *Thymus vulgaris*, which is an introduced plant species from southern Europe, showed higher performances in terms of total biomass. The higher biomass of *T.*

*vulgaris* was mainly due to its higher shoot growth whereas both species were similar in root growth parameters. On the other hand, we found higher responses of the native species to *PSB* treatments, i.e., the phosphorous treatment significantly increased root growth for *T. daenensis* as compared to its effect on *T. vulgaris*. Higher biomass allocation to above-grounds can usually increase plant sensitivity to periodical droughts (Hoffmann and Kluge-Severin, 2010), and this may be the case for *T. vulgaris* in the study area. Therefore, during the wet periods and under the irrigating conditions, cultivation of *T. vulgaris* can produce higher biomass and hence provides higher incomes for the farmers. In contrary, under the drought periods and natural field conditions, we may expect higher survival and persistence of the native *Thymus* species.

## Conclusion

Although there are numerous studies on the effects of biofertilizer on plant growth, most studies have been carried out in croplands with irrigation. In this research, we found that both *Thymus* species can be grown in pots and be successfully transplanted and established within the natural vegetation of rangelands. Seedling transplantation can reduce competition and planting date to plant harvesting, hereby can increase the chance of establishment and farmers' income. For the rainfed farming, seed sowing is usually advised to be in November when it promotes root higher plant allocation to root media that are so critical for plant growth and establishment in the arid areas and within the natural vegetation. Under the exceptionally wet years of the study, the introduced *Thymus* species (*T. vulgaris*) performed better than the native species (*T. daenensis*). However, we expect that *T. daenensis* will outperform *T. vulgaris* during the normal and dry periods, especially if PSB fertilizer is applied in its root media. A higher concentration of thymol constituent in plant tissues may offer advantages for plants with lower aboveground biomass. Therefore, future studies should consider conducting simultaneous comparisons of plant growth and thymol concentration under various cultivation and/or biofertilizer treatments on *Thymus* species.

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**Authors contributions**
All authors have contributed equally to prepare the paper.

**Availability of data and materials**
The data that support the findings of this study are available from the corresponding author upon reasonable request.

**Conflict of interests**
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.



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