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Development of a Lethal Drought Stress in Different Mycorrhizal *Alnus incana* Seedlings

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Master of Science Thesis
Wood Material Science

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Sammi Alam: Development of a Lethal Drought Stress in Different Mycorrhizal *Alnus incana* Seedlings
Master of Science thesis, 34 p.
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February, 2018

Abstract

Arbuscular mycorrhiza (AM) and ectomycorrhiza (EM) have the wide range of dominance in different environmental stress conditions like drought, frost, salinity etc. Both AM and EM play important roles in plant cells and mechanisms during drought stress. The hypothesis of the study was AM tolerate better drought than EM in drought condition. To elicit the relative importance of these mycorrhizae on plant's response and to get a lethal stress condition during very dry environment, grey alder plants were inoculated with these mycorrhizae and some were left without mycorrhizal inoculation (NM). All plants were exposed to drought in the controlled environment to visualize the significant plant's performance. Growth and development of plants, their moisture status, leaf temperature status, leaf drooping time and seedling lifetime in drought were recorded. By following this way plant's responses and mechanisms in different mycorrhizal treatments were investigated. The experimental results indicated that the plant height was smaller in AM than EM and NM seedlings. EM and NM had also the positive effect on plant diameter than AM at the same time. Soil moisture was not significantly affected by the treatments. No significant effect was found in leaf temperature between the treatments. However, leaf drooping was not affected significantly by the treatments but the seedling lifetime of plants was affected positively by AM than NM whereas, EM plants had no significant effect in the seedling lifetime in comparing to AM. AM plants showed more tolerance than EM as the seedling lifetime of EM and NM were not different from each other. These results give some satisfactory supports to the hypothesis of this experiment that AM can tolerate better drought than EM.

Keywords: Mycorrhiza. Arbuscular mycorrhiza. Ectomycorrhiza. Drought. *Alnus incana*. Growth. Soil moisture. Leaf temperature. Leaf drooping. Seedling lifetime.

Acknowledgement

First of all, I would like to thank my supervisor Dr. Tarja Lehto, University of Eastern Finland. Through her delightful supervision, good-hearted help, support, and effort, I was successfully able to conclude my thesis. I am appreciative of her considerable counseling and endurance in this work. Her every email and discussion regarding the work was very efficient and helpful to get ahead in my thesis.

I would also like to thank my co-supervisor Dr. Jouni Kilpeläinen for his enormous help and guidelines during the entire experiment. He helped me a lot by favoring valuable time and information in the technical site of data analysis and always kept his door open for me to solve any problem during the whole process. I am also thankful to my friend Aitor Barbero Lopez for his help during the experiment conducted. I am obliged to Dr. Mauritz Vestberg at MTT Laukaa for providing me the materials needed for my research work.

I am also very grateful to Dr. Antti Haapala and Dr. Kirsi Mononen for their valuable teaching directions and instructions in every part of the master degree program of Wood Materials Science. I am also filled with gratitude to the University of Eastern Finland and Faculty of Science and Forestry for their contribution and opportunity offered me to do the research work and finalize my degree program.

I want to give thanks to my parents for their prayer and my husband for great support and motivation throughout the whole period of my study at the University of Eastern Finland especially during the writing progress in thesis work. Finally, I would like to say thanks to my siblings for their appreciable encouragement that helped me a lot to continue stability with my thesis work as well my daily life together.

Thank you.

Sammi Alam

UEF, February 2018

Abbreviations

EM	Ectomycorrhiza
AM	Arbuscular Mycorrhiza
NM	Non-Mycorrhiza

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1 Introduction

It is estimated that there are a lot of significant effects of ectomycorrhizas (EM) and arbuscular mycorrhizas (AM) on different types of plant species at different environmental conditions. The on-going issue about the effect of EM and AM has created an increasing undivided attention in the possible changes of internal mechanisms that plants bring down to the surface. A lot of significant research has been made on mycorrhiza and plants responding in favorable and also in unfavorable weather conditions. This research of EM and AM now takes into consideration the other factors such as how these mycorrhizae (EM and AM) help plants to develop a lethal stress condition under drought environment.

Alnus incana (Grey Alder) is a very common species of Alder that grows well across the cooler parts of Northern Hemisphere. Grey alder and the selected fungi were tested before this experiment conducted to know whether they form mycorrhizas together or not as only in some specific woody plants the criteria of dual mycorrhizal system occurred at the same time, and they literally show their dominance into different types of mycorrhizae and have various functional mechanisms during the environmental stress conditions. There are only a very poor number of tree species present in our environment that can form both ectomycorrhizae and arbuscular mycorrhizae including some woody plants e.g. *Alnus*, *Eucalyptus, Salix, and Populus* can form both AM and EM in their roots. After the test, it has been clear that this species can go under mycorrhizal colonization with both fungi. Tree species and the fungi species were selected as test species in this experiment.

AM is a kind of mycorrhiza, in which type fungus has the capability to penetrate the cortical cells (the outer most layer of root) of plant roots of a vascular plant (the higher plant that has xylem for conducting water and minerals). This soil-borne fungus has derived from the phylum Glomeromycota. AM are mostly identified by developing vesicles and arbuscules structures. This mycorrhiza has great contribution to plant roots to uptake nutrients especially N, P, S and some micronutrients. AM fungi form an interactive relationship with roots of many plant species. This symbiosis has enormous beneficial effects on both the host plants and the AM fungus. The host plants can deliver AM fungi of about 20% of their photo-synthetically fixed carbohydrates essentially recommended for the completion of their life cycle.

On the other hand, ectomycorrhiza (EM) refers to the mycorrhiza that makes a symbiotic association between fungal symbiont and plant roots. These fungi predominantly derived from the phyla Basidiomycota and Ascomycota and a few numbers are from the phylum Zygomycota. It is generated that EM has the symbiosis approximately with about 2% of different plant species. EM fungus colonization with plant species has a considerable positive effect on nutrient progression mostly mentionable for sufficient nitrogen in the terrestrial ecosystem.

The main focus of the current research was to find out a lethal drought stress in different mycorrhizal treatments colonized with grey alder species. We also wanted to find out how plants response to mycorrhizal treatments and how they show their different mechanisms under the very drought condition. This study should give us a clear overview whether mycorrhizal plants can survive in the very water limiting condition or not and how long they can exist; how much they show differences in their growth and development; does mycorrhiza help plants to be able to survive in the drought stress condition or not.

In the literature review, at first, I would like to give some general information about grey alder (*Alnus incana)* species and state some words about their habitats, growth, and development. Sequentially I am going to focus on drought and give a basic overview about drought and after that, I want to present some words by taking different effects on plants into consideration. I am also going to deliver some more details about AM and EM and also mention some significant roles of these fungi during the drought period.

1.1 Grey alder (*Alnus incana*)

Grey alder is one type of alder species is a member of genus *Alnus* and under the family of Betulaceae. 35 plant species are included under this genus; they have usually growing potentiality mostly throughout the whole state of Europe, but some are grown on an irregular basis in the westernmost parts. A number of species of this genus are great worthy from both sites of economically and ecologically.

Alnus incana, the scientific name of grey alder, taken into consideration as a tree that has demand for light and the development of this species can be very fast on the poor quality soil. It is found along with alluvial land where appears as a colony with it, coming into view up to 1,500 meters (4,900 ft), mostly available in the central Europe part. In Southern Europe sites, in the mountainous regions, it is familiar as a very regular tree (Royal Botanical Garden, Edinburgh 2008). In the north forest parts, grey alder species grows as a pioneer tree species with sufficient of daylight, but not in northernmost Finland. It is a very common species occurred at the sea level area of northern forest. This tree species also can establish a very strongest and healthy canopy by itself that can guard it against the damaging effects of outside. This tree has root nodule which is able to fix nitrogen from the air and release it back into the soil; the leaves are green even though when they fall, so these leaves can be turned into quality soil. Thus, grey alder improves the soil properties.

In case of non-fertile soils, this tree is used for afforestation purpose. It can be differed from common alder (black alder) species by their dull green leaves forming a downy structure on both surfaces of the leaves. The reproduction system of grey alder very commonly occurs from seeds or shoots. Grey alder has usage in various manners e.g. as a raw material for beautiful carpentry, in the making of different musical instruments and also for beautifying wood items. This tree wood is used for lathing and carving purpose, also it has extensive and substantial value using as firewood and smoking chips.

1.2 Drought

Drought is counted as one of the abiotic stresses can be a reason of momentous damaging effects to plant species common in the plant growth, development, and their productivity at the different stages of their life cycle. Drought is considered as a period of a very dry environment or the condition of below average precipitation found on a specific land or area that takes place continuously for an extended period of time. Drought is generally caused due to the very little amount of rainfall in an area or lack of sufficient water required on a specific land. It can lead a dangerous impact on the living environment like plants and animals. Due to lack of water in an area during drought, the environment faces to an extremely warm and dry condition that can accelerate the dehydration of water vapor proceeding very terrific drought. Drought occurs as a leading stress in the environment and brings down various harmful effects on plant growth and development (Denby and Gehring 2005). Drought stress generates too little amount water into the plant cells ensued the plants become dry up very quickly; this dried condition of the plant is called desiccation.

Drought is also considered as a significant factor affecting the yield production of the crop; it can bring down approximately more than 50% of crop production in worldwide (Wang et al. 2003). During water limiting condition or when water is shortage than the plant needs, plants are mainly disturbed in their growth and nutrition uptake (Loreto & Centritto 2008) turned to a serious disruption in the balance of their various internal system.

1.3 AM and EM

To survive in the water limiting environment plants can bring out an interaction with microorganisms and their interaction often develops as mutualism or as parasitism. However, drought can be affected in a various way by the different mycorrhizal colonization. It is proved that mycorrhizal relationship with plant species has variable impacts on host species, that ranges extensively from better nutritional condition (Marschner and Dell 1994) and also has significant effects on plants in the endurance of stress environment such as drought (Marulanda et al. 2009). AM and EM come to an interaction with plants and make colonization, develop a symbiotic relationship with the plant species and can have functional performance under the drought condition.

Plants of different species form arbuscular mycorrhizal (AM) symbiosis with the fungi of the Glomeromycota and have been widely developed as improved host species that have beneficial effects on various stress conditions. AM have the response mechanisms beneficial to plant species in reversed environmental conditions that can assist the symbiosis between plant species and AM to be more eventual and more important under drought stress condition (Shukla et al. 2013, Wagg et al. 2011). However, it is widely developed that AM enhance the nutrient uptake capacity of plants when making colonization (Borowicz 2001), also help plants to protect from the buffer in very drought environment (Smith et al. 2010). The feasibility of developing or enhancing drought resistance power in AM inoculated plants has been mostly analyzed (Augé 2001, Rapparini and Peñuelas 2014, Saia et al. 2014). AM has the influence on their host plants, this influence can be observed as a layout like AM help plants to uptake more water and nutrients by their hyphal expedition (Cho et al. 2009, Navarro et al. 2009). Moreover, during the colonization period of plants with AM fungi, photosynthetic rates of plants increase (Subramanian and Charest 1995) that has a great contribution to carbon assimilation. A lot of experiment has been conducted and widespread endeavors have been practiced still the present time to interpret the plant reponses of AM inoculation and to find out benefits of this mycorrhiza as a subsidiary to promote the water and nutrient uptake status thus to enhance drought tolerance (Smith and Read 2008, Ruiz-Sanchez et al. 2010).

The other one, EM normally form the mutualistic interaction between the fungi and Gymnosperm and also Angiosperm plants. Their interaction with the plant can build up in a soil mycelium system, making a connection with mycorrhizal roots and productive structures. The EM inoculation helps to improve soil surface with the help of enlarged extra-radical mycelia, this mechanism can simplify the nutrient uptake process for plant roots (Read and Perez-Moreno 2003, Gobert and Plassard 2008). However, EM can have a vital role to develop the plant water status mostly in case of small seedlings that might help to improve plant performance during drought (Lehto and Zwiazek 2011).

Plants usually show slow growing criteria and become stunted, leaves of some plants change to dull from the shiny appearance in drought condition. As water is the main factor determining the availability of mineral nutrients, due to lack of mineral nutrients in severe drought, plants make permanent wilting and disturb growing. Woody plants turn their leaves yellowing and face wilting, in some cases plants supposed to as dead, appearing as all or most of leaves in drooped condition. These happen due to insufficient moisture status and moisture movement from root to shoot during the drought period. At drought, cell elongation of higher plants may be restricted through the disturbing of water flow from xylem to cell, resulting in reduced plant height and growth (Nonami 1998). However, this stress condition can be alleviated through different mycorrhizal fungi that can be described by specific physiological activities of plants like transpiration, CO_2 fixation, water use (Ruiz-Lozano and Azcón 1995). From one study it has resulted, AM fungi (*Glomus deserticola*) develop the tolerance of water deficiency in plants with having very little growth reduction of 9% (Ruiz-Lozano et al. 1995). Insufficient water status makes a disturbance to Stomatal conductance (gs) and transpiration during dry environment. Stomatal conductance refers to a measure of a degree of opening of stomata leading mechanism of CO_2 absorption and transpiration. An experiment interpreted that the rate of stomatal conductance, transpiration was higher in AM inoculated rice plants than non-AM plants at dry period (Porcel et al. 2015) as water uptake can be increased with the help of mycelium of AM fungi allowing higher water contents rate in AM plants than the non-mycorrhizal plants (Marulanda et al. 2003). From another study revealed that gs was 24% higher in AM plants than NM plants at all moisture conditions and under severe drought, as AM has the positive influence on gs over four times than ample water condition (Augé et al. 2015). To mention the benefits of mycorrhiza in balancing water status in plants cell, Augé (2001) showed the multiple effects of AM fungi on plant water relations through various internal mechanisms like soil-root contact, the hormonal symbiosis between plant roots.

Mineral nutrition is hampered during drought, leads limiting plant growth and development. A momentous change occurs in plant physiological mechanisms due to drought especially in C and N metabolisms in the root system (Augé et al. 1992). In drought stress condition uptake of N decreases than the plant needs, this insufficient N has the influence on water status in soil and plant tissue. Due to limited moisture status in the soil, plants appearing in water deficiency and decreased N mobility occurs throughout the plant and soil that resists plant growth very quickly observed in pea plant (Mahieu et al. 2009). Mycorrhizal hyphae help plants to uptake N in a better manner. From a recent study with perennial ryegrass reported that the amount of N had been reduced in lower concentration due to drought in the presence of AM colonization than NM (Lee et al. 2012).

Due to drought, reduction in P uptake and irregular transportation to plant shoots occurs. Metabolic processes like respiration and photosynthesis of plants are restricted because of insufficient P than plant needs (Marschner 1995). Photosynthesis is the most important primary mechanism responsible for plant cell growth is inhibited by drought (Chaves 1991), that prevents plant to sustain in water limiting condition. Drought significantly affects the photosynthesis by closing plant leaf stomata, thus reduces water transpiration and decreases CO_2 absorption (Flexas et al. 2004) by plant leaves, resulting in reduced photosynthesis. This reduced photosynthesis makes reduced assimilation for plants. P deficiency is one of the main reasons for inhibiting photosynthesis, as during photosynthesis carbon is exported to the cytosol as triose-P (triose phosphate) and finally converted to sucrose. The uptake of nutrients (especially P) from fine pores can be increased by extra-radical mycelium of AM fungi as they are able to penetrate finer pores than root hairs (Khalvati et al. 2005), that directly help plants to maintain higher photosynthesis than NM plants. An estimated that limiting P is a recurrent factor limiting to plant development and it has been found that about 40% reduction in accumulated aboveground P occurs in plants due to 22% soil moisture loss (Sardans and Peñuela 2004). AM fungi can increase P uptake and water relations in plant and thereby promote plant drought tolerance that helps plants to develop their photosynthetic activities as well as growth (Li et al. 2014). Also, it has been estimated that effects of AM fungi on the growth of plant during drought episode has a close relation to improved P acquisition (Augé 2001) and AM fungi could make supplementation of about 80% of phosphate fertilizer reduction in the field experiment (Jakobsen 1995).

In drought period, K deficiency occurs due to the limitation of water in soil and plant. Potassium is present in the cytoplasm as an enormous cation, numerous physiological processes of the plant such as protein synthesis, photosynthesis, osmoregulation, enzyme activation, cell extension, stomatal behavior, etc. are related with K sufficiency. The reduced amount of K has the disturbance to the functional activities of stomata that hampers internal mechanisms of water relation and photosynthesis process, therefore reduces plant growth and development. In glycophyte species, K salts play a very important role to the osmotic potential of cells and tissues (Marschner 1995). On the other hand, the reduced amount of K uptake makes less water movement throughout the plant cells and plants become more susceptible to drought. EM during drought stress increases K concentration availability in plant roots and also in leaves especially in poplar plant (Danielsen and Polle 2014). To tolerate drought condition through maintaining sufficient K in plant AM fungi has great importance, an example from the previous experiment demonstrated that during drought stress AM fungi help the plant to uptake K+ for their osmotic adjustment in *Citrus tangerine* (Wu and Xia 2006). This osmotic adjustment helps plant to balance water uptake, cell turgor mechanisms and functions of stomata.

Furthermore, also due to lack of other nutrients during drought, plants face huge damaging effects and AM colonization is able to enhance the other nutrients uptake in plants including S, and some micronutrients like Cu and Zn that play significant value for plants to keep them more durable in drought (Cavagnaro et al. 2010, Tian et al. 2010, Latef and He 2011). Because of having such multi-characteristics of AM at the time of drought, it may help the alder seedlings to be more tolerant to drought conditions than EM, as the dual mycorrhizal species can have dominance for EM in the moist condition and also have dominance for AM in dry conditions (Gehring et al. 2006). The present study has been conducted through taking into consideration the fact of tolerance ability of plants during drought and made a focus on if mycorrhizal colonization helps plants to develop their lifespan under drought stress condition.

1.4 Aims of the study

The aim of the study was to develop a lethal drought stress in different mycorrhizal treatments performed with grey alder or speckled alder (*Alnus incana*) seedlings. The main focus of the research was to assess the responses and response mechanisms of plants of the same species infected with AM or EM fungi and NM (not inoculated with fungi) in response to drought and also to assess the survival of these plants with fungi in severe drought condition. The hypothesis for this experiment was that arbuscular mycorrhiza tolerates drought better than ectomycorrhiza.

2 Materials and methods

The experiment with Mycorrhiza and grey alder started on the 18th of March 2015 and ended on the 19th of July 2015. This experiment was performed as a part of the *Mycorrhizas in the ecosystem* project in the University of Eastern Finland, School of Forest Sciences, Joensuu Campus.

Grey alder seedlings were used to perform mycorrhizas treatments and to find out a lethal drought stress condition from this experiment. Different mycorrhizal types were used; one of them is *Paxillus involutus* B (Kokkola, Joensuu) which was an ectomycorrhizal type. In addition to *Paxillus*, there was an unidentified fungus, which was previously isolated from grey alder mycorrhizas. In case of arbuscular mycorrhiza, two different fungi such as *Glomus hoi* (MTT Laukaa) and *Rhizophagus intraradices* (Myko-Ymppi, MTT Laukaa) were used.

2.1 *Paxillus involutus*

Paxillus involutus is one of the common mushrooms which is also called as a brown roll-rim, sometimes common roll-rim, brown-spored, also gilled mushroom. The northern hemisphere is the most common area where this basidiomycete fungus usually found. They have characteristics of brown color body shades, their fruit body normally having range up to 6 cm in height and they form a funnel-shaped cap which is about up to 12 cm in width. Genetic study of this fungus undoubtedly reports that this species fungus is gilled bolete in comparing to other typical agarics. This mycorrhiza has the symbiosis mostly with the variety of deciduous and coniferous trees. It has great value and advantages as it has been used broadly in various research sites and also in seedling inoculation program (Taylor et al. 2000).

2.2 *Glomus hoi* and *Rhizophagus intraradices*

Glomus is a genus of AM fungi, it is also considered as the mostly biggest genus of AM fungi because of having 85 species along with it. All species under this genus can have a symbiotic relationship with plant species. This genus is derived from the family of Glomeraceae and the division of Glomeromycota. *Glomus hoi* species is one of the very important species under this genus and is often considered as obligate symbionts, as it forms the mycorrhizal relationship with plant roots to complete their life cycle. This species is usually developed throughout the all terrestrial habitats also in deserts, the area covered with grass, crop growing lands, tropical forests, and some Arctic regions of Europe, Asia, and North America. They have a lot of advantages to their host plants and also have benefits to nutrient uptake mechanisms. *Glomus hoi* species also has the ability to tolerate drought and can perform well to increase disease resistance ability. This species has been found in some pot cultures with transplanted plants of sea coast or desert, forests, and roadsides but in case of northern areas, this species has been found in a cultivated field at the MTT Laukaa Research and Elite Plant Station in Central Finland (Vestberg et al. 2005).

Rhizophagus intraradices is an arbuscular mycorrhizal fungus under the same family of Glomeraceae and division Glomeromycota. This fungus can play a vital contribution in agricultural and horticultural sectors as they make colonization with plant species and have great advantages on plants. They are available almost in all soil areas, especially in the area of soil covered with available host plants and also in forests and in lands covered with grass. Also, it has been found to colonize new plants in colonized condition through its spores and hyphae (Klironomos and Hart 2002). This fungus has been found to represent a helpful role in phosphorous uptake in case of different plant species that can bring momentous development of soil aggregation.

2.3 Pot Selection and Plant Growing

A “semi-aseptic” growing manner, i.e. other fungi than the studied ones are eliminated, was applied. Washed pots (165-cm^3 plastic pots, Ray Leach Cone-tainer SC-10, Stuewe & Sons, Corvallis, OR) were sterilized in 70% ethanol (diluted with MilliQ water) overnight for this experiment. Filter fabric (sterilized also in ethanol) was put on the bottom of the pot to fill holes in the pot. The pots were filled with acid-washed perlite to 1 cm from the top.

Seeds were surface sterilized before sowing on the pots. The seeds in stainless steel tea sieves (“tea balls” that are opened/closed like pliers) were left in tap water overnight. Next day, one drop of Tween 80 was added on the seeds and the seeds were then shaken for 5 minutes. 30% H_2O_2 was added to the seeds. The seeds were kept in hydrogen peroxide for 15-20 minutes and shaken occasionally. After that, the seeds were rinsed for 5 times in sterilized deionized water.

All pots were arranged in a randomized block design with 50 replicates in the growth room, 3 treatments were applied to conduct this study. 8 seeds were sown in each of perlite filled pot and dressed with a thin layer of soil at the top. Seedlings were grown in a growth chamber (Conviron GR77, Controlled Environments, Winnipeg, MB, Canada), 20 hours a day at 22 °C in 70 % relative humidity, 4 hour night at 17 °C in 80 % relative humidity. Cooling/warming rate was 5 °C h-1. Day/night irradiance was about 350/0 µmol m-2 s-1 PAR from incandescent lamps (60 W, Oy Airam, Finland) and fluorescent tubes (VHO 215 W, Sylvania

Cool White, Sylvania, USA). The germination conditions were 22 °C, 90 % RH, only fluorescent tubes, 20- h day, 4-h night. At the beginning and end of a day, the light intensity level was changed stepwise during 2 h.

The trees received deionized water regularly before the fungus inoculation. AM cultures were mixed 1:1 volume before inoculation with plants. Ectomycorrhiza and arbuscular mycorrhiza for the inoculation were prepared by the following form:

-EM: 3 fungi pieces (5 mm x 5 mm) from Hagem agar dishes + 2 ml killed AM inoculum mixture

-AM: 3 Hagem agar pieces + 2 ml AM inoculum mixture.

-NM: 3 Hagem agar pieces + 2 ml killed AM inoculum mixture

After 3 weeks growth seedlings were accompanied with EM (ectomycorrhiza) or AM (arbuscular mycorrhiza) or NM (non-mycorrhiza) fungi inserted in the pots. This was regarded as the day 1 in the experiment. After the inoculation, the seedlings were watered nearly every day with the amount of 50 ml of water. After 3 weeks of inoculation, plants were fertilized 5 days in a week with nutrient solution containing Kekkilä irrigation fertilizer N-P-K 17-4-25 and all other nutrients. At first, the nutrient solution contained 20 mg N/l. The amount of fertilizer was increased during the experiment time until 50 mg N/l. Pot trays were relocated weekly to minimize possible effects of different locations in the growth room.

After 4 weeks of fungi inoculation, extra plants were removed from every pot. Extra seedlings were cut with the help of scissors and the most healthy-looking plant was left in each pot for their better growth. From 6 weeks of inoculation, the height of all plants was measured weekly with the help of a ruler and a digital caliper was used for measuring the diameter. Watering was stopped after 12 weeks of seeds sowing to create a drought environment to 150 seedlings among all seedlings. Before two weeks of creating drought condition in first 150 seedlings, height and diameter of all plants were measured daily and it continued to those 150 seedlings till the experiment completed.

2.4 Other parameters measurement

The following parameters were examined during the drought period:-

Soil moisture was calculated from the soil part comprised of pot + veil + perlite + AM inoculum + agar + soil water. The weight of the perlite + AM inoculum + agar + water was found by subtracting the pot + veil weight. The weight of the dry perlite + dry AM inoculum was subtracted to get the weight of the water.

The moisture status (%) was calculated by the following formula:

The water content (%) = 100 * weight of the water (g) / weight of (dry perlite + AM inoculum)

It was calculated from dry day 0 to end of the experiment. Leaf temperature was measured by an infrared thermometer from dry days 0-5. Leaf temperature was measured to get an idea of the water condition of plants: plants those have enough water have their stomata open for transpiration and due to evaporating water taking energy this keeps the leaf temperature lower than when there is no evaporation from drying plants that close their stomata. Drooping of leaves was counted by counting the number of drooped leaves in each plant. By counting this it was found that which mycorrhizal plants droop their leaves earlier than others and thus the time until leaf drooping in drought condition was calculated. Due to severe drought, plants loosed cell water with time and they never ceased water loss, as a result, all the leaves and stems loosed their green color and became dried; plants were considered as dead when all the leaves were brittle or fallen. The number of the dead plant was counted to find out the survival time until plant death in the drought condition, finally, a re-watering was done to all the seedling plants to check if they all are already dead.

2.5 Statistical analysis

All the data for result analysis were put one by one in the IBM SPSS 21 version file. The height and diameter of plants were analyzed for each measurement day. One way anova and post hoc test (LSD) was used to get the differences separately for each day of measurement.

Soil moisture was analyzed with the data for dry days 0, 2, 4, 8, 16. Leaf temperature was analyzed in dry days 0-5, soil moisture was used as the covariate. One way anova, General linear model, and covariate were used to analyze soil moisture, leaf temperature, time in dry days before drooping of leaves and seedling lifetime.

3 Results

3.1 Effect of mycorrhizal treatments on height

From the eight measurements of plant height, five measurements were taken before drought occurring and results showed that the height of EM and NM plants increased more than AM plants. Three measurements were taken after drought, height was also increased in relation to time and same results were found as before drought in comparison of mycorrhizal treatments. During before and after drought period, a significant difference was observed in diameter of seedlings between EM and AM (pairwise $P \leq 0.008$) and between NM and AM treatments (pairwise $P \leq 0.001$) and no significant effect was not found between EM and NM treatments (pairwise $P \leq 0.480$) (Figure 1).

3.2 Effect of mycorrhizal treatments on diameter

In diameter, five data were analyzed in total, two of them were before the drought and it was observed that the diameter of plants increased for EM and NM plants in comparing to AM. Three measurements were taken after the drought started and same results were noticed as before drought. During before and after drought, a significant difference was observed in diameter of seedlings between EM and AM (pairwise $P \leq 0.002$) and NM and AM treatments (pairwise $P \leq 0.001$) and no significant effect was found between EM and NM treatments (pairwise $P \leq 0.485$) (Figure 2).

3.3 Effect of mycorrhizal treatments on soil moisture and leaf temperature

Five days measurement after the drought started, were analyzed to find out the effect on soil moisture and it was not significantly affected any of the treatments, there was no significant difference found between EM and AM (pairwise $P \leq 0.933$), between EM and NM (pairwise $P \leq 0.938$) and between AM and NM (pairwise $P \leq 0.992$) (Figure 3).

Leaf temperature of all treatments was normally increased as the drought increased from the drought day 0-5 and no significant difference was found between EM and AM (pairwise $P \leq 0.658$), between EM and NM (pairwise $P \leq 0.597$) and between AM and NM (pairwise $P \leq 0.356$) (Figure 4).

3.4 Effect of mycorrhizal treatments on time until leaf drooping

During the drought period, no significant difference was not found for leaf drooping time between EM and AM (pairwise $P \leq 0.355$), between EM and NM (pairwise $P \leq 0.461$) and between AM and NM (pairwise $P \leq 0.112$) (Figure 5).

3.5 Effect of mycorrhizal treatments on seedling lifetime

The most remarkable significant result was found after analyzing the seedling lifetime for mycorrhizal treatments during the drought, where significant difference was observed between AM and NM (pairwise $P \leq 0.031$) but EM did not significantly differ from AM (pairwise $P \leq 0.286$) and from NM (pairwise $P \leq 0.231$) (Figure 6).

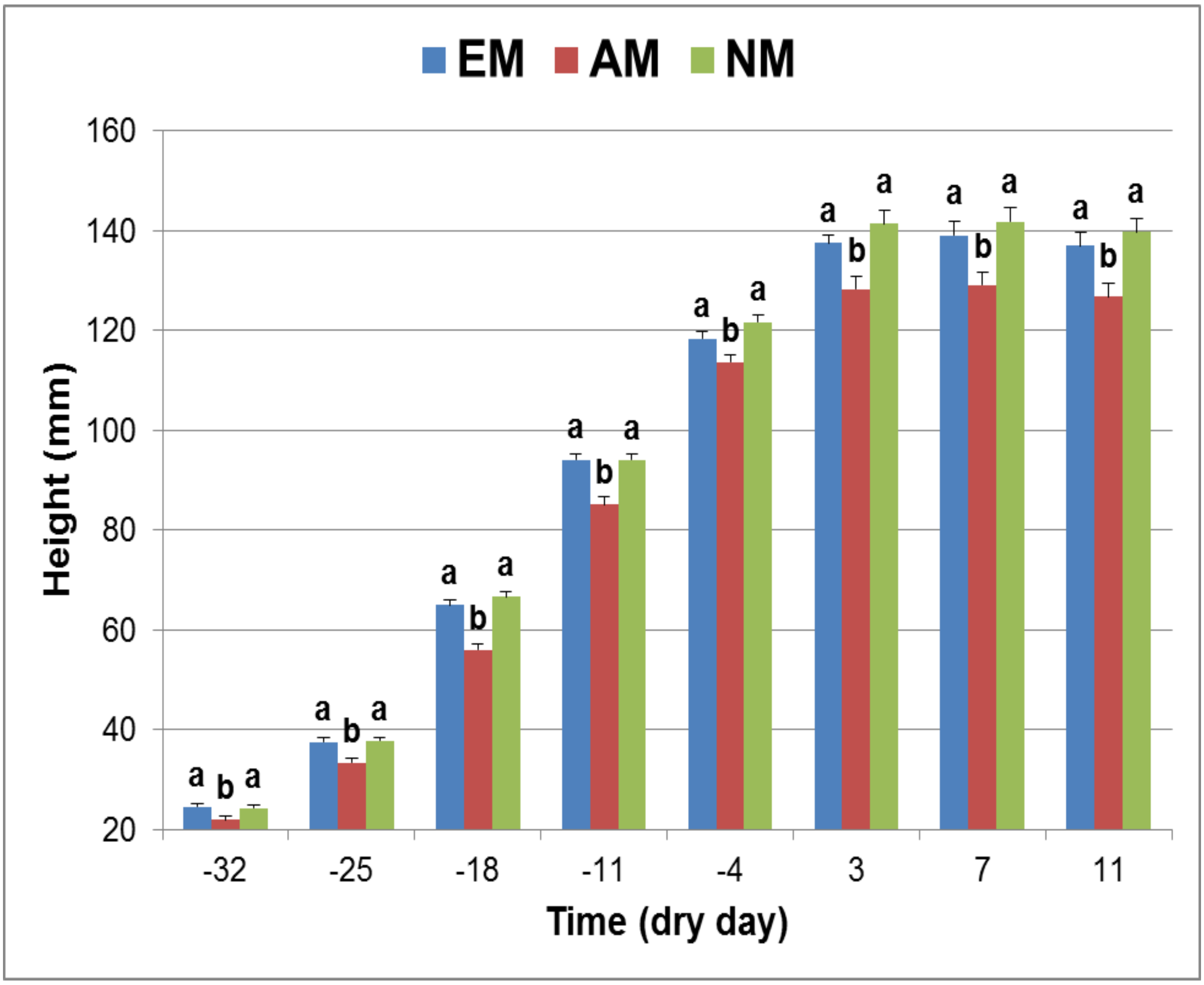

Figure 1. Mean height (+SE) of alder plant with different mycorrhizal treatments, n=50. Different letters indicate significant differences between the treatments within a day ($P \leq 0.05$).

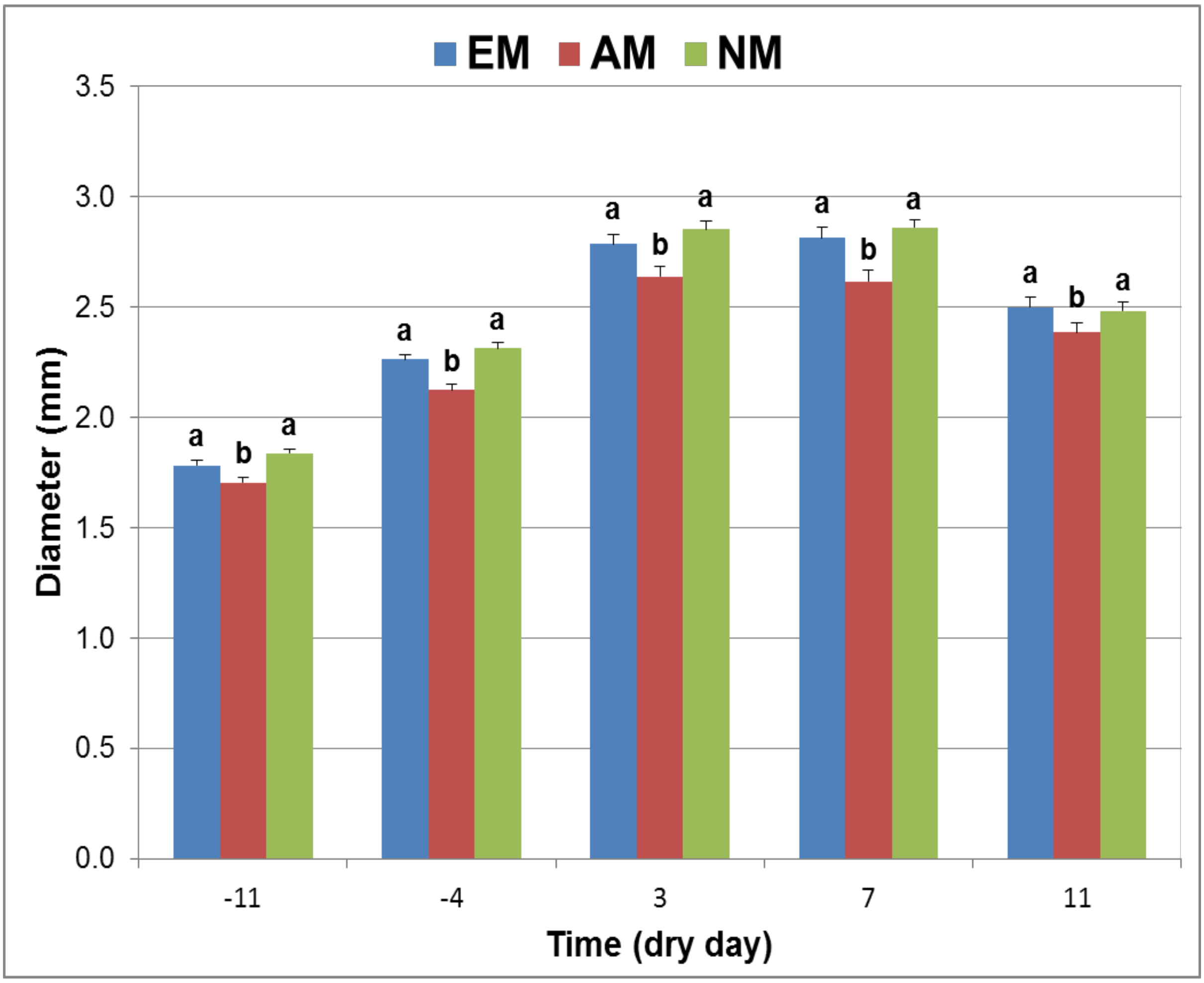

Figure 2. Mean diameter (+SE) of alder plant with different mycorrhizal treatments, n=50. Different letters indicate significant differences between the treatments within a day ($P \leq 0.05$).

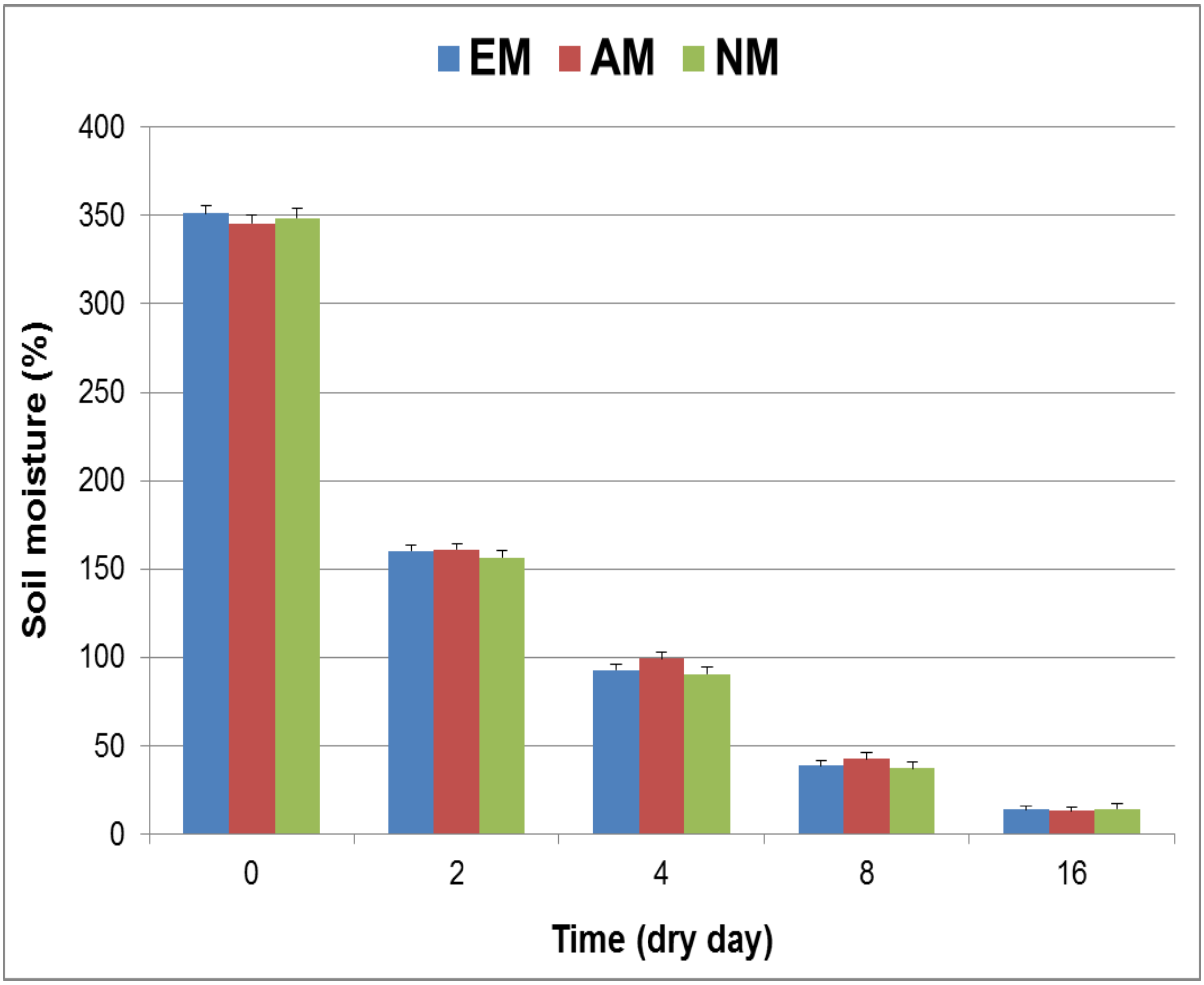

Figure 3. Mean percentage (+SE) of soil moisture in alder plant with different mycorrhizal treatments, n=50. No significant differences between mycorrhizal treatments.

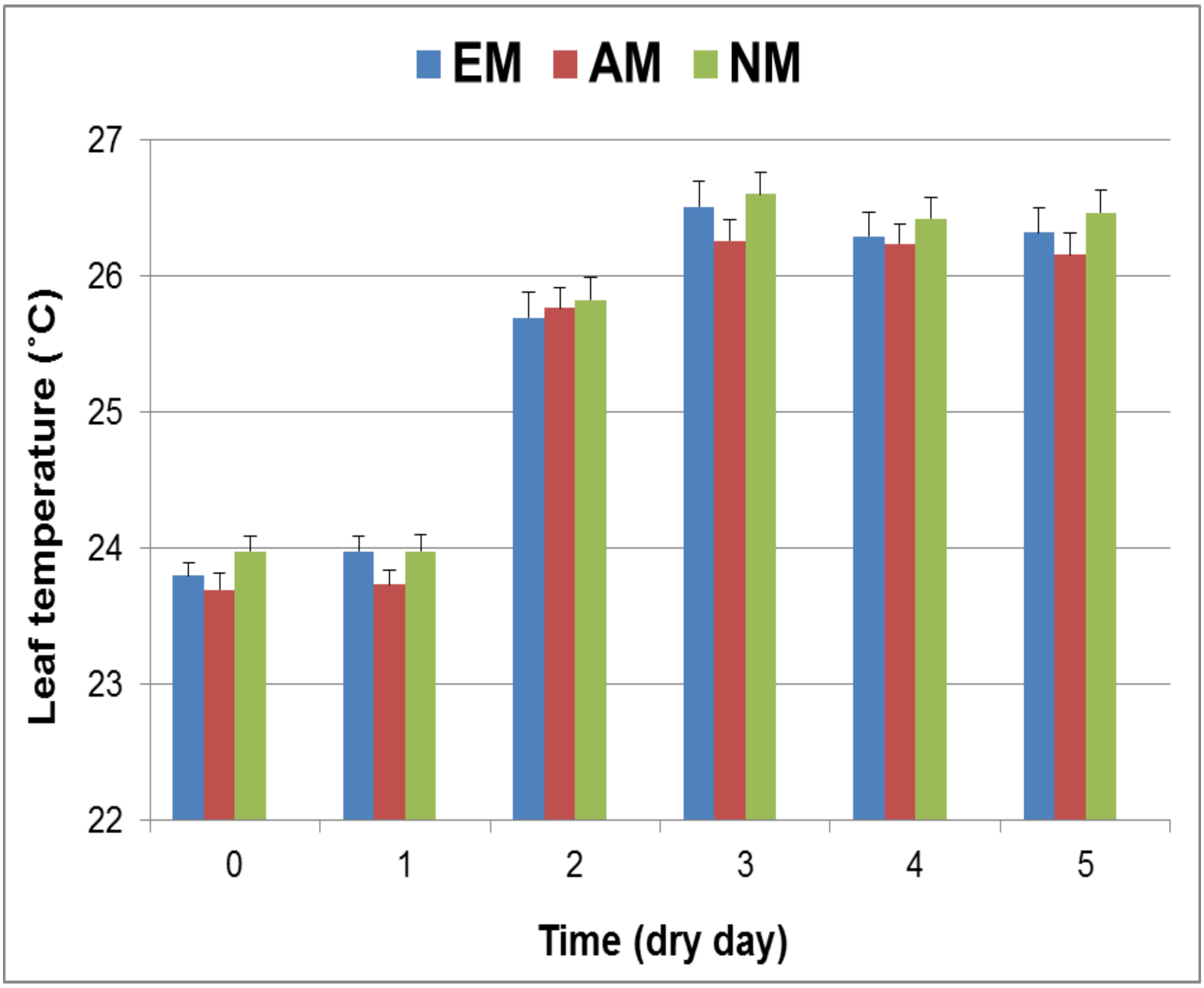

Figure 4. Mean leaf temperature (+SE) of alder plant with different mycorrhizal treatments, n=50. No significant differences between mycorrhizal treatments.

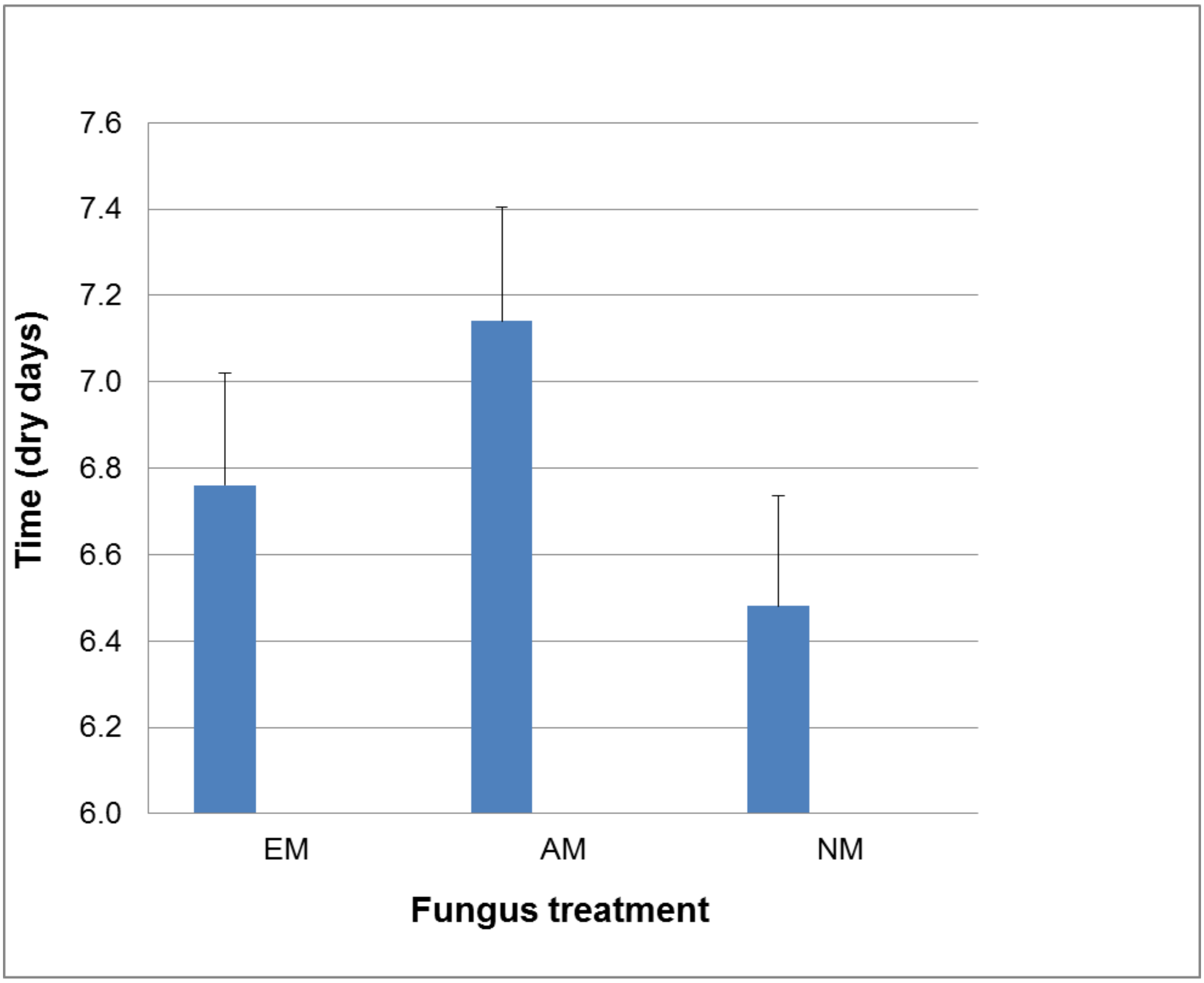

Figure 5. Mean time (+SE) in dry days until leaf drooping in alder plant with different mycorrhizal treatments, n=50. No significant differences between mycorrhizal treatments.

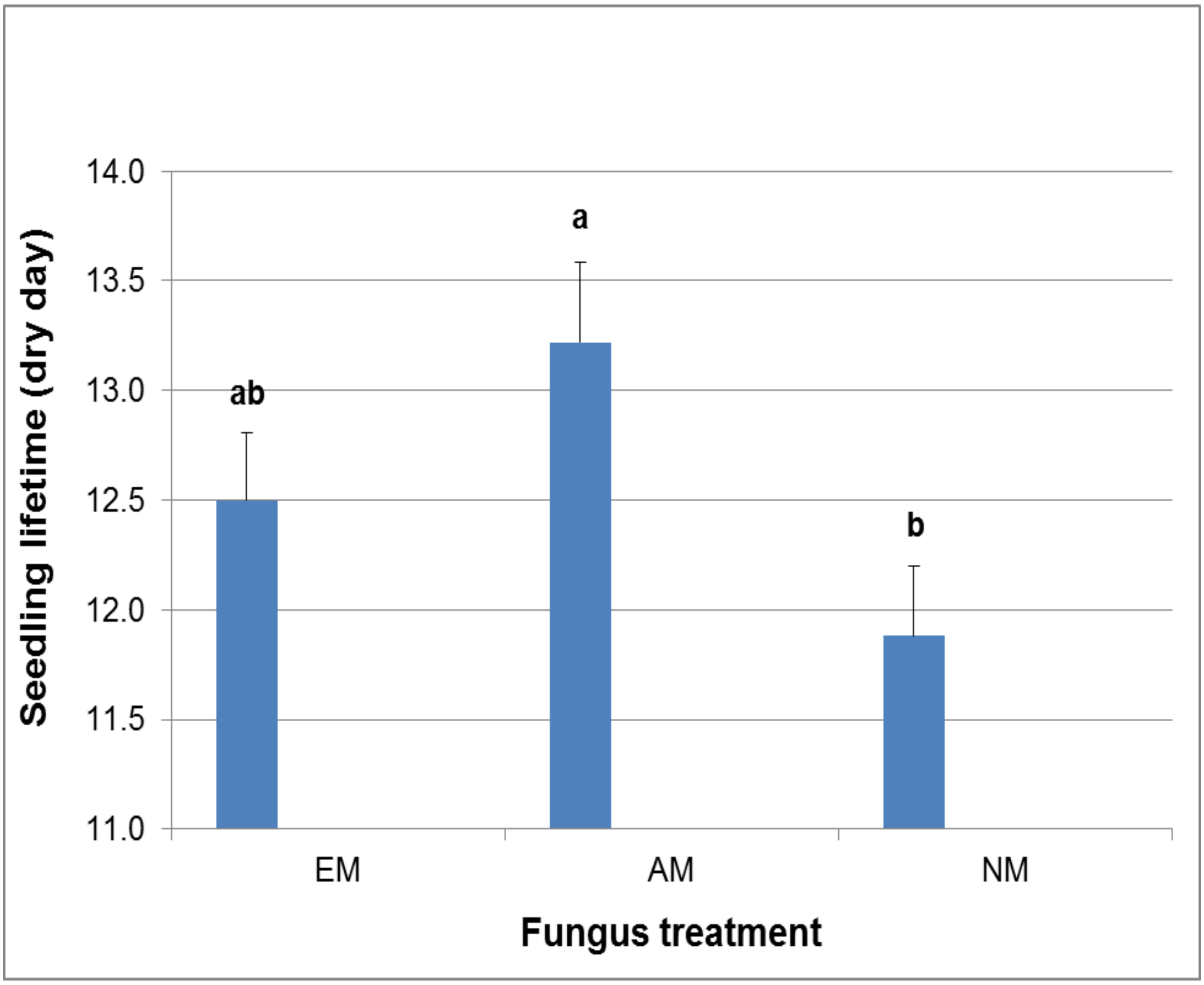

Figure 6. Mean seedling lifetime (+SE) in dry days until the plant death with different mycorrhizal treatments, n=50. Different letters indicate significant differences between the treatments ($P \leq 0.05$).

4 Discussion

Drought significantly makes water loss from the plant tissues and thereby affects the plant growth and development unfavorably. The main objective of the present study was to find out the different mycorrhizal effect on the plant under drought and also to develop a lethal stress condition at drought environment. In this study, the role of three different mycorrhizal treatments on alder plant was tested in a few specific parameters to see how the plant performs with these mycorrhizae and what can be the effects of those treatments in drought stress condition. The mycorrhizal colonization may improve the plant mechanisms during drought and develop their tolerance to severe drought stress that can be a good utilization in future research through modifying the present estimation.

The height and diameter regarding plant growth in different mycorrhizal treatments during drought stress from this study demonstrated that in the both before drought and during drought period EM inoculated alder plants had the positive growth rate than the AM inoculated plants. To elucidate more clearly, this study showed EM plants increased the plant height and diameter than the AM plants which is incompatible with report that AM inoculation play important part to improve seedlings growth which has been elicited through larger plant height due to larger water uptake status and greater amount of chlorophyll content (Fan & Liu 2011). However, this study result is not generalized and inconsistent with most of the previous studies given clearance that AM fungi play the beneficial role to improve plant growth than NM (Augé 2001, Bolandnazar 2009, Cho et al. 2009, Latef & He 2011). Also opposite to other findings estimated in most of the pot experiment with AM fungi showed progressing in plant growth (Shukla et al. 2013, Rapparini & Peñuelas 2014). Another estimation resulted about 80% of studies with mycorrhizal colonization had the same report in plant growth that AM plants showed higher growth characteristics than NM plants (Augé 2001) which are not in line with present results that NM plants showed the increased rate of growth than AM during the time of drought.

With the increasing of time in dry environment plants were subjected to lose their entire moisture, all the seedlings of different mycorrhizal treatments gradually loosed their cell moisture and no significant effect was found on soil moisture for any of those treatments. This result could be inconsistent with earlier study elucidated that AM fungi have the ability to collect water through hyphae and to increase the water uptake ability of plants (Ruiz-Lozano & Azcón 1995). Another opposite estimation of better water uptake through AM fungi has been demonstrated as AM fungi play a notable character to keep the balance of host water relationship in an effective manner (Augé 2001). As plants loosed water and became dry with the increasing of leaf temperature, all the treatments had no significant effect on leaf temperature.

At the same time running with experiment, no difference was found for leaf drooping in dry days for any of the mycorrhizal treatments which is incoherent with the previous investigation suggested AM inoculated plants drooped their leaves in a lower range of number than the NM plants that have been reported in case of wheat plants (Ellis et al. 1985). The present study results also may not in line with another study results revealed from an estimation, higher green leaf area (27.5%) was observed in AM maize plants in comparing to NM maize plants under drought stress condition (Subramanian et al. 1995).

Furthermore, no significant difference was found for the seedling lifetime between EM and NM plants. On the other hand, the result was not significantly differed between EM and AM treatments, but in this study continuous drought turned out to plant death and a very significant difference was found for AM fungi on the survival time of plant seedlings in comparing to NM plants which can be supported by the findings of better drought tolerance of AM than EM (Allen et al. 1995). This increased seedling lifetime in AM plants is well agreed with previously investigated findings presented that, AM seedlings exhibited higher survival rate than NM seedlings in case of *Artemisia tridentata* during very dry environment where soil dried below the range of soil Ψ (water potential) -2.5 Mpa to -3.8 Mpa (Stahl et al. 1998). This also agrees with the other findings of Fan & Liu (2011), who found that under drought condition AM fungi inoculated plants had greater tolerance to drought than NM plants. The findings of the current study is also rational with the findings of some previous research showing the AM plants ability to tolerate stress environment like drought (Cuming et al. 2007, Cho et al. 2009, Debiane et al. 2009, Porras-Soriano et al. 2009, Latef and He 2011).

The reasons of this result may be consistent with some results of previous studies addressed that plants can defend them from the mischievous consequences of drought with the help of AM fungi (Augé 2001, Abdel-Fattah et al. 2002, Ruiz-Lozano 2003). To support these things it has been already elucidated that AM fungi play a vital role in the favor of plants during drought stress (Augé 2001, Ruiz-Lozano 2003, Brown et al. 2012). Another study has recently declared AM fungi develop the plants lifetime during drought stress condition (Sun et al. 2017) as they enhanced the water use efficiency in host plants (Jensen et al. 1996, Tardieu et al. 1999) and also accelerated the status of nutrition available for plants (Augé 2001). Another explanation from Bartels (2001) may be a reason of more survivability of AM that AM fungi protect plants from oxidative stress and eliminate the reactive oxygen species during the drought that could be very efficient approaches for plants to tolerate drought. To make a connection with beneficial effects of AM fungi during the drought period, AM plants can deposit more organic solutes of lower molecular masses those are important for plants to tolerate drought stress as this helps plants to enhance water uptake in the very dry environment (Wu et al. 2006).

5 Conclusion

In conclusion, the present results suggest that during the drought period, height and diameter of alder plants were increased by EM and NM treatments as compared to AM. However, soil moisture retention capacity was not affected by any treatments during drought. No difference was found in leaf temperature for different mycorrhizal treatments. Leaf drooping was not affected by any treatments at all during the whole drought period. Although no significant difference was found in the seedling lifetime for EM plants from AM plants, the survival ability of seedlings was significantly increased by AM inoculation in comparing to NM plants. This revealed that AM can tolerate better dry weather than the NM treatments, as NM did not differ from EM this could be indirectly supportive to AM has better tolerance in drought than EM. Therefore, to explain in a different way, AM improved a lethal stress condition in drought in comparing to EM and NM, which supports the hypothesis of this study that AM can tolerate drought better than EM. In a word, depending upon the AM fungi colonization, plants adopted all the harmful effects of enhanced drought condition and survived for a long time than the other treatments. In principle, this study shows that EM fungi play more vital role on rapid growth of plant than the AM during drought but AM are more tolerant to drought than EM fungi, as mechanisms of water absorption by root and nutrient uptake in the host plants can be more flexible and easier by AM fungi to the plants.

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