



The influence of arbuscular mycorrhizal fungi on drought stressed selected varieties of wheat (*Triticum aestivum* L.)

Fatma Ali¹, Tabassum Yaseen^{1*}, Rani Gul², Kamran Akbar¹, Muhammad Imran³ and Khushnood Ur Rahman^{3*}

¹Department of Botany, Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan

²Department of Agriculture Bacha Khan University, Charsadda, Khyber Pakhtunkhwa, Pakistan

³Department of Botany, Islamia College, Peshawar Khyber Pakhtunkhwa, Pakistan

*Corresponding authors email: Khushnood Ur Rahman (drkhushnood@icp.edu.pk); Tabassum Yaseen (tabassumyaseen@bkuc.edu.pk)

Abstract

Drought stress conditions adversely affect the crop production, but fortunately mycorrhiza help the crops in coping with the conditions, and the impact should be determined. For the same purpose two wheat varieties were introduced to the arbuscular mycorrhizal (AM) fungus and the impacts were calculated in the form of grain production, and mineral acquisition. (*Triticum aestivum* L.) Cultivars that have been cultivated in the field in both well-watered and water-stressed environments. Results showed that, mycorrhizal plants had better biomass and grain yields than non-mycorrhizal plants. in comparison moderate or severe water stress treatments, mycorrhizal plants had considerably greater total dry weight and leaf chlorophyll concentrations than non-mycorrhizal plants. Throughout the experiment, plants were treated to low-level water stress and severe water stress (Mild (50%) and severe (30%) water deficit regimes). The effect of water levels on wheat Growth parameters (vegetative stage), as in control the mean value of number of leaves (6.4 ± 1.527), plant height (23 ± 2.6) and root length (2.67 ± 0.57) while compared with the treated crop (*Triticum aestivum* L.) Number of leaves (4.33 ± 0.577), plant height (15 ± 1) and root length (1.66 ± 0.577) there were clear difference that Arbuscular mycorrhizal inoculated plants were more pronounced effect than the other treatments and control. By comparison the plants having Mycorrhizal association vegetative stage the means of plant height (23.33 ± 1.154), No of leaves (9.33 ± 1.527), root length (4 ± 0) was higher than non- treated plants havin plant height (20 ± 1), No of leaves (7.66 ± 1.15), and root length were (3.33 ± 1.154). the identified fungal species were (*Glomus* spp. *Acaulospora* spp. *Sclerocystis* spp) and root colonization were also observed root colonization was high in inoculated plants than non-inoculated plants. © 2022 Department of Agricultural Sciences, AIOU

Keywords: Arbuscular, Mycorrhizal fungi, Water stress, Wheat

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Introduction

Arbuscular mycorrhizal fungi (AMF) is the most effected agent for the facilitation of indigenous plants to well grow in stressful conditions by the complex metabolic adaptation between the plant and the AMF and thus boost process of photosynthesis and other physiological processes like water uptake (Birhane et al., 2012). Different studies reported that fungal symbiosis combat plant stresses namely drought, salinity, herbivory, heat, metals, and infections (Abdel-Salam et al., 2018). 90% of the plant flora, which includes of Angiosperms, bryophytes, pteridophytes etc can form association with AMF (Ahanger et al., 2014). AMF make vesicles, arbuscules, and hyphae in the roots of all these plants while spores and hyphae in the rhizosphere soil. The formation of hyphal network of AMF with the net of plan roots increases the chance of nutrients absorption due to increased surface area and thus a fruitful plant growth possible (Bowles et al., 2016). AMF by increasing the process of translocation empowers the nutrition uptake (Rouphael et al., 2015). Soil health is influences on positive side by the AMF presence in the soil as it has good effects on the soil texture. A good quality of soil and their structure and plant morphology as

well as caused by AMF (Thirkell et al., 2017) also AMF can improve the process of decomposition of soil organic matter (Paterson et al., 2016). In addition, mycorrhizal fungi can affect the hair and atmospheric CO₂ of plants by enhancing the "flow" effects and migration of photoassimilates from aboveground parts and roots. Given the need for AMP and the progress of research and its application in agriculture, this review focuses on the role of AMP as a biofertilizer, regulating plant growth and development, as well as better appropriation and nutrition, stressful conditions and AMP levels, can improve plant growth in a stressful situation.

The local steppe limited (semi-arid or covered with grass or with shrubs or with both) has an impact on Charsadda. Throughout the year, there is very little rain in Charsadda. According to Koppen-Geiger climate, classification the temperature here averages 22.5 °C. The average annual rainfall is 460 mm. June is the hottest month of the year, with an average temperature of 33.3 degrees Celsius. The average temperature in January is the lowest of the year. The temperature is 10.4 °C June is the driest month, with only 11 mm of rain. August has the most precipitation, with an average of 82 mm. There is a 71 mm difference in precipitation between the driest and

wettest months. The average temperature varies by 22.9 degrees Celsius throughout the year. The lands of Charsadda are fertile and agricultural and farmers are very active. Major crops of Charsadda Tobacco, Sugarcane, Sugarbeet, Wheat and Corn. The main vegetables for Charsadda are Potato, Tomato, Cabbage, Brinjals, Okra and Spinach. The main fruits of Charsadda are Apricot, Citrus, Plum, Strawberry and Pears. Mycorrhiza is a symbiotic association between roots of higher plants with fungi, the fungus colonize the roots of the host plant, either intracellularly in the case of arbuscular mycorrhizal fungi (VAM) or extracellularly in the case of ectomycorrhizal fungi (ECM). They are an essential component of soil life and soil chemistry (Kanyagha, 2008). AMF can symbiotically interact with almost all the plants that live on the Earth. They are found in the roots of about 80-90% of plant species (mainly grasses, agricultural crops and herbs) and exchange benefits with their partners, as is typical of all mutual symbiotic relationships (Wang & Qui, 2006). About 80% of all terrestrial plants, including most agricultural, horticultural and hardwood crop species are able to establish this mutualistic association (Yaseen et al., 2011). Rhizosphere is the volume of soil under the influence of roots and roots itself this area more complex environment from the physical, chemical and biological viewpoint, than the aboveground area, around the aerial part rhizosphere is under the influence of root and microflora (Currie et al., 2011). Depending on their morphology and the involved plants and fungus species, the beneficial effects of mycorrhizae fungus on plants performances and soil health are essential for sustainable management of agricultural ecosystems (Smith & Read, 2008).

Various crops form arbuscular mycorrhizae, in which arbuscules are formed by repeated tree-like branching of hyphal filaments inside root cells (Pozo & Azcón-Aguilar, 2007). The fungal species that make up mycorrhizae take up 25% of the production of plant photosynthesis and can provide P and N up to 80% of the plant needed (Aragno, 2005). The mycorrhizae are symbiotic association, during which specific fungi are colonizing plants rootlets. In this type of relation, pathogenetic and lesion of root structures are normally missing and the plant blocks the fungal invasion. In this relation, plants are providing organic compounds for the fungal symbiont, and receive, in exchange, inorganic nutrients absorbed by hyphae. Due to the small size of hyphae, the absorptive surface and the explored volume of soil are very large, and the formation of root hairs is no longer necessary (Meyer et al., 2010). Some mycorrhizal species are considered common and spread in different habitats, and other species considered rare are forming mycorrhizae only with some host plants (Ene et al., 2010), (Fodor 2010) find the effect of Arbuscular mycorrhiza (AM) fungal inoculation on growth, production and nutrient uptake in two types of cowpea (*Vigna unguiculata*). AM-vaccinated plants are made without plants that can be controlled in terms of growth, production limits and nutrient uptake. AM fungal inoculation has a major impact on cowpea production due to growth, crop height, and number of nodules, mycorrhizal dependence and number of flowers per plant.

Water stress affects almost every aspect of plant growth and body composition. Crop responses to water scarcity depend on a variety of factors, such as duration and pressure, growth phase and time exposure to stress (Condon et al., 2004). Because of their sedentary lifestyle, plants turn to a number of adaptive strategies in response to various abiotic pressures such as high salts, dehydration, cold and heat, which ultimately affect plant growth and production (Iturbe-Ormaetxe et al., 1998). Drought and water scarcity alter Chl levels and components, as well as harm agricultural plants photosynthetic system (Gill et al., 2003). Recent years, plant inoculation with beneficial bacteria and fungus has got a lot of interest (Arzanesh et al., 2011). Symbiosis between plant roots and fungi is a method that can be used to increase the absorption of nutrients and water by plants in a stressful situation (Abdul-Wasea & Elhindi, 2011). Symbiosis in AM infected plants has been found to alter growth as well as contribute to better tolerance to biotic and abiotic challenges (Augé, 2001). Furthermore, under drought stress, the AM injected wheat cultivars showed enhanced growth, grain production, and nutrient absorption. Drought acclimation improved external hyphal growth and soil aggregation in mycorrhizal-treated plants (Davies et al., 1992). Under drought stress condition, AMF can show the positive role in the growth of corn and wheat (Miransari & Smith, 2007). AMF improves nutrition transport from the roots to the host plant and also increase tolerance to biotic and abiotic stresses (Martin et al., 2007).

Materials and Methods

Methodology of soil sampling and root extraction

Different plant roots and soil samples of wheat plants were collected during 2016-2018 from four pots randomly. Thinning was done at various host growth stages. 2-3 healthy wheat plants were collected at each replicated pot along with rhizospheric soil and roots at vegetative stages. After scraping away the top 1cm layer of soil, roots and rhizosphere soil were dug out with a trowel at a depth of 0-15 cm, and varied concentrations were added to the treatment after every week. Collected samples were then processed, pooled and homogenized and evaluated as per protocol. Rhizospheric soil (about 200 g) were air dried for 2 weeks, and then stored in sealed plastic bags at room temperature. The wheat (*Triticum aestivum*) seed that is summer crop and source is NARC Islamabad. Sowing in pots for one month and started our field work. Seeds of *Triticum aestivum* were obtained from National Agricultural Research Centre (NARC), Islamabad, Pakistan. Sowing was done on 27 June, 2020 in clay pots (Fig. 1-3).

Preservation of roots

70% Formalin Acetic Acid (F.A.A.) solution were used for the preservation of plant roots (70 ml alcohol and 30ml distilled water). For staining fungal structures, the method described by (Phillips & Hayman, 1970) was adopted, with minor changes for non-pigmented roots. Preserved roots were cleaned thoroughly with tap water

before being boiled to remove the host cytoplasm and most of the nuclei with 5% KOH for 5–10 minutes (Fig. 2). Tap water was used for cleaning of roots and then dried on

filter paper. Afterwards, they were dried for 3 to 5 minutes with 0.025 percent acid fuchsin.



Fig. 1 Treatments comparison

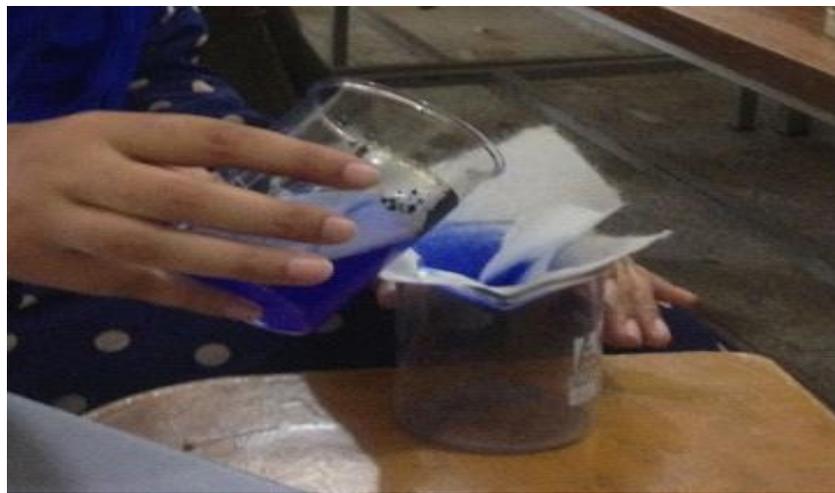


Fig. 2 Staining of roots



Fig. 3 Extraction of spores from soil suspension

Water treatments

Plants were given 200 ml water for the first week, for the second week 100 ml water, for the third week 50 ml water, and for the fourth week 40 ml water was given to plants. From two weeks to the end 30 ml water was applied to the

plants and then the results were recorded. The plants were thinned three times; weak plants were removed only 4 healthy plants left in each pot at the harvesting stage.

Assessment of root colonization

The (Giovannetti & Mosse, 1980) technique was used for root colonization. Root colonization was done by using technique of Giovannetti & Mosse (1980). It is + slide method. The roots were cut out 1 cm long for microscopic study. Morphological structure of AMF entophyte was studied that was recorded in %age. The following formula was used for counting of root infection:

$$\% C = \text{TRC} / \text{TRE} \times 100$$

Extraction of spores

Rhizospheric soil samples were collected of wheat at vegetative stages. 300 g (50 g from each replicated pot 3 treated and 3 control). At a depth of 15-20 cm, a sample of rhizospheric soil was randomly collected from the replicate pots and wet sieving and decanting technique of Gerdemann & Nicolson (1963) was applied. The spores were extracted from the soil samples using wet sieving procedures, sieving techniques including different sizes; 70 μm , 140 μm and 400 μm (Fig. 3). 15 g of treated soil was mixed in 30 ml water and 15 g of control mixed in 30 ml water. Then the solution was shaken and kept for 24 hours to get uniform mixture. The solution was filtered via sieves to remove big particles, and the little residue was placed onto petri plates to investigate spores. The solution was passed carefully from 70 μm , 140 μm , and 400 μm sieves. Depending on the soil texture or extraction goal, the number of sieves can be decreased or increased. The residue was collected on filter paper in petri plates from each sieve and inspected under a microscope independently. Spores were collected using a needle and kept on a slide in a glycerin drop. Spore was estimated by using the method of Stahl & Christensen (1982). Microphotographs of the spores were taken at various magnifications (4x, 10x) the spores were identified with the help of keys (Schenck & Perez, 1990).

Physiochemical analysis of soil

Soil samples were randomly taken from our replicated pots using the procedure of (Jalaludin & Anwar, 1991). Six soil samples of 50 g of rhizospheric soil were taken from our replicated pots randomly (13 August 2018) and dissolved into 100 ml water and cover the beaker for 24 hours. When dried then soil samples were processed for analysis. The texture of the soil was determined by Bouyoucos Hydrometer method as described by Hussain (1989). Organic matter (0.04) was recorded in the study area followed by Walkley- Black method (Nelson & Sommers, 1982). Solubridge conductivity metre was used to assess the electrical conductivity (0.15) of saturated extract (Richards, 1954). pH meter was used to determine the pH (7.76) of saturated paste as advised by Pieczynski et al. (2013).

Results and Discussion

The results show that agronomic parameter of wheat cultivar was significantly increased in control as compare

to treatment at different water regimes i.e., 200 ml, 100 ml, 50 ml and 30 ml. In vegetative stage as in control, the mean of plant height was 23 cm, root length 2.66 cm and number of leaves 6.33 was high as compared to treated plant height 15 cm, root length 1.66 cm and number of leaves 4.33, respectively (Fig. 4). Our result agreed with Jatoi et al. (2011) who stated that water deficiency affect the plant height of wheat cultivar. Leaf survival required for absorption is determined by RWC rather than leaf water potential. However, the production of new leaves depends on the availability of water. When comparing the effects of water stress on leaf number, the highest leaf number was found in the control plants. Our results are also supported by Spollen et al. (2013). Drought stress causes roots to continue to expand in search of water, but the development of airy organs is restricted. This adaptability to dry environments is seen in the distinct growth responses of shoots and roots to drought. In fruiting stage there were clear differences between control and treatment. The mean value of plant height was 23.33 cm, root length 4 cm and number of leaves (9.33) were observed in control, while the plant height (7.66 cm), root length (20 cm) and number of leaves (3.33) were recorded in treatment (Fig. 5). Shao et al. (2007); Alishah & Ahmadikhah (2009) recorded similar results who reported that response of plants or phases of plant growth made a diverse water stress effect. Our results were also confirmed by Wang et al. (2012) that root system plays a fundamental role in crop water absorption. The comprehension of the genetic and the physiological adaptive mechanisms controlling water stress tolerance is an essential aspect in plant biology. For water deficit tolerance, plants need to take out as much humidity as possible from the soil. Therefore, establishment of a profound and profuse root system is needed for the extraction of water from the soil and also the competition with weeds. When comparing the effects of water stress on root length, the highest root length was found in the control plants.

Plates of root colonization in potted experiment (water level) arbuscular mycorrhizal fungal spore density

In the current study, wheat was selected as an experimental plant. There were three type of AMF spores investigated in water stress and control (Fig. 6) the *Glomus*, *Sclerocystis* and *Aucluspora* were observed during our study in both water stress and control conditions. The highest values of spores were found in control as compared to water stress. In control, the *Glomous* were dominant AMF spore having mean value (16), *Sclerocystis* (9) and *Aucluspore* (6), similarly the mean value of *Glomus* was the highest in treatment i.e., *Glomus* (12), *Sclerocystis* (7) and *Aucluspora* (5) were observed in our study. Our result agrees with Raei & Weisany (2013) who stated that Arbuscular Mycorrhizal fungi (AMF) inoculation increased N and P uptake and also enhanced uptake of potassium (K) than that of non-inoculated plants and also our result supported with Manila & Nelson (2013) who reported that AMF spore density was affected due to soil nutrient i.e., P, Ca and soil pH.

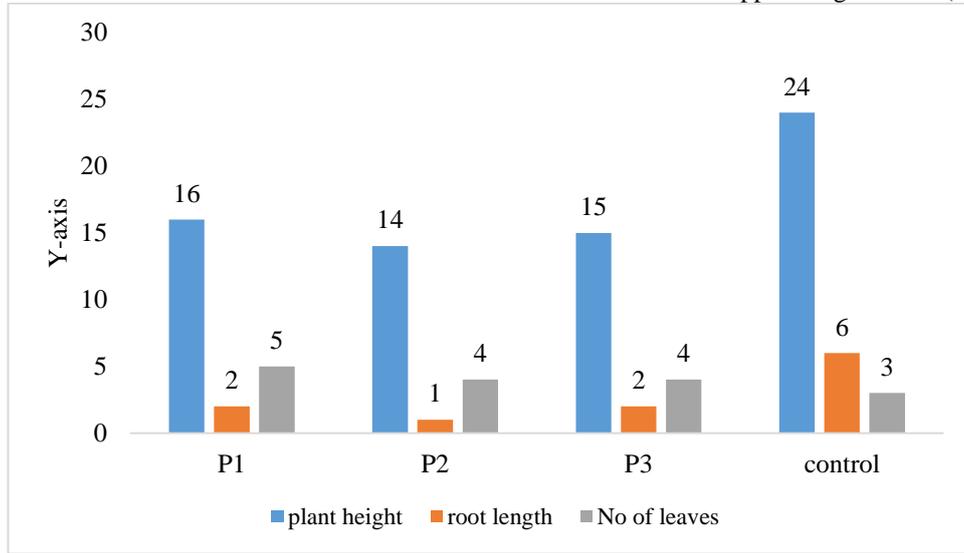


Fig. 4 Effect of different treatment on growth parameter (cm) of wheat crop at vegetative stage

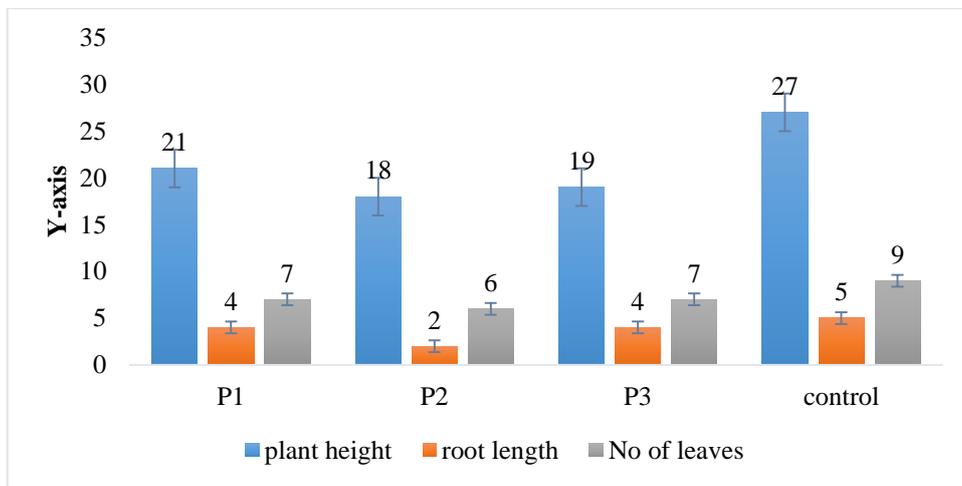


Fig. 5 Effect of different treatment on growth parameter of wheat crop at fruiting stage

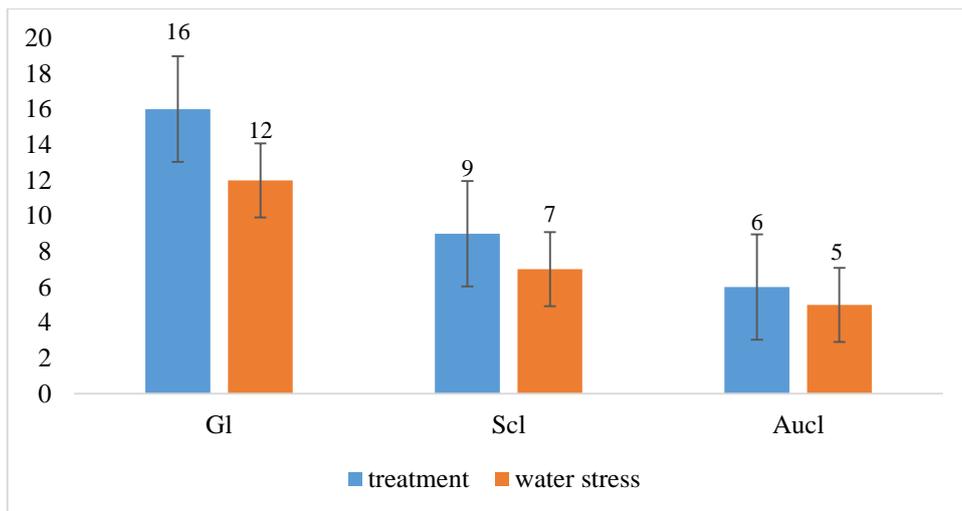


Fig. 6 Mycorrhizal Spores density in our field of district Charsadda
 Gl. = Glomus; Scl. = Sclerocystis = Ac = Acaulospora

In current study wheat was selected as an experimental plant, the study observed that root infection is caused by fungi. Four types of root infection were recorded (Table 1) i.e., arbuscules, vesicles, internal and external hyphae. In present study, the total root infection in wheat plant having mean value of arbuscules were 15, vesicles were 12, internal hyphae were 9 and external hyphae were 8 in treatment. Similarly, arbuscules were 11, vesicles were 9, internal hyphae were 8 and external hyphae were 6 in control. Our result clearly shows that arbuscular mycorrhiza is more in treatment as compared to control. Our result is line with the result of Song et al. (2010) who stated that arbuscular mycorrhiza fungi are also known to vary their response to the mineral environment of soil. The individual dominant root infection is arbuscules followed by external and internal hyphae (Table 1). Our result agrees with the finding of Nzanza et al. (2011) who reported that AMF spore density and root colonization were different from season to season.

Table 1 Arbuscular mycorrhizal fungal root colonization

Table 2 Physiochemical analysis of soil

Texture class	WHC	CaCo ₃	Organic matter	P	Clay	Silt	Sand	pH	EC
Silt loam	24.68%	11.62	0.04	4 ppm	14	53.2	32.8	7.76	0.15

WHC = Water holding capacity; EC = Electric conductivity; P = Phosphorus; ppm = Parts per million

Conclusion

Arbuscular Mycorrhizal Fungi played a significant role in better growth of wheat crop even in drought stress conditions. The influence of micronutrients, bio fertilizer and N, P, K and S application were found on the yield of wheat crop. All micronutrients and bio fertilizer inoculate returned considerably higher in yield and nutrient uptake during drought stress condition.

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Colony	Control	Water stress
External hyphae	6.7 ± 0.57	7.33 ± 0.57
Internal hyphae	7.33 ± 0.57	8.33 ± 1.52
Vesicles	9 ± 1.00	12.66 ± 1.52
Arbuscules	11.66 ± 1.52	15 ± 1.73

Physio-chemical analysis of soil (Fertility evaluation)

The soil was analyzed for the physicochemical investigations. The results of the investigations confirmed that the water holding capacity was 24.68% whereas the CaCo₃, organic matter and phosphorous were recorded as 11.62, 0.04 and 4 ppm respectively. The soil was also analyzed for the texture, which showed that the soil is clay; the pH and electric conductivity were recorded as 7.76 and 0.15, respectively (Table 2). The results confirmed that the soil is not good for the growth of plant cultivations. The results also showed that the soil has fewer mineral contents, which were the basis for the mycorrhizal association present in the soil.

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