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Research Article
Role of Arbuscular Mycorrhizal Fungi on Plant Growth and Reclamation of Barren Soil with Wheat (*Triticum aestivum* L.) crop

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Abstract

Background and Objective: In many semi-arid and barren regions of the world, drought confines the crop productivity. Inoculations of plant with Arbuscular Mycorrhizal (AM) fungi are effective in improving crop production under barren-conditions. This study will investigate the importance of Arbuscular Mycorrhizal (AM) fungi in crop productivity and restoration and reestablishment of degraded ecosystems. **Materials and Methods:** This study was conducted to determine the effects of 3 different indigenous AM fungi i.e., *Glomus mosseae*, *Glomus fasciculatum* and *Gigaspora decipiens* either single and in combination inoculation on growth enhancement of wheat (*Triticum aestivum* L.) grown in the pot experiment under barren soil conditions. **Results:** Various parameters like plant height, root length, shoot biomass, root biomass, fruit size and AM colonization were found to be maximum in *G. mosseae* (alone) and *G. mosseae*+*G. fasciculatum* treatments, whereas other parameters like photosynthetic pigment were found to be highest (1.69 mg g^{-1}) in combined inoculation of *G. mosseae*+*G. fasciculatum* treatment respectively (site II). **Conclusion:** Experimental results showed that AM fungi treated barren soil improved their physicochemical properties as compared to non-mycorrhizal treatment. This result re-affirms the prime necessity of mycorrhiza in semi-arid conditions. Thus the introduction of mycorrhizal fungi in barren land is a key tool to improve the quality of soil and plant growth.

Key words: Arbuscular mycorrhizal, barren, degraded soil, crop productivity, reclamation, restoration

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Present world demands the production of superiority food in a most sustainable way causing least possible damage to the ecosystem. Barren land sites are considered to be worldwide problem, because these restricts plant growth and production in many parts of the world especially in arid and semi-arid environment. The great challenge for the coming decades will be the task of ever-increasing food production with less water, particularly in countries with limited water and land resources[1]. Critical water shortages are developing in the arid and semi-arid regions as existing water resources are entirely exploited[2].

The total land area of Rajasthan is 3,42,239 sq. km out of which 45.25% is characterized as wasteland. Large portion of this land was productive but due to man-made deforestation, cattle pressure and improper water management, they have turned out to be wasteland[3]. These barren soils are generally characterized by poor soil structure, lowest water-holding capacity, organic matter lack and nutrient deficiency. The loss of farmable land due to urbanization and drought (abiotic) stress directly affects the food requirement of the world population, there is a need to use the barren land for food production[4].

A recent discussion on conventional versus organic production systems highlighted how the green revolution helped to meet the needs of an ever-increasing world population but at the prize of environmental pollution[4,5]. The agricultural sustain ability could be viewed as highest plant production with minimum soil loss. The establishment of plant cover is the most important step in restoration of degraded areas. Many researchers have indicated that Arbuscular Mycorrhizal (AM) fungi are capable of alleviating the adverse effects of drought on plant growth[5].

The conventional agriculture has traditionally focused on the potential of mycorrhizae fungi to improve crop yield. The endo-mycorrhizal fungi form of the symbiotic relationship with the root of 70-90% of all known vascular plant species. The AM fungi colonize the roots of host plants and make absorption services for the plant[6]. Various studies have demonstrated that plants associated with AM fungi show increased uptake of various materials from the soil, including water and nutrients compared to non-VAM plants[7]. As an outcome, AM fungi improve their host plants ability to grow under conditions of deficiency of water or in mineral deficient soils[8]. Cereal grains are of indispensable importance for the carbohydrate and protein supply in human and animal nutrition. Wheat is a cereal grain, originally from the Levant region of the near East but now cultivated worldwide. Wheat (*Triticum aestivum*) belongs to family Poaceae. Poaceae (previously known as *Gramineae*) is often well thought-out to be the most important of all plant families to human economies[9].

Therefore, the reason of the present study was to investigate the effect of AM fungal species *Glomus mosseae*, *Glomus fasciculatum* and *Gigaspora decipiens* on plant growth, nutrient uptake and in improving soil properties of pot-grown wheat subjected to barren soil conditions.

MATERIALS AND METHODS

Experimental site and soil preparation: The experimental area is located in the Jaipur, the capital city of the state of Rajasthan, India is situated in the Eastern border of Thar Desert, a semi-arid land (coordinates 26° 55' 19.45'' N and 75° 46' 43.98'' E). The bio-climate can be described as Mediterranean hot semi-arid type with an average annual rainfall of 650 mm and the elevation above sea level 431 m (1417 ft.). The average of maximum and minimum temperatures ranged between summer 25-45°C and winter 5-22°C, respectively during the experimental period.

The experiment was set up in the soil collected from barren lands which were subjected to pot trials. The soil samples were collected from both barren sites, i.e., site-I Tonk road (near Chaksu) and site-II Delhi road (near Amer/Kukus) located in the Jaipur district (India). The soil was collected at a depth of 0-30 cm from the sites air-dried and passed through a 3 mm sieve for further experiment. The soil samples were filled in pots is one of the most significant tools to determine the status of plant nutrients available in a field. In general, most of plants grow by absorbing nutrients from the soil. Their availability to do this depends on the nature of the soil. The air dried and sieved soil samples were analyzed for various parameters like pH (digital pH meter), Chloride ion, Heavy metals, Organic Carbon by Chromic Acid Method[10], Nitrogen by Kjeldahl Method[11], Phosphorous estimated by Olsen's Method[12] at the soil testing laboratory, Agriculture Research Center Durgapura, Jaipur. Soil physico-chemical characteristics before pot trials of both sites (I and II) are presented in Table 3.

Isolation of AM fungi and mass multiplication: The AM fungi were isolated from the plant roots and their rhizospheric soil of wheat plant cultivated from the field by 'Wet sieving and decanting technique'[13]. Collected VAM fungi spores were identified with the help of identification manual of Scheneck and Perez[14] and spores of common species of VAM were identified using synoptic keys of the genera and species of

Zygomycetous mycorrhizal fungi by Trappe[15], on the basis of conventional morphological characters i.e., color, size, shape, cell wall structure and type of hyphal attachment.

Three dominant AM fungi were isolated i.e., *Glomus mosseae*, *Glomus fasciculatum* and *Gigaspora decipiens* for the mass multiplication. Single and pure culture of every selected dominant AM fungus was raised by "Funnel Technique" of Menge and Timmer[16] using *Sorghum bicolor* as host for 2 months. Thus mass culture of specified VAM species was obtained through pure culturing[16].

Selected host plant: Wheat (*Triticum aestivum* L.) was selected as the host plant for this study. In India, wheat, the second essential food crop is grown on 27 million hectares out of the total 114 million hectares of land underfarming[17]. The variety was used Raj-3077, because this variety of Wheat most commonly grown by the farmers in Jaipur region. Wheat seeds were obtained from Agriculture Research Center Durgapura, Jaipur Division, India.

Experimental setup and design: The pot culture experiment was carried out in an "Open air conditions" to know the response of Wheat (*Triticum aestivum* L.) plant with AM fungi (Dec, 2014- March 2015). The earthen pots (25×25 cm) were taken and filled with air-dried sterilized soil (3-4 kg) collected from barren soil of both sites I and II. The root system of regularly well infected sorghum seedlings together with adhering soil were finely chopped and used as the starter inoculums. The pots were filled with 5-10% (w/w) of the inoculums of each AM fungi (alone and combined) as a layer of 1-2 inches below the soil level and surface sterilized (0.05% sodium hypochloride) seeds of wheat (*Triticum aestivum* L.) were planted (sowing) into a 5 cm depth of soil. Four to five seeds were sown in each pot. Thinning was done and only two to three plants pot^{-1} were allowed to grow. The plants were maintained with regular watering[18].

The following treatments were conducted to know the response of VAM on *Triticum aestivum* L. the inoculation with alone and combined VAM fungi.

T_1: Inoculated with *Glomus mosseae* (alone)
T_2: Inoculated with *Glomus fasciculatum* (alone)
T_3: Inoculated with *Gigaspora decipiens* (alone)
T_4: Inoculated with *Glomus mosseae+Glomus fasciculatum* (combined)
T_5: Inoculated with *Glomus mosseae+Gigaspora decipiens* (combined)
T_6: Inoculated with *Glomus fasciculatum+Gigaspora decipiens* (combined)
T_0: Control (without AM fungi)

Three replicates of each treatment were maintained. The VAM inoculated plants were harvested after 90 days (3 months) of growth and observations were taken.

Analysis of various growth, biochemical and yield parameters: Plants were harvested after 90 days by uprooting them from each treatment of AM fungi pot trial combination and various morphological and physiological parameters were measured. After harvest the roots, shoots and fruit size, weight were taken separately to determine fresh weight (biomass) and then placed in an oven to dry at 42-45°C to 48 h until a constant dry weight was obtained[19].

The photosynthetic pigment (chlorophyll) was estimated by using Arnon[20] method by using 80% acetone as solvent. The same physio-chemical parameters were studied on various treatment of the soil samples remained in pots after uprooting the plants.

Mycorrhizal root colonization was studied by 'Rapid clearing and staining method' of Phillips and Hayman[21]. Percentage AM colonization of roots was calculated using the equation:

$$\text{Colonization (\%)} = \frac{\text{Number of root segments with VAM}}{\text{Total number of root segments examined}} \times 100$$

The root segment was considered mycorrhizal even if 1 of the 3-4 structures, i.e., hyphae, arbuscules, vesicles or spores were present.

Statistical analysis: All determinations of plants, biochemical parameters and measurements were conducted using 3 replicates. The value for each sample was calculated as the Mean±SE. Statistical analyses was carried out using Microsoft Excel 2007.

RESULTS

In this present investigation, an attempt was to analyze and study the morphological as well as biochemical parameters of test plant treated with different combination of AM fungal species, the morphological and biochemical parameters of host plant in reference to plant height, weight (fresh and dry) and quantitative estimation of chlorophyll contents in VAM treated plant were analyzed and compared with control.

Plant height: All the different plant growth parameters considerably increased in all the inoculated treatments in comparison to uninoculated control (Table 1). The change in

Table 1: Plant height and mycorrhizal colonization (%) of Site-I and Site-II pot trials winter crop (wheat) plants

	Plant height						Mycorrhizal colonization (%)	
	Root length (cm)		Shoot length (cm)		Fruit size (cm)			
Treatments	Site I	Site II	Site I	Site II	Site I	Site II	Site I	Site II
Control (T_0)	6.66±0.02	6.73±0.15	46.23±0.92	44.70±0.55	7.40±0.10	7.43±0.15	Nil	Nil
T_1	11.24±0.01	11.16±0.20	63.58±1.01	63.83±1.22	8.23±0.32	8.16±0.11	93	90
T_2	11.00±0.09	10.76±0.35	60.56±1.72	58.20±3.73	7.90±0.08	7.82±0.11	88	86
T_3	9.56±0.05	8.13±0.15	50.56±0.86	50.76±1.12	7.65±0.03	7.57±0.06	62	58
T_4	9.10±0.01	9.53±0.30	62.43±0.83	60.46±2.54	7.89±0.01	7.83±0.11	90	87.5
T_5	9.06±0.20	8.26±0.47	58.66±1.75	56.40±0.79	7.83±0.15	7.73±0.25	82	83
T_6	8.73±0.23	8.36±0.15	52.80±3.30	49.93±2.72	7.61±0.10	7.55±0.08	79	72

Data represents an average of 3 replicates indicates Mean±SE, T: Treatment with diff. VAM fungi spp.,

Table 2: Plant Biomass (Fresh and dry weight) and total chlorophyll content of pot trials Site-I and Site- II wheat plants

		Plants fresh weight				Plants dry weight				
Sample sites	Parameters treatments	Leaf (g)	Stem (g)	Root (g)	Fruit (g)	Leaf (g)	Stem (g)	Root (g)	Fruit (g)	Total chlorophyll (mg g^{-1}) fresh wt.
Site I	T_1	1.36±0.01	4.56±0.20	1.05±0.05	3.39±0.07	0.43±0.01	1.47±0.02	0.81±0.01	1.41±0.01	1.63±0.03
	T_2	1.28±0.01	4.20±0.09	0.92±0.01	3.28±0.02	0.42±0.03	1.42±0.02	0.79±0.03	1.37±0.01	1.66±0.01
	T_3	1.05±0.04	3.38±0.01	0.86±0.01	3.10±0.10	0.28±0.02	1.30±0.01	0.57±0.02	1.31±0.02	1.39±0.01
	T_4	1.23±0.05	4.23±0.05	0.82±0.02	3.43±0.41	0.41±0.03	1.43±0.01	0.78±0.03	1.40±0.02	1.69±0.02
	T_5	1.16±0.05	3.97±0.14	0.81±0.00	3.24±0.04	0.38±0.03	1.39±0.00	0.70±0.02	1.37±0.02	1.51±0.01
	T_6	1.02±0.07	3.56±0.47	0.78±0.03	3.27±0.30	0.33±0.04	1.36±0.02	0.67±0.01	1.36±0.03	1.46±0.03
	T_0 (Control)	0.88±0.01	3.09±0.10	0.65±0.08	2.96±0.05	0.20±0.01	1.16±0.01	0.40±0.02	1.22±0.03	0.98±0.02
Site II	T_1	1.36±0.04	4.51±0.16	1.09±0.09	3.37±0.03	0.41±0.01	1.48±0.02	0.81±0.01	1.41±0.01	1.63±0.05
	T_2	1.29±0.07	4.02±0.08	0.95±0.02	3.25±0.02	0.39±0.03	1.46±0.02	0.81±0.02	1.39±0.03	1.67±0.03
	T_3	1.10±0.08	3.42±0.04	0.75±0.03	3.18±0.02	0.30±0.01	1.30±0.01	0.53±0.05	1.32±0.02	1.39±0.01
	T_4	1.26±0.05	4.26±0.06	0.82±0.02	3.24±0.04	0.38±0.01	1.43±0.04	0.77±0.04	1.41±0.02	1.68±0.03
	T_5	1.18±0.10	4.06±0.13	0.77±0.04	3.22±0.02	0.36±0.05	1.39±0.02	0.68±0.01	1.38±0.01	1.55±0.02
	T_6	1.08±0.11	3.59±0.48	0.78±0.04	3.20±0.00	0.32±0.04	1.36±0.01	0.64±0.04	1.36±0.00	1.55±0.03
	T_0 (Control)	0.84±0.02	3.02±0.11	0.66±0.07	2.92±0.06	0.19±0.01	1.14±0.01	0.40±0.02	1.21±0.02	0.96±0.02

Data represents an average of 3 replicates indicates Mean±SE, T: Treatment with diff. VAM fungi spp.

plant height was highest in host plants Site-II treated with *G. mosseae* (63.83 cm) followed by *G. mosseae+G. fasciculatum* (62.43 cm) and least in control. The longest roots were also recorded site-I treated with *G. mosseae* (11.24 cm) followed by Glomus fasciculatum (11.0 cm) and the highest mycorrhizal colonization find out site-I treated with *Glomus mosseae* (93%).

Analysis of the present investigation showed that soil inoculated with AM fungi increased plant growth (Table 1), nutrient uptake and yield parameters of wheat (*Triticum aestivum* L.) crops.

Plant biomass: Biomass of all the AM inoculated plants of wheat increased significantly in terms of fresh and dry weight[18]. The shoot and root biomass (fresh and dry) was recorded to be maximum in treated with *G. mosseae* (Table 2) followed by *G. fasciculatum* and *G. mosseae+G. fasciculatum* plants and lowest in control. Minaxi *et al.*[22] were found that the maximum enhancement in root length is due to the network of mycorrhizal mycelia, able to extend deeper in soil to invade nutrient depletion zone.

Total chlorophyll pigments: The role of chlorophyll in photosynthesis is vital during photosynthesis, chlorophyll captures the sun light and creates (sugar) carbohydrates and energy, which allows the plant to grow. The different treatment of AM inoculated plants showed increased level of chlorophyll content. The highest total chlorophyll (1.69 mg g^{-1}) was found in combination of *G. mosseae+G. fasciculatum* treatment respectively (Site I) followed by treated of *G. mosseae+G. fasciculatum* (1.68 mg g^{-1}) (Table 2).

Comparative analysis of physico-chemical properties of pot trial soils in before and after AM fungi treatments of both sites[23] in Table 3. In AM fungi treated pot soil organic carbon, phosphorus, nitrogen, potassium, calcium and magnesium contents were greater than the non-mycorrhizal plants pot soil at all harvests (Table 3), Such increases in soil nutrient contents in response to the mycorrhizal effects were highly associated, respectively, with the level of each mycorrhizal infection[24].

The soil quality was influenced by the AM fungal treatments compared to control and the highest was observed

Table 3: Biochemical and soil nutrient characteristics (0-10 depth) of experimental soil at the time of initiation of pot trial and after pot trial

Soil source	Sites	Parameter/ treatments	pH	Ec (dS m^{-1})	Organic carbon (%)	N (kg ha^{-1})	P (kg ha^{-1})	K (kg ha^{-1})	Zn (ppm)	Fe (ppm)	Cu (ppm)	Mn (ppm)
Initial of trial	Site I	S1	8.01	0.26	0.11	87	16	197	0.41	3.9	0.27	2.63
	Site II	S2	8.2	0.29	0.09	84.66	14	203	0.38	3.78	0.28	2.67
After pot exp.	Site I	T_1	8.1±0.07	0.13±0.02	0.25±0.02	126±2.64	31±1.53	266±1.73	0.86±0.00	4.67±0.01	0.37±0.02	3.50±0.02
		T_2	8.3±0.15	0.08±0.00	0.24±0.01	124±2.51	29±1.52	263±2.51	0.88±0.01	4.64±0.02	0.38±0.02	3.48±0.02
		T_3	7.9±0.32	0.11±0.01	0.20±0.03	117±3.21	23±1.73	235±3.78	0.62±0.01	4.40±0.02	0.33±0.02	3.16±0.02
		T_4	7.8±0.32	0.13±0.01	0.27±0.02	127±2.00	32±1.52	263±2.08	0.86±0.01	4.66±0.01	0.38±0.01	3.51±0.01
		T_5	8.3±0.15	0.12±0.01	0.24±0.03	123±1.52	28±2.00	259±1.52	0.81±0.02	4.61±0.03	0.36±0.02	3.42±0.02
		T_6	8.0±0.32	0.14±0.01	0.23±0.02	122±1.73	26±2.08	249±2.08	0.78±0.01	4.55±0.01	0.35±0.01	3.35±0.05
		T_0 (Control)	8.1±0.15	0.10±0.02	0.13±0.01	97±3.21	17±1.73	208±3.00	0.44±0.01	3.84±0.02	0.28±0.01	2.83±0.02
	Site II	T_1	8.0±0.05	0.10±0.02	0.24±0.02	125±2.00	31±1.73	262±2.08	0.90±0.01	4.64±0.01	0.40±0.02	3.48±0.01
		T_2	8.1±0.30	0.12±0.01	0.26±0.01	125±3.60	28±1.52	260±2.51	0.86±0.01	4.65±0.01	0.41±0.01	3.46±0.03
		T_3	8.3±0.15	0.10±0.02	0.17±0.03	115±3.51	22±1.73	237±2.64	0.65±0.02	4.40±0.01	0.34±0.02	3.13±0.01
		T_4	8.1±0.35	0.11±0.02	0.25±0.01	126±3.05	30±1.15	262±2.08	0.88±0.01	4.63±0.01	0.40±0.01	3.49±0.01
		T_5	8.4±0.05	0.12±0.03	0.22±0.01	122±2.64	27±1.52	259±2.30	0.84±0.01	4.60±0.26	0.35±0.02	3.41±0.01
		T_6	7.8±0.25	0.13±0.01	0.20±0.03	120±1.15	24±2.51	251±1.00	0.80±0.02	4.54±0.02	0.33±0.01	3.33±0.02
		T_0 (Control)	8.2±0.05	0.08±0.00	0.11±0.01	94±2.64	16±1.52	208±3.60	0.46±0.01	3.81±0.02	0.30±0.02	2.86±0.01

Data represents an average of 3 replicates indicates Mean±SE, T: Treatment with diff, VAM fungi spp.,EC: Electrical Conductivity, N: Nitrogen, P: Phosphorus, K: Potassium, Zn: Zinc, Fe: Iron, Cu: Copper, Mn: Manganese

in *G. mosseae+ G. fasciculatum* treatment respectively (Site I) (0.22%) followed by *G. mosseae+G. fasciculatum* (0.18%) Site II treated soil (Table 3). Such an increase in carbon pool is due to the strong influence of mycorrhizal fungi on the release of compounds from living roots because, these fungi can affect plant carbon metabolism while representing a sizeable sink for plant derived carbon[25].

In this study, inoculation with AM fungi provided an important enhancement to yield. The enhancement in plant growth and biomass yields due to inoculation with AM fungi was higher for wheat grown comparative to without AM inoculated under barren conditions.

DISCUSSION

This study examined the effects of AM fungi colonization on a winter crop wheat plant. The results showed that AM fungi played vital roles in growth and nutrition absorption under barren soil conditions[8], results achieved were positive as AM Fungi present in the inoculums colonized host in greater levels compared to non-inoculated plants and increased parameter of growth or soil nutrient.

A number of research articles can be deciphered from the literature[5,26], which shows the role of AM fungal species alone and/or in combination in enhancing plant growth under stress conditions[26]. The AM fungal species of *Glomus mosseae* was the most efficient for its ability to increase plant growth, soil nutrient and level of active arbuscular formation. This result of the study were consistent with previous reports of the reported that growth and yield of mycorrhizal and non-mycorrhizal *Triticum aestivum* L. crop under soil stress condition[5,27].

In addition to their interactions with plants, these AM fungi show symbiotic interactions in the soil environment. These interactions may be vital for sustainable agriculture because they mainly depend on biological processes rather than on agrochemicals to maintain plant growth and development as well as proper soil health under barren conditions[28]. Therefore, the present research has revealed another factor, under barren conditions, that appears to enhance in overall effects of AM Fungi on plant and soil biology.

CONCLUSION

The results confirm that AM fungi alleviate the detrimental effect of drought environment through improved water and nutrient uptake by AM hyphae and colonized roots of wheat plants. This cumulative effect increases the soil

quality and growth performance of the mycorrhizal inoculated plants compare to non-mycorrhizal under barren soil condition. Therefore, this aspect seeks more attention from the researchers to unveil the mechanism of drought-stress alleviation by AM fungi.

SIGNIFICANCE STATEMENTS

The symbiosis of indigenous Arbuscular Mycorrhizal (AM) fungi is protecting wheat host plant and improves soil quality from the effect of drought stress (under barren soil). This research will help to farmers for restoration of their barren land by the treatments with indigenous AM fungi.

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