



RESEARCH ARTICLE

# Effect of *Glomus intraradices* spore abundance of the inoculum on percent mycorrhizal colonization and growth of *Vigna mungo* (L.) Hepper

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## Abstract

Arbuscular mycorrhizal (AM) fungi are well known symbiotic microorganism found to improve the growth of host plant by mobilizing immobile nutrients, mainly phosphorus, from the soil. However, the effect of AM fungi on host plant growth depends on the percentage mycorrhizal colonization, whereas it is not clear that the percent mycorrhization impacts by AM fungal spore abundance of the inoculum. Therefore, the current investigation was conducted to know the effect of percent mycorrhization of *Glomus intraradices* on the growth of blackgram inoculated with varied numbers of AM fungal spores *via* seed biotization (1 to 10 AM fungal spores per seed). Percent mycorrhizal colonization and plant growth characteristics of blackgram were recorded after 10, 20 and 30 days of sowing (DOS). Our results are revealed that the percentage of mycorrhizal colonization significantly influenced based on the availability of AM fungal spore richness of the biotized seeds, which leads to altered crop growth. Percent mycorrhizal colonization in the roots of blackgram increased with increasing AM fungal spore abundance per seed and it ranges from 10 to 70 %. Moreover, mycorrhizal colonized plants recorded higher shoot and root length, leaf area, leaf area index, shoot and root biomass production as well as chlorophyll content over control, conversely it was increased further with increasing percent mycorrhizal colonization, which is directly proportional to the richness of the AM fungal spores per seed. Therefore, AM fungal spore abundance is one of the governing factors that influence percent mycorrhizal colonization in roots of plants besides AM fungal and plant species and soil condition.

## Keywords

Blackgram *Glomus intraradices*, arbuscular mycorrhizal fungal spore, percent mycorrhizal colonization, plant growth

## Introduction

Blackgram, *Vigna mungo* (L.) Hepper is an important leguminous pulse crop extensively grown in India. It is highly nutritious and protein rich food grain legume, provide nutrients rich complete human diet. Blackgram dal contains more than 20% protein, which is complementary to vegetable protein and also supplements protein in the cereal based low protein diet. Besides, it contains sugars, dietary fibers, starch, minerals, vitamins and essential amino acids (1, 2). Interestingly, leguminous pulse crops including blackgram sustain their growth by establishing a symbiotic association with *Rhizobium*, which fix atmospheric nitrogen in the root nodules (3-7). In addition to *Rhizobium*, AM fungi also form a symbiotic association with leguminous crops (8).

AM fungi belonging to the phylum *Glomeromycota*, is a unique symbionts that forms association with almost 80 % of terrestrial plant species (9). AM fungi-plant interaction promotes plant growth and nutrition by improving the uptake of immobile inorganic nutrients primarily phosphate and other nutrients such as ammonia as well as micronutrients from the soil (10-12). In turn, the plant provides around 20% of photosynthetically assimilated carbon to their symbiotic partner, AM fungi (13). Moreover, AM fungi also helps the plant to absorb moisture from the soil (14). This beneficial fungus brings physiological changes in the host plants by regulating the production of endogenous phytohormones besides supplying nutrients and water (15-16). A plethora of findings confirmed that AM fungi colonized plants exhibited a greater growth rate by increasing leaf area, chlorophyll content, root length and photosynthetic rate as well as phytochemical constituents such as sugars, proteins, phenols, tannins and flavonoids (17-22). Besides, it also enhance plant's resistance to various stresses including drought, salinity, herbivorous insect, temperature, heavy metal toxicity and phytopathogen (23-26).

Considerably, AM fungi delivers comprehensive benefits to the host plants with the lack of host specificity even though it has shown low species diversity (27). However, AM fungi mediated benefits to the host plants varied by numerous factor including AM fungal and host plant species, percent mycorrhizal colonization, growth stages of the crops and nutrient status of the soil (28). Percent mycorrhizal colonization is one of the factors largely responsible for plant growth and health improvement, which depends on infective propagules availability and dynamics; it has been evaluated through the estimation of AM fungal spore abundance in soil (29), roots colonization (30), extra radical mycelium length and total viable propagules (31). However, the effect of availability and abundance of AM fungal propagules on percent mycorrhizal colonization and growth of plants remain elusive and need to be studied. It was (32) reported that *Astragalus parrowianus* Boiss. and *Alopecurus arundinaceus* Poir. with the highest number of AM fungal spores in rhizosphere recorded maximum percent root colonization than other plant species with the lowest number of AM fungal spores, which includes *Crepis sancta* L. Babcock, *Thymus fedtschenkoii* Ronneger, *Alcea tholozani* Stapf., *Verbascum cheiranthifolium* Boiss., *Anchusa italica* Retz., *Scandix pectin-veneris* L., *Turgenia latifolia* L. Hoffm., *Silene conoidea* L., *Centaurea depressa* M. Bieb., *Hyoscyamus niger* L. and *Achillea tenuifolia* (Lam.). Moreover, it was proved that percent mycorrhizal colonization positively correlated with the number of AM fungal spores availability in the rhizosphere soil (32). With this background, the current study was conducted to evaluate the effect of AM fungal spore availability and abundance on percent mycorrhizal colonization and growth of blackgram.

## Materials and Methods

### AM fungal inoculum, blackgram seeds and soil sources

AM fungal inoculum, *Glomus intraradices* was obtained from the Biofertilizer Production and Quality Control Unit,

Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. Then, it was multiplied in the medium containing vermiculite and soil (10:1) mixture using maize. AM fungal spores were extracted from the vermiculite based carrier material by using a method wet sieving and decanting and used as a source of AM fungal inoculum (33). Blackgram (variety CO6) seeds were obtained from the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore and used for the study. For growth media, topsoil (15-20 cm) red was collected from the farm of the Department of Pulses, Tamil Nadu Agricultural University, Coimbatore and used as a substrate for growing plants. The collected red soil is sandy loam texture with a pH of 8.4, electrical conductivity (EC) of 0.16 ds m<sup>-1</sup>, 9.32 % nitrogen, 1.94 % phosphorus, 14.32 % potassium and 0.73 % organic carbon.

### Seed biotization with AM fungal spore and plant growth condition

Blackgram seeds (CO6) were surface sterilized using 10% sodium hypochlorite for 10 min followed by rinsed with sterile distilled water for 5 times. Then, the surface sterilized seeds were biotized with AM fungal spore (1-10 spores/seed) using 2% carboxyl methyl cellulose (CMC) as a sticker. In detail, a liquid suspension containing 200 numbers of AM fungal (*G. intraradices*) spores was mixed with 200 numbers of seeds to coat approximately 1 AM fungal spore per seed. Similarly, seeds were coated with up to 10 spores per seed. After biotization, seed associated AM fungal spore population was examined and verified microscopically. Biotized seeds were then dibbled in 2 kg plastic pots filled with steam-sterilized (121 °C for 30 min with 15 psi) red soil and sand mixture (2:1). After seed germination, all the pots were equally irrigated with 100 ml of water and 100 ml Hoagland's nutrient solution alternatively once in 2 days interval.

### Experimental design

A pot culture experiment was conducted at the Department of Agricultural Microbiology, Tamil Nadu Agricultural University, Coimbatore. The current investigation was carried out with eleven treatments and three replications by following a Completely Randomized Design (CRD). Treatments viz., 0 GS - Control without AM fungal spores, 1 GS - one AM fungal spore per seed, 2 GS - two AM fungal spores per seed, 3 GS - three AM fungal spores per seed, 4 GS - four AM fungal spores per seed, 5 GS - five AM fungal spores per seed, 6 GS - six AM fungal spores per seed, 7 GS - seven AM fungal spores per seed, 8 GS - eight AM fungal spores per seed, 9 GS - nine AM fungal spores per seed and 10 GS - ten AM fungal spores per seed. After 10, 20 and 30 days of sowing, plants were harvested and used to assess AM fungal colonization and plant growth parameters.

### Chlorophyll content

The chlorophyll content of leaf tissue was estimated based on the procedure of Arnon (34). About 100 mg of fresh leaf tissue was macerated in 5 ml of 80% acetone and then centrifuged for 10 min at 3000 rpm. This step was repeated till all the chlorophyll was extracted in the solvent. Then, the extracts were pooled and the volume was made up to

10 ml with 80% acetone. The intensity of color was measured at 665 nm in a digital spectrophotometer (Spectramax® i3X). Acetone (80%) was used as a blank. The chlorophyll content was denoted as mg g<sup>-1</sup> of fresh leaf tissue material.

### Plant growth parameters

Plant growth parameters such as shoot and root length (cm plant<sup>-1</sup>), total leaf area (cm<sup>2</sup> plant<sup>-1</sup>), and leaf area index were recorded after 10, 20 and 30 DOS. Then, the shoot and root system were separated and oven dried at 70 °C for 4 hr. The dry weight of the shoot and root system were recorded and expressed as g plant<sup>-1</sup>.

### Mycorrhizal colonization

Mycorrhizal colonization was examined with freshly collected root samples by following the standard procedure (35). First, roots were gently washed with running tap water to remove soil particles. Then it was treated with 10% KOH solution for 30 min in a boiling water bath. The alkaline solution was discarded and the roots were washed with distilled water followed by treated with 2% HCl solution. After, roots were stained with 0.05% trypan blue (lactic acid: glycerol: water at 2:2:1) for 12 hr and the AM fungal infection was observed under the microscope. Mycorrhizal colonization in roots of blackgram was expressed as a percentage (%).

### Statistical analysis

Statistical analysis was performed using SPSS (version 16.0) and Microsoft Excel (2010) to compare the effect of *G. intraradices* spore abundance and availability on percent mycorrhizal colonization and growth of blackgram. One way analysis of variance (ANOVA) was done for the data of percent mycorrhizal colonization and plant growth parameters. The mean value of the treatments was compared using Duncan's multiple range test (DMRT) at P=0.05 and stated as the mean with standard error (mean ± SE). Correlation analysis was performed to ascertain the relationship between the abundance of AM fungal spores on percent mycorrhizal colonization and their effect on growth of blackgram.

## Results and Discussion

The well-known fact is that mycorrhizal inoculation significantly improved plant growth and health, however it depends on the percentage of mycorrhizal colonization (24, 28). The percent mycorrhization is generally based on the abundance of infective propagules such as spores, extra radical hyphae and infected roots in the AM fungal inoculum (35, 36). Nevertheless, it is remain a need to study the impact of availability and abundance of AM fungal spores on percent mycorrhization and plant growth. In the current study reveals that the richness of AM fungal spores of biotized seeds had a significant impact on the percent mycorrhizal colonization and growth of blackgram.

### Mycorrhizal colonization

Percent mycorrhizal colonization in the roots of blackgram was significantly influenced by the availability of AM fungal

spore richness per seed. The initiation of mycorrhizal infection was noticed within 10 DOS of biotized seeds with more than three AM fungal spores/seed and the colonization ranges from 10 to 26.7%. Even though, seeds biotized with one AM fungal spore recorded 13.33 and 20.00 % mycorrhizal colonization after 20 and 30 DOS respectively. The percent mycorrhizal colonization increased with increasing AM fungal spores per seed from 10 to 30 DOS (Table 1; Fig. 1). Plants inoculated with 10 AM fungal

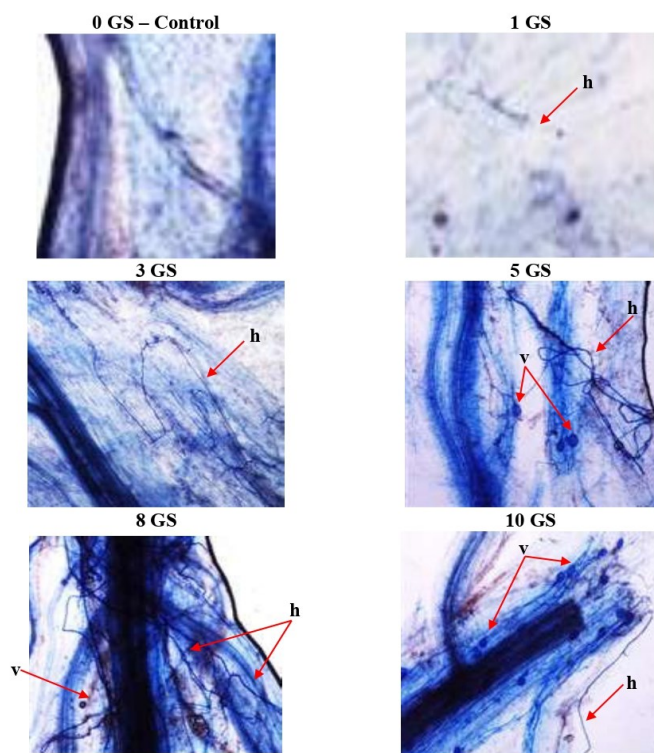
**Table 1.** Impact of AM fungal spore abundance on percent mycorrhizal colonization in blackgram at 10, 20 and 30 days after sowing

Treatments	AM fungal spore population (Number of spores seed <sup>-1</sup> )	Mycorrhizal colonization (%)		
		10 DAS	20 DAS	30 DAS
0 GS	0.00 (±0.00)	0.00 (±0.00) <sup>d</sup>	0.00 (±0.00) <sup>e</sup>	0.00 (±0.00) <sup>e</sup>
1 GS	1.00 (±1.00)	0.00 (±0.00) <sup>d</sup>	13.33 (±3.33) <sup>ef</sup>	20.00 (±5.77) <sup>d</sup>
2 GS	2.00 (±1.00)	0.00 (±0.00) <sup>d</sup>	10.00 (±0.00) <sup>fg</sup>	26.67 (±3.33) <sup>cd</sup>
3 GS	3.00 (±1.00)	10.0 (±5.77) <sup>c</sup>	20.00 (±0.00) <sup>def</sup>	23.33 (±3.33) <sup>d</sup>
4 GS	4.00 (±1.00)	10.0 (±0.00) <sup>c</sup>	23.33 (±3.33) <sup>cde</sup>	36.67 (±3.33) <sup>c</sup>
5 GS	5.00 (±2.00)	20.0 (±5.77) <sup>ab</sup>	33.33 (±6.67) <sup>bcd</sup>	63.33 (±3.33) <sup>ab</sup>
6 GS	6.00 (±1.00)	16.7 (±3.33) <sup>bc</sup>	26.67 (±8.81) <sup>cde</sup>	53.33 (±3.33) <sup>b</sup>
7 GS	7.00 (±1.00)	26.7 (±3.33) <sup>a</sup>	36.67 (±3.33) <sup>abc</sup>	63.33 (±6.67) <sup>ab</sup>
8 GS	8.00 (±2.00)	20.0 (±0.00) <sup>ab</sup>	26.67 (±3.33) <sup>cde</sup>	63.33 (±3.33) <sup>ab</sup>
9 GS	9.00 (±2.00)	20.0 (±0.00) <sup>ab</sup>	43.33 (±3.33) <sup>ab</sup>	70.00 (±5.77) <sup>a</sup>
10 GS	10.00 (±1.00)	20.0 (±0.00) <sup>ab</sup>	46.67 (±3.33) <sup>a</sup>	66.67 (±3.33) <sup>ab</sup>
<i>Df</i>		22	22	22
<i>F</i>		11.400	11.812	33.141
<i>P</i>		0.001	0.001	0.001
<i>R</i> <sup>2</sup>		0.838	0.843	0.938

Values are mean of three replicates ± standard error (n=3); values followed by the same letter in each column are not significantly different from each other as determined by DMRT ( $p \leq 0.05$ ). 0 GS - Control without AM fungal spores, 1 GS - one AM fungal spore per seed, 2 GS - two AM fungal spores per seed, 3 GS - three AM fungal spores per seed, 4 GS - four AM fungal spores per seed, 5 GS - five AM fungal spores per seed, 6 GS - six AM fungal spores per seed, 7 GS - seven AM fungal spores per seed, 8 GS - eight AM fungal spores per seed, 9 GS - nine AM fungal spores per seed and 10 GS - ten AM fungal spores per seed.

spores/seed recorded higher colonization of 46.67 ±3.33% after 20 DOS followed by seeds biotized with nine AM fungal spores (43.33 ±3.33%). However, maximum infection percentage (70.0 ±5.77%) was observed after 30 DOS in the treatment having 9 AM fungal spores per seed followed by seeds biotized with 10 AM fungal spores per seed (66.67 ±3.33 %), which is statistically on par with the treatments of 8, 7 and 5 AM fungal spores per biotized seed. Our results indicated that the initiation and percent mycorrhizal colonization in roots of plants completely depended on





**Fig. 1.** Different AM fungal spore density and mycorrhizal colonization in blackgram at 30 days after sowing (DAS). 0 GS, Control without AM fungal spore; 1 GS, one AM fungal spore per seed; 3 GS, three AM fungal spores per seed; 5 GS, five AM fungal spores per seed; 8 GS, eight AM fungal spores per seed; 10 GS, ten AM fungal spores per seed. (h), hyphal thread; (v), vesicle

the availability of AM fungal spore abundance of the inoculum. Moreover, the correlation analysis proved a positive correlation between percent mycorrhizal colonization and the number of AM fungal spores of inoculation ( $r^2 = 0.656$ ; Table 2). Similar to our study, it was also observed a positive correlation between AM spore density and percent root colonization in sugarcane (37). It was also reported that *Astragalus parrowianus* and *Alopecurus arundinaceus* recorded significantly higher mycorrhizal colonization with the highest number of spores in their rhizospheric soil (32). On the contrary, it was observed an inverse relationship between spore density and colonization in four perennial trees from a lowland tropical rain forest (38).

**Table 2.** Correlation coefficients between AM fungal spore abundance, percent mycorrhizal colonization, and blackgram growth parameters

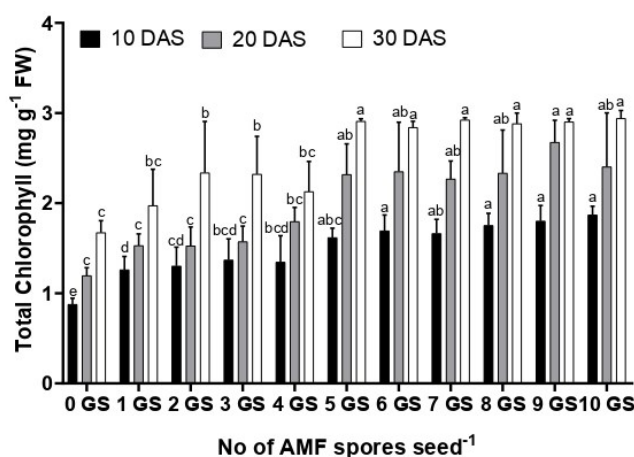
Variables	AM fungal Spore density	Mycorrhizal colonization	Chlorophyll	Leaf area	Leaf area index	Shoot length	Root length	Shoot biomass	Root biomass
Spore density	1								
Mycorrhizal colonization	0.656**	1							
Chlorophyll	0.587**	0.865**	1						
Leaf area	0.253 <sup>†</sup>	0.742**	0.778**	1					
Leaf area index	0.251 <sup>†</sup>	0.741**	0.776**	1.000**	1				
Shoot length	0.442**	0.872**	0.808**	0.853**	0.852**	1			
Root length	0.410**	0.841**	0.799**	0.876**	0.874**	0.912**	1		
Shoot biomass	0.352**	0.710**	0.779**	0.841**	0.840**	0.722**	0.779**	1	
Root biomass	0.355**	0.838**	0.867**	0.914**	0.913**	0.870**	0.891**	0.882**	1

<sup>†</sup>. Correlation is significant at the 0.05 level (2-tailed); \*\*Correlation is significant at the 0.01 level (2-tailed).

### Host plant growth

Mycorrhizal colonization improves plant growth by increasing chlorophyll content, leaf area, shoot and root

length, total dry matter production and photosynthetic rate as well as phytochemical constituents such as sugars, proteins, phenols, tannins and flavonoids (17, 21), as a result of supplying nutrients mainly phosphate and water from the soil (10), but it depends on percent colonization, soil fertility, plant and fungal species, age of crops and environmental factors (28). Percent mycorrhizal colonization is one of the most important factors that significantly influence the growth of crops directly or indirectly (39). In the current study, the total chlorophyll content of leaves of AM fungal colonized blackgram was recorded significantly higher over a non-AM fungal plant, however, it was significantly influenced by percent mycorrhizal colonization, which was directly correlated with AM fungal spore density of biotized seeds (Fig. 2). Plants inoculated with 10 AM fungal spores per seed recorded higher chlorophyll content after 10 (1.87 mg g<sup>-1</sup> FW) and 30 DOS (2.94 mg g<sup>-1</sup> FW), however, 9 AM fungal spores treated plants recorded signifi-

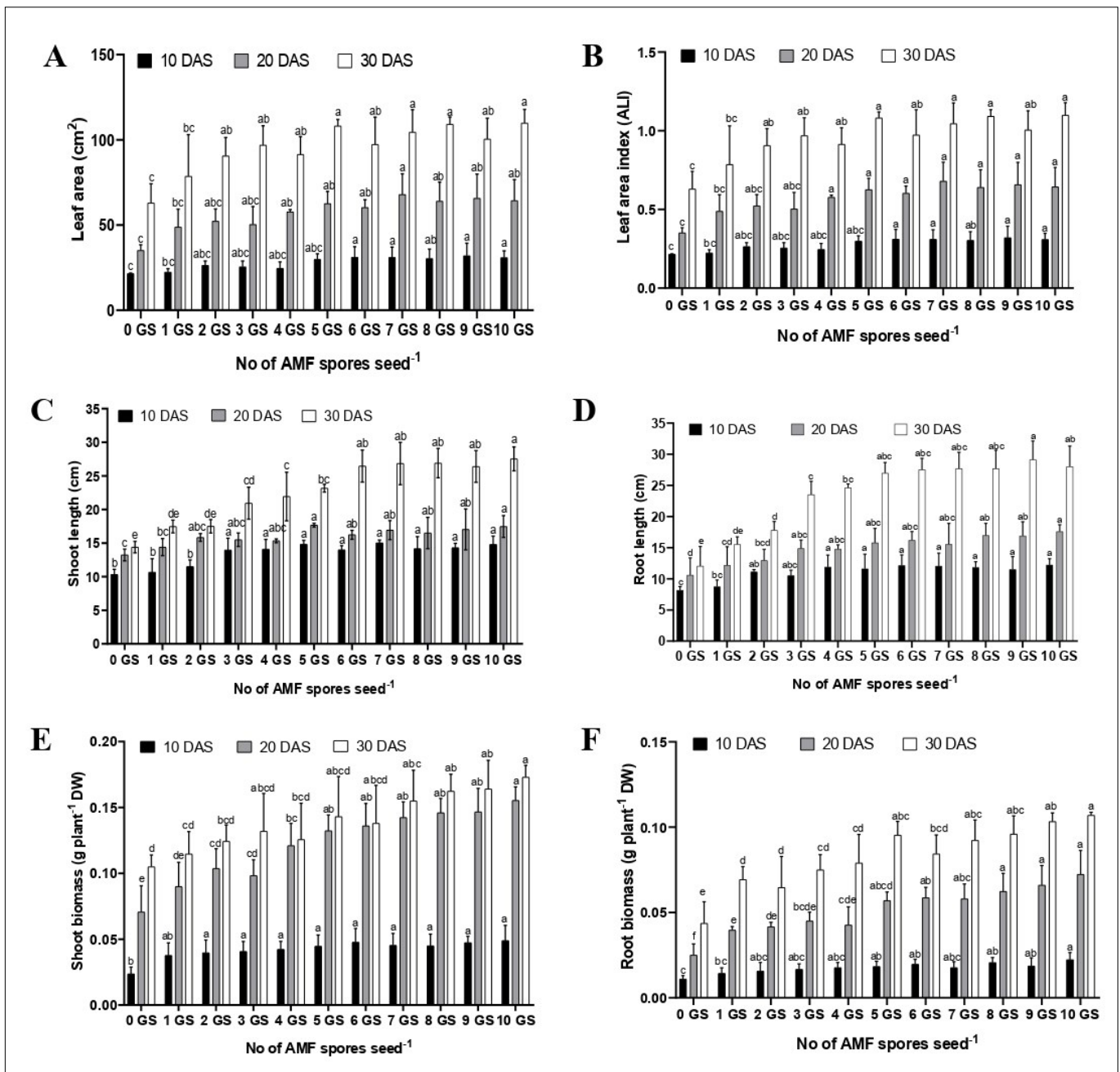


**Fig. 2.** Impact of AM fungal spore abundance and percent mycorrhizal colonization on total chlorophyll content of blackgram at 10, 20 and 30 days after sowing. Data in the figure are expressed as mean  $\pm$  SE. Mean values followed by the same letter do not differ significantly at  $P \leq 0.05$  by DMRT. 0 GS - Control without AM fungal spores, 1 GS - one AM fungal spore per seed, 2 GS - two AM fungal spores per seed, 3 GS - three AM fungal spores per seed, 4 GS - four AM fungal spores per seed, 5 GS - five AM fungal spores per seed, 6 GS - six AM fungal spores per seed, 7 GS - seven AM fungal spores per seed, 8 GS - eight AM fungal spores per seed, 9 GS - nine AM fungal spores per seed and 10 GS - ten AM fungal spores per seed.

cantly higher chlorophyll content after 20 DOS (2.68 mg g<sup>-1</sup> FW). Moreover, the total chlorophyll content of plants inoculated with 5 to 10 AM fungal spores was found to be on

par with each other after 10, 20 and 30 DOS, except 7 spores after 10 DOS and 9 spores after 20 DOS. Uninoculated control plants recorded a minimum of 0.88 mg g<sup>-1</sup> FW and a maximum of 1.68 mg g<sup>-1</sup> FW at 10 DOS and 30 DOS respectively. These results indicated that percent mycorrhizal colonization significantly influenced the total chlorophyll content of leaves of blackgram. Furthermore, it was confirmed by a positive correlation between chlorophyll content of blackgram plants, percent mycorrhizal colonization ( $r^2 = 0.865$ ; Table 2) and abundance of AM fungal spores per seeds ( $r^2 = 0.587$ ). These results are supported by another study (40), two strains of *Funneliformis mosseae* and *Diversispora tortuosa* inoculated *Gleditsia sinensis* Lam. plants with greater than 75 % root colonization registered higher chlorophyll concentrations over a control.

Leaf area is an important growth parameter determining plant productivity, which affects light interception as well as light use efficiency (41). AM fungal inoculated plants found to improve total leaf area, which in turn increased photosynthetic ability and biomass production (40). In the current study, compared to un-inoculated control plants, leaf area (LA) and leaf area index (LAI) were significantly higher in AM fungal inoculated blackgram plants, however it was increased with increasing AM fungal spore abundance per seed and percent mycorrhizal colonization (Fig. 3 A and B) and recorded a maximum of 31.13, 67.95, and 109.90 cm<sup>2</sup> plant<sup>-1</sup> LA in 6, 7 and 10 AM fungal spores per seed inoculated plants after 10, 20 and 30 DOS respectively, 0.32, 0.68 and 1.10 LAI in 9, 7 and 10 AM fungal spores applied plants after 10, 20 and 30 DOS respec-



**Fig. 3.** Impact of AM fungal spore abundance and percent mycorrhizal colonization on leaf area (A), leaf area index (B), shoot length (C), root length (D), shoot biomass (E) and root biomass (F) of blackgram at 10, 20 and 30 days after sowing. Data in the figure are expressed as mean  $\pm$  SE. Mean values followed by the same letter do not differ significantly at  $P \leq 0.05$  by DMRT. 0 GS – Control without AM fungal spores, 1 GS – one AM fungal spore per seed, 2 GS – two AM fungal spores per seed, 3 GS – three AM fungal spores per seed, 4 GS – four AM fungal spores per seed, 5 GS – five AM fungal spores per seed, 6 GS – six AM fungal spores per seed, 7 GS – seven AM fungal spores per seed, 8 GS – eight AM fungal spores per seed, 9 GS – nine AM fungal spores per seed and 10 GS – ten AM fungal spores per seed.

tively. One AM fungal spore inoculated plant recorded maximum of 63.00 cm<sup>2</sup>plant<sup>-1</sup> LA and 0.63 LAI after 30 DOS. The LA and LAI of 10 AM fungal spores per seed inoculated plants were statistically on par with 5, 7 and 8 AM fungal spores inoculated plants. As a result, it was clearly indicated that percent mycorrhizal colonization had a significant impact on LA and LAI of blackgram. Moreover, the correlation analysis proved that the percent mycorrhizal colonization positively influenced the LA ( $r^2 = 0.742$ ) and LAI ( $r^2 = 0.741$ ). Similarly, another study (42) also reported that the tea plants inoculated with *Claroideoglossum etunicatum* recorded higher total leaf area, which had around 40.23 % colonization, compared to other species of AM fungi *Diversispora spurca* and *Diversispora versiformis* inoculated plants, which registered only 24.94 and 15.12 % colonization respectively.

In the current study, AM fungal inoculation significantly improved shoot and root length of blackgram over control and it was increased significantly with increasing AM fungal spore density per seed and percent mycorrhizal colonization (Fig. 3 C and D). Plants inoculated with 10 AM fungal spores per seed registered significantly higher shoot and root length after 10, 20, 30 DOS (14.80, 17.67 and 27.53 cm plant<sup>-1</sup>shoot length respectively; 12.23 and 17.60 cm plant<sup>-1</sup>root length respectively) compared to other AM fungal inoculated treatments and un-inoculated control. Moreover, the correlation study revealed a positive relationship between percent mycorrhizal colonization and shoot and root length of plants ( $r^2 = 0.872$ ; 0.841). Our results are supported by another study (42) reported that the tea plants inoculated with *Claroideoglossum etunicatum* could record higher plant height with greater mycorrhizal colonization (40.23 %) compared to other AM fungal species such as *Diversispora spurca* and *Diversispora versiformis*.

The percentage of plant root length colonized by AM fungi is generally correlated with plant biomass production (i.e. ratio of biomass of plants with high mycorrhizal colonization to plants with a low rate of colonization) by increasing the transfer of nutrients to plants (39, 43, 45). In the present study, shoot and root biomass production were significantly influenced by percent mycorrhizal colonization and AM fungal spore density (Fig. 3 E and F). Shoot and root biomass production were increased with increasing AM fungal spore population and recorded a maximum of 0.049, 0.155 and 0.173 g plant<sup>-1</sup> shoot biomass and 0.022, 0.072 and 0.107 g plant<sup>-1</sup> root biomass production at 10 AM fungal spores inoculated plants after 10, 20 and 30 DOS respectively. The root biomass production had a significant difference among the AM fungal spore inoculation from 1 to 10 per seed. Moreover, a positive correlation between percent mycorrhizal colonization, shoot and root biomass production ( $r^2 = 0.710$ ; 0.838) clearly indicated that plant biomass production was significantly enhanced by increasing percent mycorrhizal colonization. Similarly, *Gleditsia sinensis* Lam. inoculated with two strains of *Funneliformis mosseae* and *Diversispora tortuosa* recorded greater values for seedling height and dry biomass production (40). The plants with greater percent root length colo-

nization could receive more nutrients (such as phosphorus, P) from their mycorrhizal symbionts, leads to greater plant growth and health (39).

## Conclusion

The current investigation reveals that the percentage of mycorrhizal colonization in blackgram depended on the availability of AM fungal spores abundance in the inoculum required per seed. Although, LA, LAI, chlorophyll content, shoot length, root length and shoot and root dry biomass production were improved in AM fungal colonized blackgram plant, still it was significantly influenced by percent mycorrhizal colonization and inoculation of AM fungal spore abundance per seed. Moreover, it also implicated that maximum mycorrhizal colonization was attained within a shorter period with the optimum number of AM fungal spore abundance of biotized seeds. Besides it, we also need to investigate the importance of AM fungal spores availability to attain maximum and efficient mycorrhizal colonization in all agricultural importance crops, which leads to improving crop growth and production, through a sustainable agriculture system. Thus, the results are suggested that AM fungal spore abundance is one of the governing factors that influence percent mycorrhizal colonization in roots of plants, resulted in influencing host plant growth and productivity.

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## Authors contributions

SA and TK conceived the idea, planned the work and drafted the manuscript. SA carried out the experiments and statistical analysis. All authors read and approved the final manuscript.

## Compliance with ethical standards

**Conflict of interest:** Authors do not have any conflict of interests to declare.

**Ethical issues:** None.

## References

1. Saeed SMG, Ali SA, Ali R, Sayeed SA, Mobin L, Ahmed R. Exploring the potential of blackgram (*Vigna mungo*) flour as a fat replacer in biscuits with improved physicochemical, microstructure, phytochemicals, nutritional and sensory attributes. *SN Applied Sciences*. 2020; 2(12):1-17. <https://doi.org/10.1007/s42452-020-03797-6>
2. Sarangapani C, Devi RY, Thirumdas R, Trimukhe AM, Deshmukh RR, Annapure US. Physico-chemical properties of low-pressure plasma treated black gram. *LWT-Food Science and Technology*. 2017;79:102-10. <https://doi.org/10.1016/j.lwt.2017.01.017>



3. Allito BB, Ewusi-Mensah N, Logah V, Hunegnaw DK. Legume-rhizobium specificity effect on nodulation, biomass production and partitioning of faba bean (*Vicia faba* L.). Scientific Reports. 2021;11(1):1-13. <https://doi.org/10.1038/s41598-021-83235-8>
4. Muthini M, Awino R, Kirui KC, Koech K, Jalloh AA, Njeru EM. Optimizing Rhizobium-legume symbiosis in smallholder agroecosystems. In: Guleria P, Kumar V, Lichtfouse E (eds) Sustainable Agriculture Reviews 45. Springer, Cham. 2020;159-77. [https://doi.org/10.1007/978-3-030-53017-4\\_8](https://doi.org/10.1007/978-3-030-53017-4_8)
5. Sindhu SS, Sharma R, Sindhu S, Sehrawat A. Soil fertility improvement by symbiotic rhizobia for sustainable agriculture. In: Panpatte D, Jhala Y (editors) Soil fertility management for sustainable development. Springer, Singapore. 2019; 101-66. [https://doi.org/10.1007/978-981-13-5904-0\\_7](https://doi.org/10.1007/978-981-13-5904-0_7)
6. Sharma K. Impact of different Rhizobial strains on physiological responses and seed yield of Mungbean [*Vigna radiata* (L.) Wilczek] under field conditions. Legume Research: An International Journal. 2021;44(6):679-83. <https://doi.org/10.18805/LR-4339>
7. Stella M, Sharma R, Nema S, Ramakrishnan R, Kumar A. Genetic characterization and diversity of Rhizobia isolated from root nodules of green gram (*Vigna radiata* L.) found in Central Plateau of India. Legume Research: An International Journal. 2021;44(3):353-61.
8. Wang X, Feng H, Wang Y, Wang M, Xie X, Chang H, Wang E. Mycorrhizal symbiosis modulates the rhizosphere microbiota to promote rhizobia-legume symbiosis. Molecular Plant. 2021;14(3):503-16. <https://doi.org/10.1016/j.molp.2020.12.002>
9. Smith S, Read D. Mycorrhizal symbiosis. 3<sup>rd</sup> edition Academic Press. San Diego. CA. 2008.
10. Chandrasekaran M. A meta-analytical approach on arbuscular mycorrhizal fungi inoculation efficiency on plant growth and nutrient uptake. Agriculture. 2020;10(9):370. <https://doi.org/10.3390/agriculture10090370>
11. Kim SJ, Eo JK, Lee EH, Park H, Eom AH. Effects of arbuscular mycorrhizal fungi and soil conditions on crop plant growth. Mycobiology. 2017;45(1):20-24. <https://doi.org/10.5941/MYCO.2017.45.1.20>
12. Qi S, Wang J, Wan L, Dai Z, Du D, Egan S, Moles AT. Arbuscular mycorrhizal fungi contribute to phosphorous uptake and allocation strategies of *Solidago canadensis* in a phosphorous-deficient environment. Frontiers in Plant Science. 2022; 13: 831654-831654. <https://doi.org/10.3389/fpls.2022.831654>
13. Basyal B, Emery SM. An arbuscular mycorrhizal fungus alters switchgrass growth, root architecture and cell wall chemistry across a soil moisture gradient. Mycorrhiza. 2021;31(2):251-58. <https://doi.org/10.1007/s00572-020-00992-6>
14. Allen MF. Mycorrhizal fungi: highways for water and nutrients in arid soils. Vadose Zone Journal. 2007;6(2):291-97. <https://doi.org/10.2136/vzj2006.0068>
15. Zhang F, Wang P, Zou YN, Wu QS, Kuča K. Effects of mycorrhizal fungi on root-hair growth and hormone levels of taproot and lateral roots in trifoliolate orange under drought stress. Archives of Agronomy and Soil Science. 2019;65(9):1316-30. <https://doi.org/10.1080/03650340.2018.1563780>
16. Zhang J, Bi Y, Song Z, Xiao L, Christie P. Arbuscular mycorrhizal fungi alter root and foliar responses to fissure-induced root damage stress. Ecological Indicators. 2021;127:107800. <https://doi.org/10.1016/j.ecolind.2021.107800>
17. Balestrini R, Brunetti C, Chitarra W, Nerva L. Photosynthetic traits and nitrogen uptake in crops: which is the role of arbuscular mycorrhizal fungi ? Plants. 2020;9(9):1105. <https://doi.org/10.3390/plants9091105>
18. Manoharan P, Pandi M, Shanmugaiah V, Gomathinayagam S, Balasubramanian N. Effect of vesicular arbuscular mycorrhizal fungus on the physiological and biochemical changes of five different tree seedlings grown under nursery conditions. African Journal of Biotechnology. 2008;7(9):3431-36.
19. Juntahum S, Ekprasert J, Boonlue S. Efficiency of arbuscular mycorrhizal fungi for the growth promotion of sugarcane under pot conditions. Sugar Tech. 2022; 1-10. <https://doi.org/10.1007/s12355-022-01129-z>
20. Moustakas M, Bayçu G, Sperdouli I, Eroğlu H, Eleftheriou EP. Arbuscular mycorrhizal symbiosis enhances photosynthesis in the medicinal herb *Salvia fruticosa* by improving photosystem II photochemistry. Plants. 2020;9(8):962. <https://doi.org/10.3390/plants9080962>
21. Wu YH, Wang H, Liu M, Li B, Chen X, Ma YT, Yan ZY. Effects of native arbuscular mycorrhizae isolated on root biomass and secondary metabolites of *Salvia miltiorrhiza* Bge. Frontiers in Plant Science. 2021;12:66. <https://doi.org/10.3389/fpls.2021.617892>
22. Shamizi N, Yarnia M, Mohebalipour N, Fara-marzi A, Ajalli J. The effect of mycorrhizal species on the growth, essential oils, yield and morpho-physiological parameters of Lemon Balm (*Melissa officinalis* L.) under water-deficit conditions in Tabriz region. Plant Science Today. 2022;9(2):228-35. <https://doi.org/10.14719/pst.1338>
23. Adeyemi NO, Atayese MO, Sakariyawo OS, Azeez JO, Sobowale SPA, Olubode A, Adeoye S. Alleviation of heavy metal stress by arbuscular mycorrhizal symbiosis in *Glycine max* (L.) grown in copper, lead and zinc contaminated soils. Rhizosphere. 2021;18:100325. <https://doi.org/10.1016/j.rhisph.2021.100325>
24. Chen J, Zhang H, Zhang X, Tang M. Arbuscular mycorrhizal symbiosis mitigates oxidative injury in black locust under salt stress through modulating antioxidant defence of the plant. Environmental and Experimental Botany. 2020;175:104034. <https://doi.org/10.1016/j.envexpbot.2020.104034>
25. Fiorilli V, Vannini C, Ortolani F, Garcia-Seco D, Chiapello M, Novero M, Bagnaresi P. Omics approaches revealed how arbuscular mycorrhizal symbiosis enhances yield and resistance to leaf pathogen in wheat. Scientific Reports. 2018;8(1):1-18. <https://doi.org/10.1038/s41598-018-27622-8>
26. Rivero J, Lidoy J, Llopis-Giménez Á, Herrero S, Flors V, Pozo MJ. Mycorrhizal symbiosis primes the accumulation of antiherbivore compounds and enhances herbivore mortality in tomato. Journal of Experimental Botany. 2021;72(13):5038-50. <https://doi.org/10.1093/jxb/erab171>
27. Chen M, Arato M, Borghi L, Nouri E, Reinhardt D. Beneficial services of arbuscular mycorrhizal fungi from ecology to application. Frontiers in Plant Science. 2018;9:1270. <https://doi.org/10.3389/fpls.2018.01270>
28. Hussain HA, Qingwen Z, Hussain S, Hongbo L, Waqqas A, Li Z. Effects of arbuscular mycorrhizal fungi on maize growth, root colonization and root exudates varied with inoculum and application method. Journal of Soil Science and Plant Nutrition. 2021;21(2):1577-90. <https://doi.org/10.1007/s42729-021-00463-7>
29. Mangan SA, Eom AH, Adler GH, Yavitt JB, Herre EA. Diversity of arbuscular mycorrhizal fungi across a fragmented forest in Panama: insular spore communities differ from mainland communities. Oecologia. 2004;141(4):687-700. <https://doi.org/10.1007/s00442-004-1684-2>
30. Sielaff AC, Polley HW, Fuentes-Ramirez A, Hofmockel K, Wilsey BJ. Mycorrhizal colonization and its relationship with plant performance differs between exotic and native grassland plant species. Biological Invasions. 2019;21(6):1981-91. <https://doi.org/10.1007/s10530-019-01950-w>
31. Rubio R, Borie F, Schalchli C, Castillo C, Azcón R. Occurrence and effect of arbuscular mycorrhizal propagules in wheat as affected by the source and amount of phosphorus fertilizer and fungal

- inoculation. *Applied Soil Ecology*. 2003;23(3):245-55. [https://doi.org/10.1016/S0929-1393\(03\)00045-3](https://doi.org/10.1016/S0929-1393(03)00045-3)
32. Khakpour O, Khara J. Spore density and root colonization by arbuscular mycorrhizal fungi in some species in the northwest of Iran. *International Research Journal of Applied and Basic Sciences*. 2012;3(5):977-82.
  33. Gerdemann JW, Nicolson TH. Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*. 1963; 46: 235-44. [https://doi.org/10.1016/S0007-1536\(63\)80079-0](https://doi.org/10.1016/S0007-1536(63)80079-0)
  34. Arnon DI. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology*. 1949;24(1):1. <https://doi.org/10.1104/pp.24.1.1>
  35. Phillips JM, Hayman D. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*. 1970;55(1):158-61. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
  36. Dhumal KC, Shinde BP. Impact of chemical properties of soil on spore density, colonization and distribution of native arbuscular mycorrhizal fungi associated with *Capsicum annuum* L. *Journal of Applied Biology and Biotechnology*. 2020;8(05):59-67.
  37. Sivakumar N. Effect of edaphic factors and seasonal variation on spore density and root colonization of arbuscular mycorrhizal fungi in sugarcane fields. *Annals of Microbiology*. 2013;63(1):151-60. <https://doi.org/10.1007/s13213-012-0455-2>
  38. Louis I, Lim G. Spore density and root colonization of vesicular-arbuscular mycorrhizas in tropical soil. *Transactions of the British Mycological Society*. 1987;88(2):207-12. [https://doi.org/10.1016/S0007-1536\(87\)80216-4](https://doi.org/10.1016/S0007-1536(87)80216-4)
  39. Treseder KK. The extent of mycorrhizal colonization of roots and its influence on plant growth and phosphorus content. *Plant and Soil*. 2013;371(1):1-13. <https://doi.org/10.1007/s11104-013-1681-5>
  40. Wang J, Fu Z, Ren Q, Zhu L, Lin J, Zhang J, Yue J. Effects of arbuscular mycorrhizal fungi on growth, photosynthesis and nutrient uptake of *Zelkova serrata* (Thunb.) Makino seedlings under salt stress. *Forests*. 2019;10(2):186. <https://doi.org/10.3390/f10020186>
  41. Weraduwege SM, Chen J, Anozie FC, Morales A, Weise SE, Sharkey TD. The relationship between leaf area growth and biomass accumulation in *Arabidopsis thaliana*. *Frontiers in Plant Science*. 2015;6:167. <https://doi.org/10.3389/fpls.2015.00167>
  42. Shao YD, Zhang DJ, Hu XC, Wu QS, Jiang CJ, Xia TJ, Kuča K. Mycorrhiza-induced changes in root growth and nutrient absorption of tea plants. *Plant, Soil and Environment*. 2018;64(6):283-89. <https://doi.org/10.17221/126/2018-PSE>
  43. Liese R, Leuschner C, Meier IC. The effect of drought and season on root life span in temperate arbuscular mycorrhizal and ectomycorrhizal tree species. *Journal of Ecology*. 2019;107(5):2226-39. <https://doi.org/10.1111/1365-2745.13181>
  44. Cesaro P, Massa N, Cantamessa S, Todeschini V, Bona E, Berta G, Lingua G. Tomato responses to *Funneliformis mosseae* during the early stages of arbuscular mycorrhizal symbiosis. *Mycorrhiza*. 2020;30(5):601-10. <https://doi.org/10.1007/s00572-020-00973-9>
  45. Birhane E, Gebretsadik KF, Taye G, Aynekulu E, Rannestad MM, Norgrove L. Effects of forest composition and disturbance on arbuscular mycorrhizae spore density, arbuscular mycorrhizae root colonization and soil carbon stocks in a dry afro-montane forest in Northern Ethiopia. *Diversity*. 2020;12(4):133. <https://doi.org/10.3390/d12040133>

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