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Investigating the Effects of Arbuscular Mycorrhizal fungi in improving growth of Mung Bean

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Abstract

Arbuscular Mycorrhizal fungi (AMF) through symbiotic association with host roots increase water and nutrients supply of host plant getting carbohydrates from host plant in return. This study aimed to investigate the effects of AM fungi on growth and growth related parameters of mung bean. To achieve the aims, the methodology included soil analysis, determining spore density and diversity, root colonization by AM fungi, growth parameters and protein in the colonized plants at vegetative and fruiting stages. This study reports spore density and AMF colonization (in tested plants) was higher in fruiting stage and lower in vegetative stage in mung bean as compared to control. The study reported Glomus had the maximum number of spores and Sclerocystis and Acoulospora showing the lowest spores number than control. Root colonization was noted to be more effective at fruiting stage than vegetative stage. Plant height observed at vegetative stage of AMF treated plants was 18.33±0.88 cm whereas 11.33±082 cm in control. Similarly, plant height at fruiting stage was 19.3 ± 1.45 cm and 12.3 ± 1.76 cm in AMF treated plants and control respectively. Importantly, the crude protein content recorded in untreated plants was 18.67±0.33% while in AMF treated plant was 23.33±1.45%. From the findings of the current study, it can be illustrated that AMF can be used as biofertilizers.

Keywords: Arbuscular mycorrhizal fungi, mung bean, improving plant growth

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1. INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) belonging to the phylum "Glomeromycota" forms symbiotic association with around 80 percent of shrubs, herbs and trees[1]; once they come into contact with the host's root, fungi enter the epidermis and in the cortical parenchyma start forming hyphae[2]. The entrance of fungi inside the root surface can occur in three ways: by forming an appressorium, from which intracellular hyphae develop; by penetrating via a radical hair; or by crossing over outer layers cells that are frequently dead and flake off [3]. In the roots, the hyphae deepen, colonized the cells abundantly and go through intensive branching, forms arbuscules with a lifetime of 7–12 days[4], serving as the site for fungus metabolite exchange and storage of reserve nutrients[5]. These fungi are obligate symbionts that develop a mutually beneficial relationship with the plant via exchange of substances, to complete its life cycle the fungus get carbon and plant receives nutritional benefits[6]. Glomeromycota are depending on its host plants for carbon to stay alive and utilized about 20 percent of carbon fixed by plant[7]. In return, the fungi increase water and nutrients supply to the host plant such as phosphate and nitrogen via extraradical and intraradical hyphae, arbuscules, and the root apoplast interface[8]. AMF has helped to improve plant growth parameters[9,10,11] and take up of various important nutrients like N and P under

challenged conditions[12,13]. The challenged condition is associated with the fact that AMF expands their hyphae in nutrient depleted regions in root zone, enable access to soil[14]. Moreover, fungal hyphae are extremely thin than roots, they can penetrate micropores and absorb more nutrients[15]. Extreme use of land can have a negative influence on biodiversity, which in response can alter operations of ecosystem according to several articles[16,17]. Significant role of these symbiotic association is the transfer of nutritional ingredients, such as, hydrocarbon, in the form of carbohydrates and lipids[18]. It is believed that AMF colonization increase nutrients intake in plants. It is clear that inoculation of AMF can significantly improve several macro- nutrients and micro-nutrients concentration, in improved photosynthate production and thus enhanced growth and development of plant[19,20]. AMF has the potential to enhance the intake of inorganic nutrients, specifically phosphate almost in all plants[21]. AMF are also highly efficient in assisting plants to absorbing minerals from the nutritionally deficient soils[22]. Except NPK, AMF interaction has been shown to significantly surge the Phyto- availability of copper (Cu) & zinc[23]. AMF enhance the surface absorbency ability of host roots[24]. Tomato treated with **AMF** had indicated larger leaf area as well as potassium, nitrogen, phosphorus, and calcium constituents, indicating increased plant growth[25].

The beneficial modifications of soil structure are a vital service provided by AM fungi in both natural and agricultural environments[26]. The compact hyphal network of extremely branched AMF mycelium forms a three-dimensional structure that traps and crosslinks soil particles without compacting the soil. Glomalin is a soil glycoprotein considered to be formed by AMF is another important stabilization agent of soil aggregates[27]. "Glomalin is not a specified chemically homogenous molecular species or gene product, rather it is a soil fraction distinguished by its extractability and immuno-reactive properties"[28]. Collectively, AM fungi, hyphal network, and its influence on plant growth and root system development[29] save the soil from erosion. The combined effects of AM fungi on soil qualities lead to increased water retention capacity, which enhance plant growth and nutrient availability[30]. Agricultural soils, on the other hand, are disturbed by agricultural practice (particularly plowing) and receive enormous amounts of fertilizer, primarily N, P, and K[31]. AM fungi pick up nutrients from the soil solution[32] allowing mycorrhizal soils to have better soil solution absorption[33,34].

Pulses are good source of vegetable protein, especially for the world's poor, who rely on vegetables for protein and energy requirements. Apart from the monopoly of five main crops grown in Pakistan (wheat, rice, cotton, sugarcane, and maize), the share of pulse crops in GDP is 2.2%[35]. Pulses are known as important minor crops along with oil seed crops, fruit orchards, vegetables, and a few other high value crops[36]. Pulses are grown on 5% of the total cultivated area[37]. In the Asian region, Pakistan is the second largest importer of pulses[38]. During 2018–19, Pakistan spent Rs. 68.2 billion to import 976.6 thousand tonnes of pulses[39]. In Pakistan, the total area under major pulses, is approximately 1.9 million hectares[40]. Pulses are considered the vital food source for about 10billion people worldwide. It belongs to family Leguminosae and consist of species used by humans and animals mostly in grains form[41]. In Pakistan, substantial cultivated crops include chickpea, mung bean, lentil and mash bean[42]. Pulses through the process of biological nitrogen fixation can increase soil fertility by improving the availability of the essential nutrients for the crop plants and mobilization of nutrients as nitrogen and phosphorus (P)[43,44]. This study aimed to examine the effect of Arbuscular Mycorrhizal Fungal inoculation on growth of mung bean, which is leading pulse crop in Pakistan.

2. MATERIALS AND METHODS

2.1 Research sites:

Experimental Net House, Department of Botany Bacha khan University Charsadda

2.2 Collection of Plants and preservation

Plants were gathered randomly during different time periods of pulse growth. After gathering of samples, they were dried under room temperature in shade in open place. The dried samples were then kept on herbarium sheet.

2.3 Physiological and chemical analysis of soil samples

Various parameters relating to soil sample collected were observed. Soil PH, lectro conductivity, Soil Texture. Organic matter and NPK

2.4 Evaluation of Roots

Mung bean roots were separated and dipped in formalin acetic acid solution to preserve it. The staining technique given was used for staining fungal structures with some modifications for non-pigmented roots[45].The root of the plant was washed with tap water segment into fragment and heated for 10 to 15 minutes in 10 percent KOH solution for the decolorization, the pigment portion of root was kept in alkali H_2O_2. After that the segment was treated with 1%HCL for about 1-2 minutes to keep the acidic acid effect for proper staining in 0.025 acetic acid fushine the root segment was kept and heated for 2- 3 minutes. Segment of about 1 cm length was randomly collected and keep under microscope for the morphological studies of AMF.

2.5 Assessment of root colonization

The technique of [46] was followed. Each of the 50 randomly chosen root segments, which were each about 1 cm long, was examined under a microscope. The VAM endophyte morphology was inspected and showed in %, and the % infection was computed using the given formula:

$$\% \text{ mycorrhizal infection} = \frac{\text{No. of infected segments} \times 100}{\text{Total No. of segments inspected.}}$$

Microphotographs of the best selected slides were taken.

2.6 Extraction of Spores

Soil sample were collected at different stages of host growth vegetative and fruiting stages of mung bean. A 100-gm sample of fine soil was taken from each of the test plants. 2 treatments x 1-varieties of green mung bean, x 3 replicates at a depth15-20 cm. Wet sieving and decanting technique of [47] was used to extract the spores from collected soil sample.100 gm of fine soil was taken, removed the debris and other particles and dissolved in water for 24 hour duration when the soil was completely settled down in the bottom of the beaker the water were passed from the different size of sieves 50, 90 and 250 µm. The remaining residues above the sieves was collected by rubbing the filter paper on the sieves and studied under the compound microscope for the fungal species diversity and density.

2.7 Mounting of spores

The Spores were collected through pin and kept on a slide in a droplet of Canada balsam.

2.8 Calculation of density of spores

The average number of spores per 100 g of soil was used to quantify density. It was estimated using a conventional formula in accordance with[48].

2.9 Identification of spores

Spores were recognized with the help of keys following[49,50].

2.10 Field Work, Experimental Layout

To assess the impact of AMF on Mung bean (*Vigna radiata*), experiments were conducted in the Experimental Net House of Botany department of Bacha Khan University throughout the succeeding summer and winter seasons. As rhizobase inoculum, pulse field soil with roots infected with arbuscular mycorrhiza and rhizospheric soil with high spore counts of three AMF species (*G. fasciculatum, G. mosseae, and G. aggregatum*) were employed. Segment the roots into 1-cm-long pieces. Before sowing, these root fragments and the soil base inoculum (rhizosphere soil) were evenly distributed in layers at depths of 3 cm and 6 cm. 180 gram of soil and mycorrhizal infected roots made up the inoculum for each pot. It was discovered that there were 290 spores in every 100g of soil.

2.11 Experiment plan, treatments, and replications

The experiment was set up using a random block design, consisting of two treatments and one variety of green mung bean for each treatment. Each treatment was reproduced three times within each pot. Ten to twenty seeds were put in each of the five pots that make up each treatment. At a depth of 5 cm below the soil surface, seeds were planted. The appropriate AMF inoculum was added to the soil for the AMF treatments, and seeds were then sown in each pot in accordance with the treatments as the basal dose. The control treatments lacked an AM fungus inoculation.

1. Control (without VAM inoculation)
2. Treated (VAM fungus)

In the net house pot under natural condition were placed. To examine the significance of different treatments, variations, and interactions, probabilities of significance were used.

2.12 Evaluation of growth Parameters

The ensuing variable were recorded.

1. Plant height
2. Leaves/plants
4. pods/plants
5. Length of pod

2.13 Plant harvest and parameters

After ripening of pulse plant, seed and soil were collected. Some Soil were packed and preserved for post analysis and the spores were extracted from each collected soil sample used Wet sieving and decanting technique of Gerdemann & Nicolson[47].

2.14 Protein Analysis

Crude proteins were determined using the standard methods of [51]. In the Tecator digestion tubes, about 300mg samples were introduced in triplicate together with 10 ml of concentrated sulfuric acid and 5000 mg of potassium sulphate, 93% copper sulphate were used as a catalyst. Acetanilide (0.1 g) underwent normal processing to extract nitrogen. In the Tecator digestion block, the digestion tubes were heated. After that, room temperature cooling was allowed for the tubes. The tubes containing the digested material were mixed with 15 ml of distilled water.

The sample was alkalined by adding the necessary volume of NaOH liquid (40 % w/v) to tubes and distilled about for 7 minutes. In a conical flask with.000l litre of boric acid (2 percent) and 3 to 4 droplets of methylene red indicator; the produced ammonia was collected. With solutions of 0.5 N sulfuric acid, the distillate was titrated. Duplicate tubes were treated for distillation and titration using 15 ml of distilled water and 5 ml of NaOH in order to determine the blank values. The % nitrogen percentage (PCP) was determined by the given formula:

$$PCP = (ml\ H_2SO_4 - blank) \times Nitrogen \times (6.25 \times 14.01)\ (Sample\ weight \times 100)$$

3. RESULTS AND DISCUSSIONS

3.1 Pre and post analysis of soil

The soil was analyzed for their physio-chemical characteristics before experiment and after cultivation of mung bean. Soil was analyzed for their texture and found it is primarily composed of silt loam in both the pre-analysis and post-analysis samples (Table-3.1). Soil is an essential component for the sustainable development of any crop. Nutrient uptake (particularly N, P) of host plant improved by AMF[52]. As a result, AMF enhanced growth rate and encouraged the growth of rhizomes. It has been noted that association of fungi with plant provides P & N, thereby promoting plant growth at P deficient soils[53]. The introduction of AMF increased soil pH. This phenomenon can be related to AMF's favorable influence on the soil bacterial community, which results in an increase in bacterial metabolites. AMF's augmentation of the soil bacterial community is critical in fostering conditions conducive to a pH rise, producing a chain reaction in which increased bacterial activity and metabolite production contribute to the observed increase in soil pH[54]. Our study is in line with[55] who reported increase in total P uptake with the pH increase. Previous studies indicated a substantial augmentation in nitrogen (N) uptake, amounting to 35% increase in AMF plants compared to non-AMF counterparts[56] This elevated N uptake is attributed to the capacity of AMF to absorb and transfer nitrogen to host plants[57] because the extensive hyphal network of AMF, extending beyond the root surface by more than 10 cm. This extended network facilitates the rapid and widespread acquisition of inorganic nitrogen from the soil. AMF produce alkaline phosphatases that break down soil substrates, rendering phosphates accessible[58]. AMF colonization also plays a role in the uptake of P and K by plants[59]. Furthermore, AMF colonization enhanced soil organic matter (SOM) by fostering a synergistic relationship with various microbial components in the soil, promote plant growth and also provide benefits to the plants[60]. The mycorrhizal association significantly enhances plant growth by improving the transfer of nutrients between the roots and shoots of infected plants. The maximum plant phosphorus (P) uptake observed in treatments involving mycorrhizal fungi was 0.093 g and 0.088 g, representing impressive increases of 402.9% and 375.67% in P uptake compared to the control treatment. In contrast, the control and treatments involving only nitrogen (N) and potassium (K) recorded P uptake was notably lower, measuring 0.018 g and 0.025 g, respectively. These findings align with previous research by[61] which indicated that the application of AMF improved the uptake of nitrogen, phosphorus, potassium, and magnesium by rice plants[62] also suggested that mycorrhiza combine application with phosphorus fertilizer showed more effective growth of corn over rock phosphate alone or control treatment. In wheat, the yield and its various components, along with the uptake of nitrogen, phosphorus, zinc, copper, iron, and

manganese by plants, experienced significant enhancement due to mycorrhiza inoculation[63.] The findings indicated that faba bean plants treated with AMF+ exhibited a greater overall uptake of phosphorus compared to those without AMF. This increased uptake potentially enhances plant growth, development, and ultimately, the crop's final yield[64]. Our results are correlated with the studies of [65] reported that in lettuce the EC are higher 2.04 dSm^{-1} in uninfected plants while in AMF treated the EC was reduced and recorded 1.41 dSm^{-1} because AMF increase root surface area. Our result is in contrast with[66] who reported that in olive plant significant increase were recorded in EC.

Table 3.1 Pre and Post Analysis of Soil.

S. No	Area	Sample id	Texture	PH	EC dSm-1	Organic Matter (%)	P (ppm)	K (ppm)	N (%)
1	Charsadda	Pre- analysis	Silt loam (no effect was observed on soil texture)	7.9	0.761	0.345	0.1552	59.8	0.0552
		Post analysis		8.1	0.616	0.034	0.0819	56.1	0.0056

3.2 AMF spore density at vegetative and fruiting stage.

The inoculation of mung bean plants with AMF appeared to significantly increase the spore density of all three AMF species compared to the control, suggesting that AMF inoculation positively influences spore production at studied stages (Table 3.2,3.3). It was seen that number of AMF spore was high during fruiting stage when compared with vegetative stage. our results are similar to[67] who documented that in vegetative stage root growth is poor. The finding of the study contradicts with[68]. Plant species in the initial stages of succession show greater susceptibility to AM root colonization and sustain higher levels of AM sporulation compared to species found in later successional stages. The amount of root colonization varied from cultivar to cultivar and from stage to stage. It was shown that root colonization was greatest during the fruiting stage and least during the vegetative stage. Our results are in line with[69] who studied that initial growth stages of AMF infection are characterized by low intensity, which steadily increases as growth stages progress. The age of the plants and other physiological factors of the plants both contributed to the steady increase in AMF colonization from the vegetative to fruiting stages. In chosen fields in various locations, dominant mycorrhizal infections were seen for the fruiting stage of Wheat plants.

Table-3.2: **AMF spore density in test crop at vegetative stage**

Variety	Spore density			
	Spore $100gm^{-1}$			
	Species			
	Treatment	Glomus	Sclerocystis	Acoulospora
Local	Control	59.33±3.38	9.30±1.60	2.60±0.80
	VAM	70.68±1.28	11.68±1.05	6.34±0.70

Table 3.3 AMF spore density at fruiting stage of mung bean

Variety	Spore density			
	Spore 100gm^{-1}			
	Species			
	Treatment	Glomus	Sclerocystis	Acaulospora
Local	Control	74.00±2.80	11.33±0.20	4.33±0.29
	VAM	86.00±0.86	12.00±0.336	6.33±0.26

3.3 AMF root colonization at vegetative and fruiting stages in studied mung bean

Data recorded suggested AMF treatment leads to a significant increase in the presence of external hyphae, internal hyphae, vesicles, and arbuscules in mung bean plants, indicating successful colonization by AMF. Presence of these structures indicates successful AM colonization, as these are typical structures formed by mycorrhizal fungi during their symbiotic association with plant roots. The impact of AMF on root colonization appears to vary between growth stages, with a more pronounced effect observed at the fruiting stage (Tables 3.4, 3.5). This colonization is a crucial aspect of the mycorrhizal symbiosis, potentially enhancing nutrient uptake and plant growth. During the current study mung bean were used and tested for mycorrhizal association at different host growth stage (vegetative and fruiting). Our findings showed that *Glomus* has the maximum number of spores, *Sclerocystis* and *Acoulospora* had the lowest spores when compared to the control. Our finding is in line with[70] who found that *Glomus* is the most common mycorrhizal fungus studied in barley field in India our finding are also in accordance with[58,59] who studied that *Sclerocystis* and *Acaulospora* are the main AMF spores. Our findings are also in consistent with[71] who found that Glomus is the most governing genus in the rhizosphere zone of *Retama raetam*.

Table 3.4 AMF root colonization at vegetative Stage in mung bean:

Varieties	AMF/ Root assessment				
Local	Treatment	External Hyphae	Internal Hyphae	Vesicles	Arbuscules
	Cont.	6.6±0.21	12.2±0.22	20±0.24	28.8±0.40
	VAM	24.2±022	36.4±0.24	42±0.46	28+0.30

Table 3.5 AMF root colonization at fruiting Stage in mung bean (*Vigna radiata)*

Varieties	AMF/ Root assessment				
	Treatment	External	Internal	Vesicles	Arbuscules

		Hyphae	Hyphae		
Local	Cont.	34.6±0.22	52.8±1.05	60±0.20	32.4±0.30
	VAM	54.2±0.96	58±0.30	80.4±0.20	60+0.20

3.4 Evaluation of growth parameters

3.4.1 Plant Height

The Plant height observed at vegetative stage of control plant was 11.33±082 cm while in AMF treated plants it was 18.33±0.88 cm. while in case of fruiting stage, the untreated plant height was 12.3 ± 1.76 cm and AMF treated plant, the height observed was 19.3 ± 1.45 cm (Figure-1). AMF+ plants in faba beans have the highest plant height compared to AMF- plants, possibly due to the positive impact of AMF application on plant absorption of water and nutrients. Increased nitrogen levels stimulate metabolic activity, leading to increased metabolites and elongation of internodes. Our results are in line with[70] shows that AMF considerably enhance plant height in soyabean as compared to the control. It can be as a result of the AMF application's response promoting root growth, which might promote plant height growth by increasing water and nutrient intake necessary for the body's metabolic process[71]. Our results are in line with[72] where significant rise in leaves number were recorded when AMF applied to *Glycine max* plants. Introducing AMF to plants can boost intake of P which is crucial for the process of photosynthesis. The author[72] reported that plants inoculated mycorrhizal have higher leaves number, plant weight, root weight and the canopy.

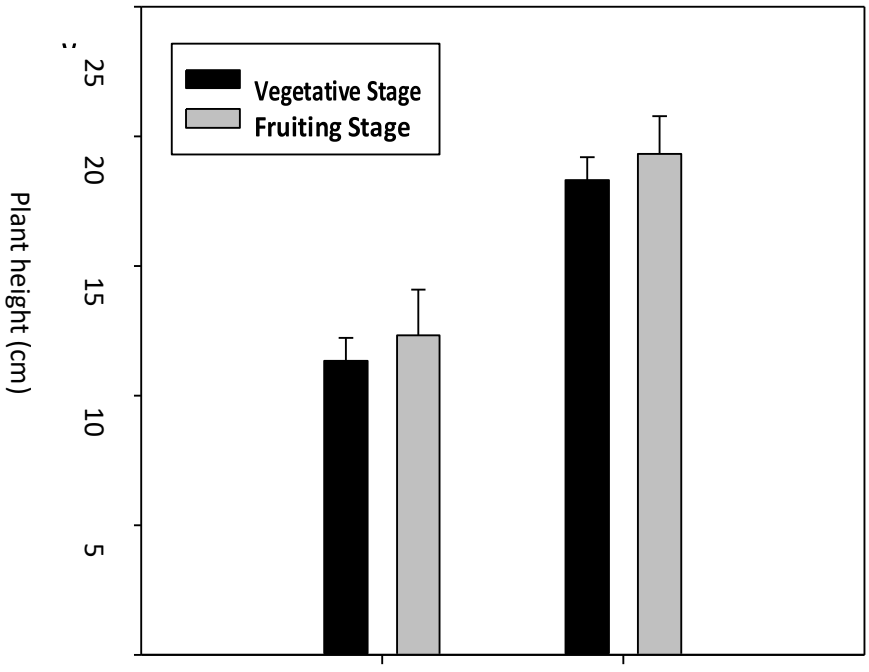

Figure-3.1: Plant height of mung bean in control and AMF inoculated plants

3.4.2 Pod per plant, pod length and protein content measured from tested plants

In the present study, pod per plant in control was recorded as 8.33±0.88 while in AMF treated plant was 10±0.58. By comparing the number of pods per plant between the control and AMF-treated groups, it can be observed that the AMF treatment led to an increase in the number of pods per plant. Similarly,pod length observed during fruiting stage was 5±0.57 cm while in AMF treated plant was 7.67±0.88 cm. Our results are also agreed with[72] who documented that the number of pods are enhanced by AMF in *Phaseolus vulgaris L*. Similarly[73] reported that peanut treated with AMF had a higher number of pods and increased weight of pods than untreated plants. This is explained by the fact that AMF inoculation significantly increases the accumulation of several macro and micronutrients, which in turn results in an increase in photosynthate production and better yields.

Result suggests that the application of AMF had a positive impact on pod length during the fruiting stage. Our result are in agreement with[64] who reported that length of pod increased in common bean (*Phaseolus Vulgaris L*) with AMF treated than control. Our results are also in agreement with[74] who found that in lentil pod length increased with treated with AMF. Protein content recorded in untreated plant was 18.67±0.33% while in AMF treated plant was 23.33±1. 45%. The result showed application of AMF resulted in enhanced protein content of tested plants (Figure-3.2). Results showed that AMF inoculum amplified crude protein of mung bean as compared to control. Maximum mean value 23.33±1.45% was documented in AMF inoculated plant than non innoculated plant. our results are in line with[75] who studied that AMF increased protein and oil content of soya bean and sunflower.

4. CONCLUSIONS AND RECOMMENDATIONS

This study investigated the effects of AM fungi on Mung bean and reported significant increase in growth, growth related parameters and overall productivity of mung bean. The study recommends application of AM fungi to agronomically important cereal crops in order to meet the food requirement for large population in Pakistan.

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AUTHOR'S CONTRIBUTION

Inzimam ul haq conducted this study as part of his M.Phil degree, Ajmal khan was the major supervisor of the Inzimam, Muhammad shakeel and Tabassum Yaseen acted as AM fungal specialists, Qayash Khan helped in manuscript proof reading.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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