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RESPONSE OF MYCORRHIZA ON PHYSIOLOGICAL AND BIOCHEMICAL PARAMETERS OF BLACK GRAM *VIGNA MUNGO* (L.) HEPPER

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Abstract: The present study was conducted to investigate the effect of different concentrations (5%, 10% and 15%) of arbuscular mycorrhiza (AM) (*Glomus mossae*) as a biofertilizer on seed germination, plant growth and proline content in nodules and leaves of black gram [*Vigna mungo* (L.) Hepper]. An experiment was carried out with the obtained seeds of black gram sown in field with and without mycorrhiza. The results obtained an overall increase in all the parameters in the inoculated plants than their comparable uninoculated ones. Mycorrhiza with the15% concentration, an *Vigna mungo* (L.) showed the higher effect on seed germination, plant growth, fresh and dry matter, protein and leghaemoglobin content as compared to 5% and10% mycorrhiza.

Keywords: *Vigna mungo* L. (Hepper), *Glomus mossae*, Protein, Proline and Chlorophyll.

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INTRODUCTION

Black gram commonly known as urad bean, a rich pulse crop, reported to be originated in India. Black gram (*Vigna mungo* L.) is a member of the family Fabaceae, is an annual and important short duration pulse crop which is cultivated both in kharif and rabi season. It is native to Central and South East Asia and is important food grain legume and rich in protein (El-Karamany, 2006). The AM fungi associated with legumes has an essential link for effective phosphorus nutrition, leading to enhanced nitrogen fixation that in turn promotes root and mycorrhizal growth (Geneva et al., 2006). Vesicular-Arbuscular mycorrhiza (VAM) has the symbiotic relationship with plant roots and certain soil fungi that play a pivotal role in nutrient management in ecosystem and protect plants against cultural and environmental stresses (Kothamashi *et al*., 2001). AM fungi helps in water regulation by extending their hyphae towards the available moisture zone for continuous water absorption and translocating them to plants. Arbuscular mycorrhiza (AM) has widespread symbiotic associations that are commonly described as the result of co-evolution events between fungi and plants where both partners are benefited from the reciprocal nutrient exchange (Sharma, 2003; Bonfante and Genre, 2008). Mycorrhizal infection has particular value for legumes because nodulation and symbiotic nitrogen fixation by rhizobia require an adequate phosphorus supply and restricted root system leads to poor competition for soil phosphorus (Carling *et al*., 1978). The beneficial effects of AM fungi with symbiotic association on the growth of plants are well known (Powell and Bagyaraj, 1984; Safir, 1987; Smith and Smith, 1996; Rajasekaran and Nagarajan, 2004). Subba Rao (1993) reported that the *Glomus mossae* enhance the accumulation of enzymes and amino acids resulting in the development of resistance in the host plants. In 1995, he also suggested that it is known to enhance the enzyme activity and arginine accumulation, which develops resistance in host plants. Arbuscular mycorrhizal fungi (AMF) are recognized for their multiple positive effects on plant growth and for contribution toward the maintenance of soil quality. Actually the full potential of this plant/AMF symbiosis has still not been fully recognized. Large numbers of AMF in soil have been used for inoculum production as alternative to chemical fertilizers (Duponnois *et al*., 2006). Root colonization and spore population of AMF may vary greatly in different plant species grown in different types of soils (Jakobsen and Nielsen, 1983). The objective of this study was to determine the response of mycorrhiza on physiological and biochemical parameters of *Vigna mungo* L.

MATERIALS AND METHODS

A field experiment was conducted between the month of March to June during the summer session of 2015. The experiment was conducted in the experimental plots in the Department of Botany, C.C.S. University Meerut. The experiments were carried out in natural conditions following standard agronomic practices. AM fungi (*Glomus mossae*) were managed to procure obtained from Indian Agricultural Research Institute, New Delhi to evaluate their inoculation response on the physiological and biochemical parameters of *Vigna mungo* (L.). The experiments were designed in four plots of equal size (1×1 M), three plots for treatment and one plot for control. Three solutions of different concentrations of mycorrhiza was prepared as 5%, 10% and 15% through by mixing 5gm, 10gm, 15gm in each 2 kg soil and then distributed in each of the plot uniformly. Fifty healthy seeds of *Vigna mungo* (L.) were used as sample size for every plot. Control plot as well as treated were irrigated with normal tap water.

The seed germination percentage was calculated after counting the difference between germinated (coming out of the soil) and non-germinated seeds (remaining inside, non emergent). After 30 days of seed sowing five plants were selected randomly from each plot and tagged for recording plant height, fresh weight/ plant, dry weight/ plant and number of nodules/ plant. Nodules were detached from the plant roots with the help of forceps. Fresh weight of nodules was measured immediately and followed by the measurement of their dry weight after drying them at 60ºC for 24 hours to obtain dry weight. Bradford (1976) method was used to determine the total protein content of nodules. Method adopted by Bates, *et al.,* (1952, 1973) was used to determine proline content of nodules. Appleby and Bergersen (1980) Method used leghaemoglobin quantity was measured spectrophotometrically as hemochromogen. Arnon (1949) Method used to determine chlorophyll content of leaves.

RESULTS AND DISCUSSION

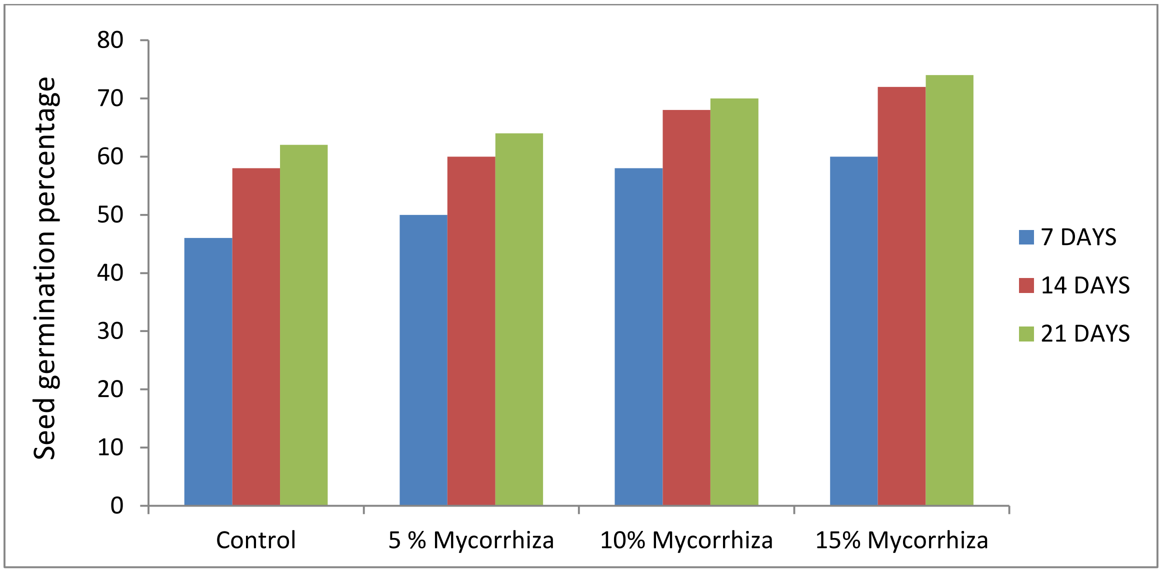
Seed germination

Germination in the 15% Mycorrhiza treated plots seeds were found higher as compared to control. Lowest difference is observed between mycorrhiza 10% and mycorrhiza 5%. The seed germination was measured at the different time intervals i.e. 7, 14, 21 days after showing. This could be due to arbuscular mycorrhizal fungi help in water regulation of plants by extending their hyphae towards the available moisture zone for continuous water absorption and translocating them into the plant (Panwar, 1991). AM symbiosis with plant provide necessary water and phosphorus for seed germination. All treated plants shown better germination as compare to control (Table 1, Fig 1).

Table 1: Seed germination percentage of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

Treatment	8 DAYS	14 DAYS	21 DAYS
Control	46	58	62
Mycorrhiza 5%	50	60	64
Mycorrhiza 10%	58	68	70
Mycorrhiza 15%	60	72	74

Figure 1: Seed germination percentage of *Vigna mungo* (L.) with the different concentrations of mycorrhiza.



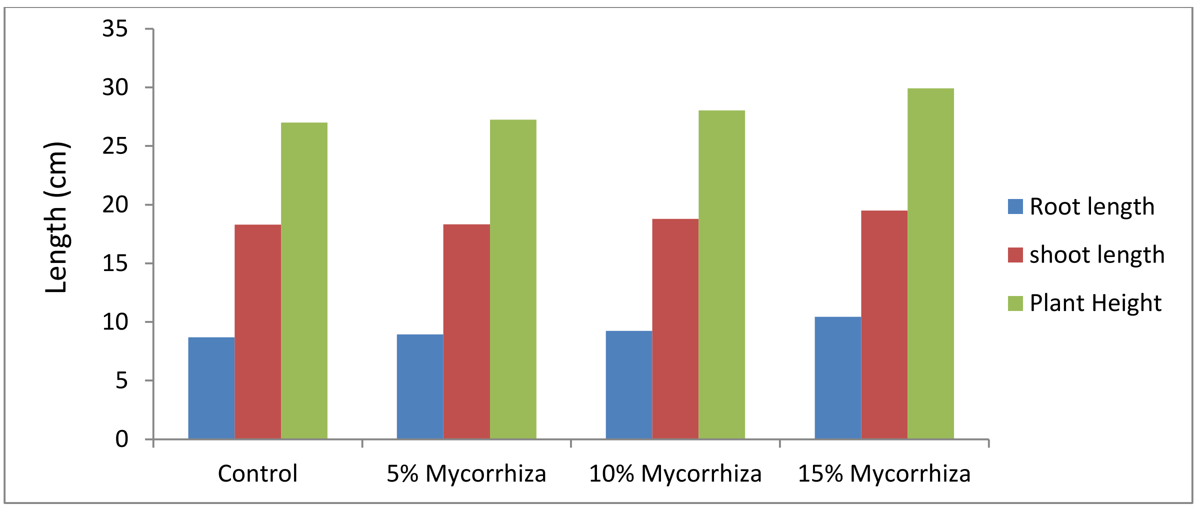
Root and Shoot lenght

The effect of *mycorrhiza* and its deifferent concentration on *Vignamungo* L. growth are given in (table 2 and figure 2). Plants treated with concentrations of mycorrrhiza 15% showed an increase in root length and shoot length when compared to control than other 5% and 10% treatments of mycorrhiza. Maximum plant root length (10.43) and shoot length (19.50) was recorded with 15% Mycorrhizal concentration (Figure 2). Plant height was increased orderly in all treated plants compared to control. Minimum plant root and shoot length was observed in control (Table 2). Significant increase in the root and shoot growth was possible due to their nutrient value required by plants in trace amount (Reichman, 2002). *Glomus mossae* of AM fungi that can either directly or indirectly increase plant growth by improving soil conditions (Kapoor and Mukerji, 1990). Graham *et al.,* 1987 and Huang *et al*., 1988 were indicated that Mycorrhizae are known to increase the root growth of infected plants and are also known to increase or cause no change in xylem pressure. Mycorrhizal plants were taller than non mycorhizal plants, the same results are supported by (Tarafdar & Marschner, 1995) that mycorrhizal plants performed better in terms of plant height. Due to the inoculation of AM (*Glomus mossae*), the mitotic activity of stem cells may enhance, resulting in taller plants in chickpea variety (Tabassum *et al*., 2012). Khanam *et al*., (2006) reported significantly higher plants in chickpea at all of the growth stages examined following dual inoculation of AM and *Rhizobium* along with added P.

Table 2: Root and shoot length of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

Treatment	Plant Length	Shoot Length	Root Length
Control	27.00	18.30	8.70
Mycorrhiza 5%	27.26	18.33	8.93
Mycorrhiza 10%	28.03	18.80	9.23
Mycorrhiza 15%	29.93	19.50	10.43

Figure 2: Root and shoot length of *Vigna mungo* (L.) with different concentrations of mycorrhiza.



Fresh and dry matter production

Fresh and dry weights of plants are given in the table 3. The maximum root and shoot fresh and dry weight were recorded in mycorrhiza 15% which were higher than other treatments and as well as with control. The minimum fresh and dry weight of root and shoot were recorded in control (table & fig 3). The experimental results suggest that a mycorrhiza produced maximum shoot and root biomass. Samelevels of results were obtained by Javaid, (2009). The interactive effect of AM and soil amendment was also significant for shoot biomass. Similar effect of AM was observed on dry weight of root and shoot. AMF are known to improve plant growth in different ways like increased phosphorous uptake, which increase in biomass of plants (Fattah and Javot *et al*., 2007). This reveals the host ability to translocate energy to shoot production due to the increased efficiency of the roots with AM fungi (Bethlenfalvay *et al*., 1982). They obtained a higher dry weight of shoots in single or AM+R inoculated plants than in uninoculated plants. The similar findings of Barakah and Heggo (1998) also correlate with these results. Many fungi and *Aspergillus fumigates* in particular enhanced the growth of mung bean and cluster bean (Tarafdar *et al*., 1992).

Table 3: Root and shoot fresh and dry weight (gm) of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

Treatment	Root		Shoot	
	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
Control	0.367	0.100	5.762	1.571
Mycorrhiza 5%	0.561	0.165	8. 235	1.851
Mycorrhiza 10%	0.563	0.166	11.783	3.196
Mycorrhiza 15%	0.979	0.199	12.47	3.522

Figure 3(i): Root fresh and dry weight (gm) of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

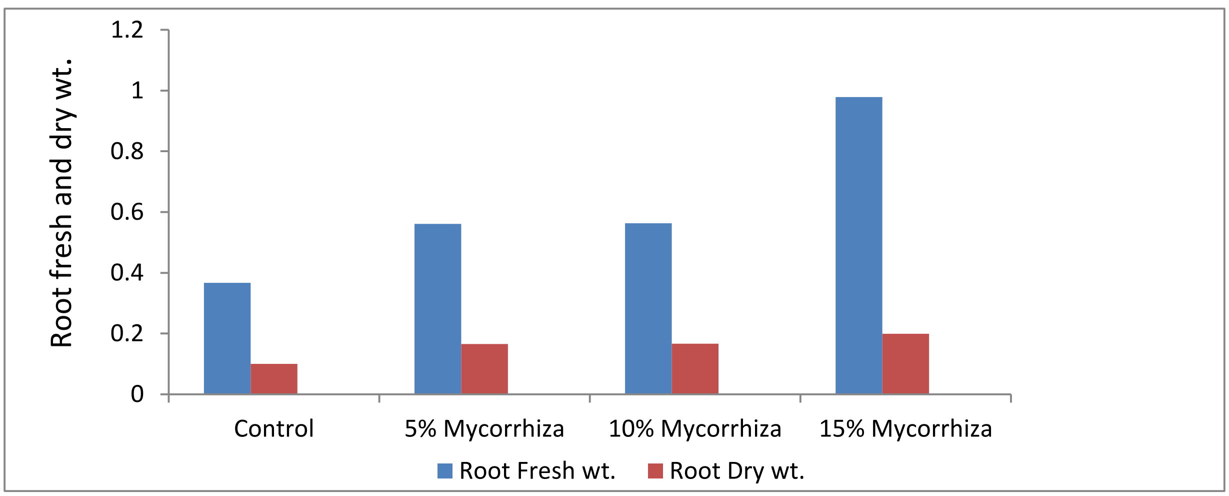
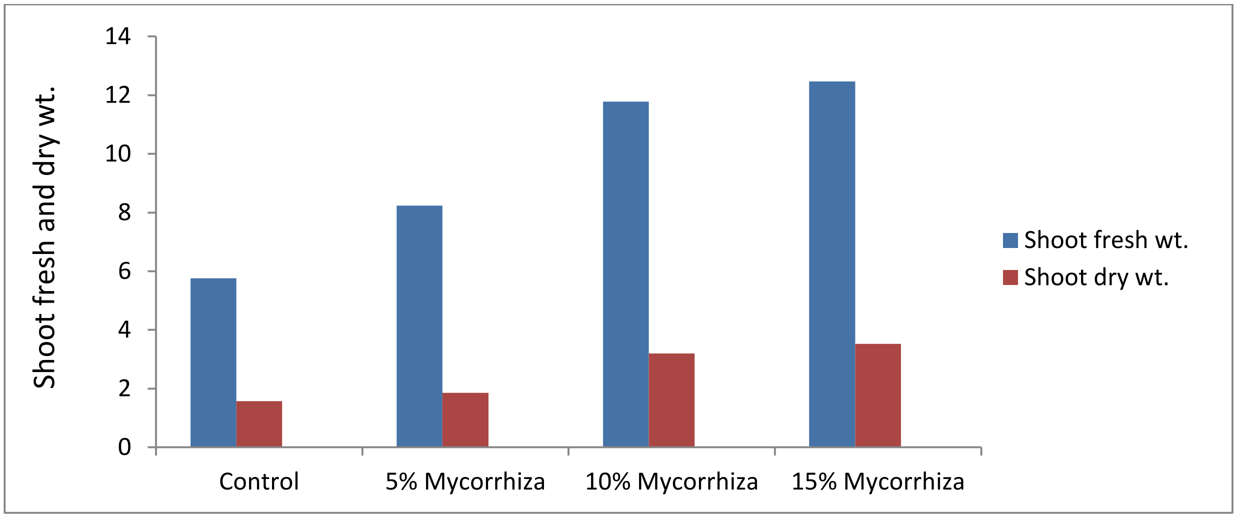


Figure 3(ii) : Shoot fresh and dry weight (gm) of *Vigna mungo* (L.) with different concentrations of mycorrhiza.



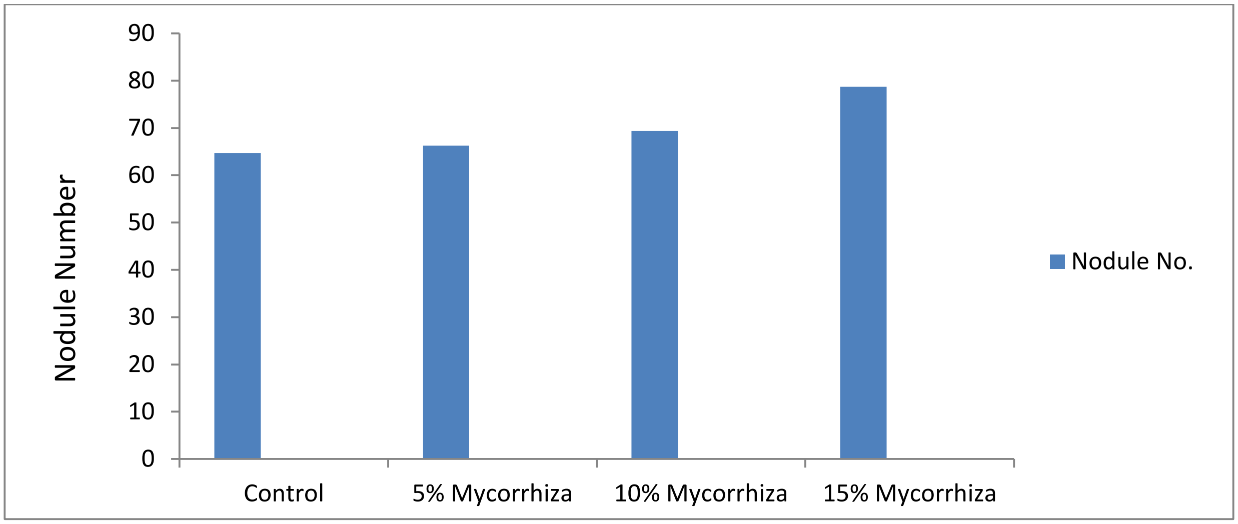
Nodulation

The numbers of the root nodules in *Vigna mungo* L. were higher in 15% *mycorrhiza* treatment as compared to other treatment. Maximum number of root nodules per plant (78.67) were found in the case where 15% mycorrhiza were used and minimum nodule numbers (64.70) per plant were observed in control (Table 4 & Fig 4). Valdenegro *et al*., (2001) indicated that all of the AM fungi had no effect on the nodule no. and on nodules biomass during the crop season except for *Glomus mossae*, which increase nitrogenise activity, AM improve P uptake, the higher p concentration in the plants benefits the bactetial symbionts and the functioning of its nitrogen, leading to increase N-fixation. AM fungi are evident to usually enhance nodulation and nitrogen fixation in legumes. Mortimer *et al*., (2008) observed that, the increase in total nitrogen in plant tissues has been linked with nitrogen fixation as a result of a higher phosphorus uptake through the AM hyphae rather than increased uptake of nitrogen from soil. AM application markedly enhanced nodule no. as well as biomass of nodules with *Bradyrhizobium japonicum*and with Phosphorus supply (Tabassum *et al*., 2012 and Fatma *et al*., 2012).

Table 4: An average nodule number of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

Treatment	Nodule no.
Control	64.70
Mycorrhiza 5%	66.24
Mycorrhiza 10%	69.33
Mycorrhiza 15%	78.67

Figure 4: Nodule number of *Vigna mungo* (L.) with different concentration Mycorrhiza.



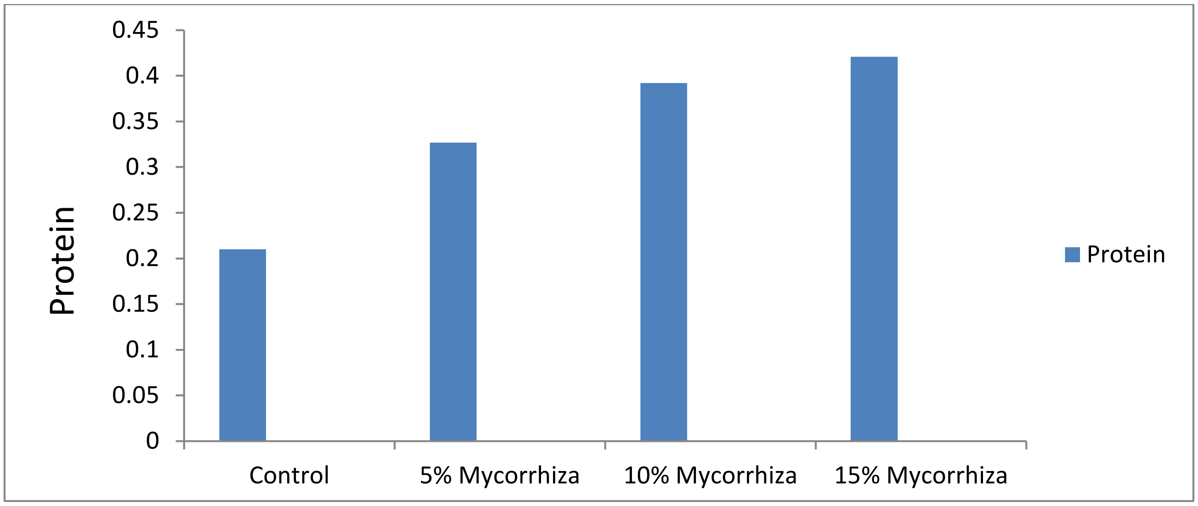
Protein Content

The effect of 15% mycorrhiza treated plants showed a greater increase in protein content than in control and other concentrations treated plants. Mycorrhiza concentrations enhanced the protein content as compared to control (Table 5). Increases in protein concentration of mycorrhiza treated plants are more frequently found (Gianinazzi-Pearson and Gianinazzi, 1995). The highest value of protein content was obtained at 15% mycorrhiza concentration. Minimum protein contents were found in control (Fig 5). Mycorrhizal treatment increased the protein contents might be due to more nitrogen and phosphorus availability to nodules.this could be linked to the higher accumulation of proteins which can enhances the root fresh and dry weights (El-samad *et al*., 2005). *Glomus mossae* effect on growth and yield of legumes shows positive effect on growth compare to non mycorrhizal plants (Avis *et al*., 2008). Maximum yield growth and productivity was observed in mycorrhizal treated plants (Satyawati et al., 2005).

Table 5: Protein content of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

Control	0.210
Mycorrhiza 5%	0.327
Mycorrhiza 10%	0.392
Mycorrhiza 15%	0.421

Figure 5: Protein content of *Vigna mungo* (L.) with different concentration of Mycorrhiza.



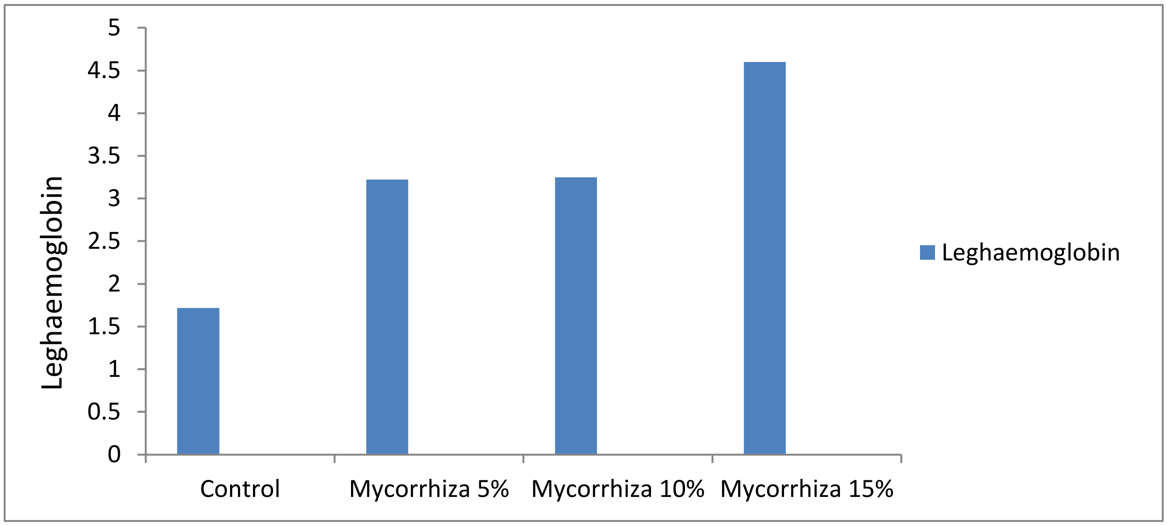
Leghaemoglobin Content

The most rich and greatest characterized nodule specific proteins are the leghaemglobin (Lb), which are articulated in the infected cells just prior to the beginning of nitrogen fixation. The Lb ammount was significantly higher in Mycorrhiza treated plants (Table and Fig 6). The leghaemoglobin content was maximum at 15% concentration of mycorrhiza when compare to 10%, 5% concentration of mycorrhiza and control. These oxygen binding heam proteins are supposed to be responsible for supporting the flux of oxygen to the nitrogen fixing bacteroids (Appleby, 1984). The pink color, of healthy and effective nodules, is due to the presence of leghemoglobin. Leghaemoglobin contribute to the higher nitrogen-fixing ability of AM plants (Evelin et al., 2009). The better nodulation might be resulted in higher content of leghemoglobin in nodular tissues. Similarly, higher leghemoglobin contented in mycorrhiza was mainly due to better root and nodules development (Sidhu et al., 1967).

Table 6: Leghaemoglobin content of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

Treatment	Leghaemoglobin mM (g.f.m.)$^{-1}$
Control	1.717
Mycorrhiza 5%	3.222
Mycorrhiza 10%	3.247
Mycorrhiza 15%	4.598

Figure 6: Leghaemoglobin content of *Vigna mungo* (L.) with different concentrations of mycorrhiza.



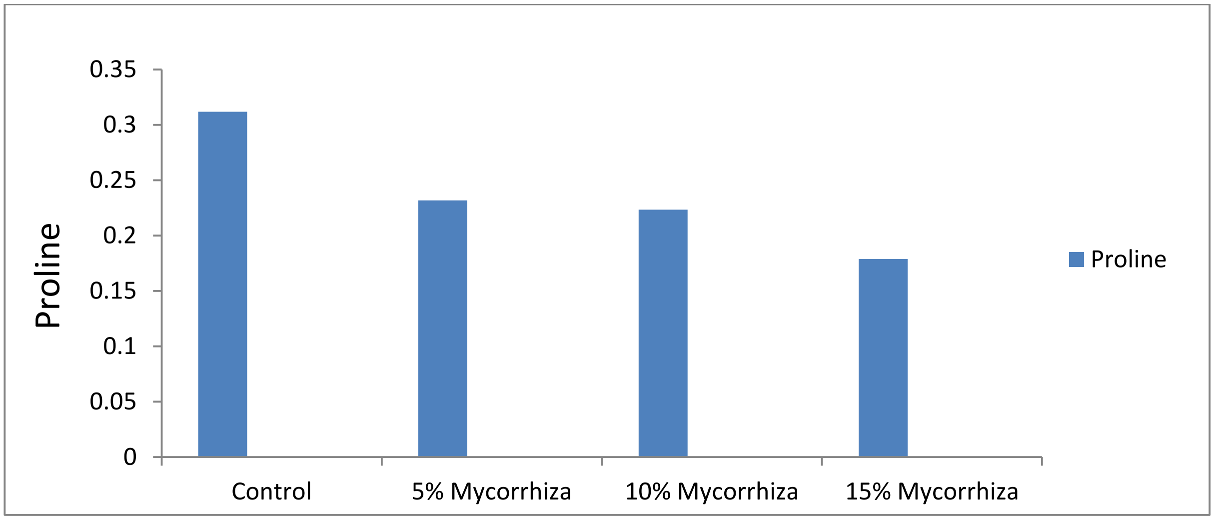
Proline Content

Higher plants accumulate higher levels of proline to cope with the harsh environmental conditions. Proline accumulation accepted as an indicator of environmental stress maximum proline content was observed in control plants and minimum proline concentration was observed in 15% mycorrhizae (Table and Fig 7). Proline act as a signalling molecule and can help plants in recovering from stress conditions (Szabados and Savoure, 2009). Increased amount of proline in control plants may be due to the nutrient deficiency in stress condition because control plants have lack P and N content in comparision to AM treated plants.

Table 7: Proline content of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

Treatment	Proline mg/g
Control	0.3119
Mycorrhiza 5%	0.2318
Mycorrhiza 10%	0.2234
Mycorrhiza 15%	0.1791

Figure 7: Proline content of *Vigna mungo* (L.) with different concentrations of mycorrhiza.



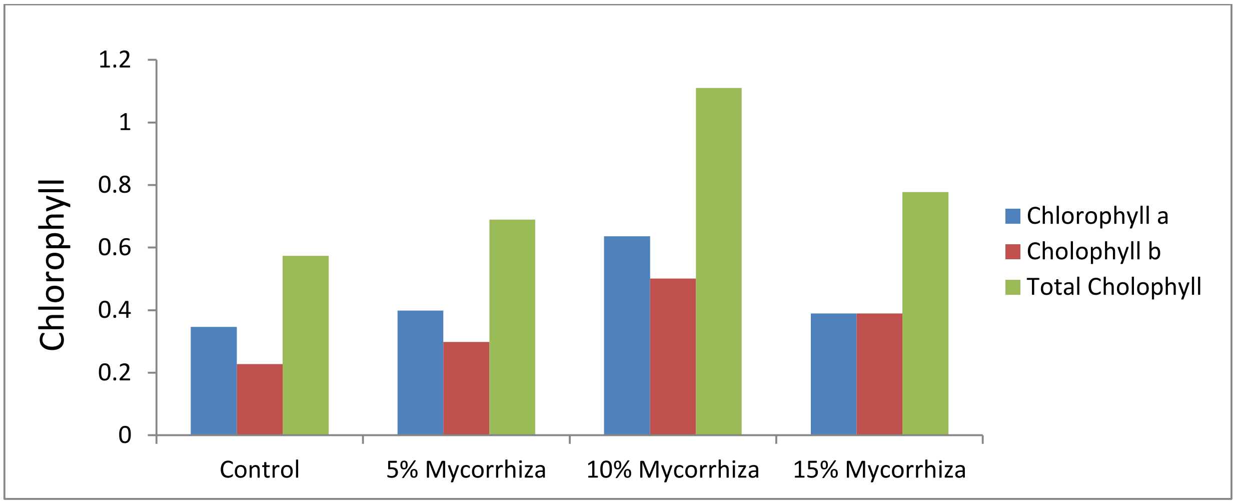
Chlorophyll content

The effect of mycorrhiza treated plants showed a greater increase in the chlorophyll content of leaves than in the control (Table and Fig 8). 10% percent concentration of mycorrhiza shows maximum chlorophyll content. Chlorophyll amount gradually lowers down with much higher concentration of mycorrhiza. AMF inoculated plants increased vegetative growth, total chlorophyll content and uptake of nutrients like nitrogen, phosphorus, potassium, calcium and magnesium in maize plants (Sitaramaiah *et al.,* 1998). AM fungi have been found to increase chlorophyll content (Demir, 2004). AM have been shown to improve the chlorophyll contents on leaves of many plants. AM fungi with *Rhizobium* might be attributed to increased rate of photosynthesis and transpiration (Sampathkumar *et al*., 2003).

Table 8: Chlorophyll content (mg/g leaves) of *Vigna mungo* (L.) with different concentrations of mycorrhiza.

Treatment	Chlorophyll a mg/g leaves	Chlorophyll b mg/g leaves	Total Chlorophyll mg/g leaves
Control	0.3463	0.2273	0.5734
Mycorrhiza 5%	0.3984	0.2983	0.6895
Mycorrhiza 10%	0.6364	0.5005	1.1097
Mycorrhiza 15%	0.3893	0.3889	0.7779

Figure 8: Chlorophyll content of *Vigna mungo* (L.) with different concentrations of mycorrhiza.



CONCLUSION

From the present study, it can be concluded that different concentrations of mycorrhiza had significant and beneficial effect on germination, plant growth, and nodular number, fresh and dry weight of *Vigna mungo* L. The increase in concentration of mycorrhiza (*Glomus mossae*) led the germination percentage and plant growth and has a positive effect on other physiological parameters. The higher concentration of mycorrhiza also exhibits the better response on all the biochemical parameters except its inhibitory effect on proline. The increase in plant growth and yield of crops like urad bean gives a clue to use AM fungi as a tool to enhance plant biomass and yield of various other crops. Thus it might be possible to use the mycorrhiza as a biofertilizer has a potential role in plant growth and development.

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