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Ecological balance of Iron ore mines land in Chhattisgarh by using vesicular arbuscular mycorrhiza fungi.

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Abstract

The State of Chhattisgarh is situated in the Mid Eastern India, bounded between North latitude 17^0 46' to 24^0 06' and East Longitude 80^0 15' to $84^0$25' and the states is geologically one of the most important terrains in the Indian shield comprising of litho logical sequence ranging in age from Archaean to Recent. Bountiful nature has bestowed Chhattisgarh with vast reserves of all important minerals. Chhattisgarh state has rich sources of mineral resource especially iron and coal. Open cast mining is the dominant form of mining. The immediate effect of open cast mining is the removal of soil and vegetation cover. The extent of damage depends on location of mining site, scale of operation, mining methods, degree of mechanization etc. The effects of mining activity as well as mining waste causes such as soil erosion, air and water pollution, toxicity, geo-environmental disasters, loss of biodiversity, and ultimately loss of economic wealth. This research paper presenting the how Vesicular Arbuscular Mycorrhiza fungi used for ecological balances. VAM fungi are types of endomycorrhizae and used as biofertilizer for revegetation of mining destroyed sites especially iron ore mines in Chhattisgarh. Mycorrhizae associate plants shows more root and shoot height, fresh and dry biomass weight, high content of soluble protein and low rate of mortality when planting in actual mining sites.

Keywords: VAM fungi, mining activity, biofertilizer, revegetation, ecological balances, etc.

INTRODUCTION

Land is one of the most important resources on which human beings depend. The rate of consumption of mineral resources is continuously increasing with the advancement of science and technology, economic development, industrial expansion, acceleration of urbanization and growth of population are create the ecological imbalances. The end result for mining activities on the surface is mining wastes and alteration of land forms which is a concern to the society and it is desired that the pristine conditions are restored (Sheoran et al., 2010). The mining disrupts the aesthetics of the landscape along with it disrupts soil components such as soil horizons and structure, soil microbe populations, and nutrient cycles those are crucial for sustaining a healthy ecosystem and hence results in the destruction of existing vegetation and soil profile (Kundu and Ghose, 1997). The effects of mine wastes can be multiple, such as soil erosion, air and water pollution, toxicity, geo-environmental disasters, loss of biodiversity, and ultimately loss of economic wealth (Wong, 2003; Sheoran et al., 2008). Mined land sites are generally known to be nutrient poor and contain soils that are in dire need of stabilization to prevent erosion. Mined land sites, however, are physically, chemically and biologically altered. Mining activity also affects many soil factors, such as pH, fertility, toxicity, bulk density and soil moisture. Mineral extraction process must ensure the return in productivity of the affected mines (Ghose 1989). This all causes the ecological imbalances, so we need to revegetate the mining sites. Revegetation is the process of replanting and rebuilding the soil of disturbed land. This may be a natural process produced by plant colonization and succession, or an artificial (manmade), accelerated process designed to repair damage to a landscape due to wildfire, mining, flood, or other cause. Conservation and reclamation efforts to ensure continued beneficial use of land resources are essential. Reclamation is the process by which derelict or highly degraded lands are returned to productivity, and by which some measures of biotic function and productivity is restored. Long term mine spoil reclamation requires the establishment of stable nutrient cycles from plant growth and microbial processes (Singh et al., 2002, Lone et al., 2008; Kavamura and Esposito, 2010).

Mycorrhiza is the most prevalent form of symbiotic (mutual) relationship between plant roots and soil fungi. Mycorrhizae contain both plant roots and fungal tissues. In this symbiotic association, the growth of plant is enhanced by the absorption of water and nutrients from the soil with the help of mycorrhiza. In return, plant supplies food / energy in the form of carbohydrates to the fungus. Due to the difficulties associated with mass production of its culture, mycorrhiza has not made much way in terms of practical application, as compared to N-fixing or P-solubilizing biofertilizers. (The terms 'Mycorrhiza comes from Greek where 'Myco' means fungus and 'rhiza' means root i.e. literally means root fungus).

Out of the many factors which affect the establishment of mycorrhizae in the soil-plant system, the important ones are the 1) Mycorrhizal-depending plant species (legumes are more mycotrophic than grasses). Such plants live in association with fungus. 2) Host specificity: These fungi are associated with a wide range of host plants. Over 80% vascular plants are associated with mycorrhiza. Such associations are thought to be mutually beneficial in many

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cases where in the host plant exchanges the photosynthates produced by it for the mineral nutrients mobilized by the fungus. 3) Photosynthesis Autotrophic host supplies carbohydrate to mycorrhiza from photosynthesis which is mainly influenced by light. 4) Soil conditions (pH, temperature etc): The efficiency of mycorrhiza is influenced by sol properties. Intensity of infection and number of spores in the soil depend on soil pH.5) Fertilizer: Excess fertilizer application may suppress the growth of mycorrhiza. 6) Pesticides/fungicides: These may be harmful for the development of mycorrhiza.7) Soil microorganisms: The abundance of N-fixing and P-solubilising microorganisms in the soil may stimulate mycorrhiza (Bhattacharyya and Tandon, 2012).

MATERIALS AND METHODS

Survey

Survey was conducted in three Iron ore mines which include Rajhara, Mahamaya and Ari-dongari iron ore mines in Chhattisgarh state.

Soil sampling

Soil samples were collected from the three regions of iron ore mines unmined area, mined area and dumped area. The sampling location sites were randomized after site facing. The soil sample was collected from ten different locations of all three areas. The core method was used to take samples from 30 cm depth of the selected site soil samples.

Analysis of soil Physio-chemical parameter

The physical parameters like temperature, pH, total dissolve solid (ppm), and conductivity (ms/cm) of soil of three different regions was checked. (Deluxe soil and water analysis kit model 172, company EI) along with their chemical parameter such as percentage oxidizable organic carbon, available phosphates (kg/ha), available potassium (kg/ha), ammonical nitrogen (kg/ha) and nitrate nitrogen (kg/ha). (Soil test kit, Himedia)

Isolation & identification of VAM

Isolation of VAM from soil sample is done by Gerdemann and Nicolson (1963) and is the most widely used procedure for the study of spores of Endogonaceous fungi in soil.

Identification of VAM: Identification of different genera of VA Mycorrhiza is done based on their morphological characteristics (Schenck N C and Perez Y, 1990; Mukerjee, K.G.; INVAM worksheet).

Propagation of mycorrhiza

The culture of VAM has been growing natural media soil and sand with ratio (1:1) in pot after three days successive sterilization in autoclave at 121^0C for 01 hour and overnight cooling. Inoculums were raised on *Zea mays* L. grown in a greenhouse.

Inoculation studies of VAM fungi

Five tree species namely, *Dulbergia sissoo, Gmelina arborea, Diospyros melanoxylon, Delonix regia, Dendrocalamus strictus,* were selected for the study. The seeds were sterilized with 0.01% of sodium hypochlorite and the percentage germination of seed was checked by Petri plate method. After germination the 14-day-old seedlings were transplanted for the study. In the green house pots of 4 kg capacity were filled with sterilized (autoclaved at 15 lb, 121° C, for three successive days) soil and sand at the ratio of 2:1 Inoculation of different AM isolates was done spreading soil inoculum (aprrox 100 g) containing about 300-350 spores in each pot separately for each treatment. Only sterilized soil was taken as control. Plants of individual tree species was transplanted in 5 replicates separately and watered as per the requirement. Uninoculated pots (control) were supplied with 25 ml of half strength of Hoagland solution every fortnight and inoculated pots were supplied with the same solution only once at the beginning of the experiment and with phosphate-free solution thereafter.

Thirty days and Ninety days old plant were harvested and comparative studies of mycorrhizal and non-mycorrhizal plant, along with parameters like percentage root infection, spore population, root length, shoot length, fresh and dry biomass, root-shoot ratio and fresh-dry biomass ratio of plant was analyzed.

Molecular study

Plants growth, sampling and protein extraction

Dulbergia sissoo (Roxb)*, Diospyros melanoxylon* (Roxb) and *Gmelina arborea* (Roxb) were grown in a sterilized soil- sand-vermiculite (1:1:1) mixture either uninoculated or inoculated with soil-inoculum (10 % v/v) of *Glomus sp.* Inoculum was raised on *Zea mays* L. grown in a greenhouse. The substrate was inoculated with soil containing hyphae, infected root fragments (90% root colonization) and spores (150 spores per 100 g soil). Plants were grown in 05 replicate in pots under controlled conditions in greenhouse. Uninoculated pots were supplied with 25 ml of Hoagland nutrient solution every fortnight; inoculated pots were supplied with the same solution only once at the beginning of the experiment and with phosphate-free solution thereafter.

Roots with the rhizosphere material mycorrhizal and non mycorrhizal were batched from several pots 90 days later and were removed by washing. One gram of fresh root of each plant means mycorrhizal and non mycorrhizal plants. Protein was extracted by 0.1 M potassium phosphate buffer (PB) at pH 7.2, containing 0.15 M NaCl, 3 mM KCl, 5 mM isoascorbic acid, 0-2 % polyvinyl polypyrrolidone and pinch of sterile silica with a mortar and pestle in ice box. Extracted protein was centrifuge at 15k rpm for 15 minutes at 4^0 C. The supernatant was kept overnight at 4^0 C. The soluble protein content was precipitated by ice chilled acetone in equal volume and centrifuged as before. The pellet was obtained suspended in 4X Laemmli buffer.

Quantification of protein contents

The protein solution was again precipitated by equal volume of 10% TCA solution and centrifuged as before. The pellet was suspended in 1N NaOH solution and soluble protein content was quantified by Bradford assay (1976). Optical density was taken at 595 nm (ELISCO SL 27).

Outplanting

Mycorrhizal treated plants were planted in Rajhara iron ore

mines.

RESULTS AND DISCUSSION

Inoculation studies

Five tree species *Dendrocalamus strictus, Gmelina arborea*, *Diospyros melanoxylon, Dulbergia sissoo* and *Delonix regia* were inoculated with *Glomus sp*., results depicted in Figure no. 01.

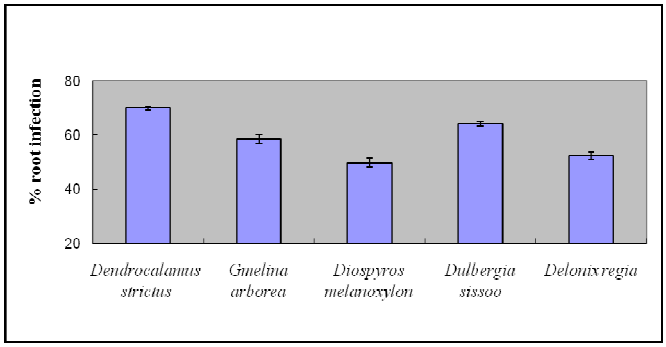

Fig 1. Percentage root infection of plants inoculated with selected VAM fungi at different stages of growth.

The maximum percentage root infection was found in the root of *Dendrocalamus strictus* (70±0.707), followed by the *Dulbergia sissoo* (64.2±0.86), *Gmelina arborea* (58.6±1.66), *Delonix regia* (52.4±1.288*) and Diospyros melanoxylon* (49.8±1.77). The VAM spore found in *Dulbergia Sissoo and Delonix regia* are high but same in number, followed by *Gmelina arborea, Diospyros melanoxylon* and the minimum value *Dendrocalamus strictus*. The results indicated a wide spectrum of infection potential of this fungus. Since low percentage of infection in forest tree species has been observed; it cannot be correlated as an efficient parameter for determination of AM infectivity and affectivity. Mycorrhizal association provides additional support to plants for nutrient uptake in deficient soil. Incidence of fairly low percentage of root infection was apparent in all five species.

The present study showed the relative effects of *Glomus sp*. on the growth of host plant. As far as symbiotic benefits were concerned most of the host plants, achieved increments in all parameters like root length (figure no. 02), shoot length (figure no. 03), fresh weight biomass (figure no. 04) and dry weight biomass (figure no. 5). Enhancement of plant growth as compared to uninoculated plants. Similar differential responses due to AM inoculations were observed in plant root length. Fresh and dry biomass of plant enhanced to the maximum over the control. All inoculated plants showed increase in fresh shoot length over the control. The maximum percentage of root infection was recorded in *Dendrocalamus strictus.*

Root length

Figure no. 02 shows comparative study of five mycorrhizal and nonmycorrhizal plants root length. The root length (cm) of mycorrhizal inoculated plant *Dendrocalamus strictus* was (21.34) compared then nonmycorrhizal plant (12.68). The root length of mycorrhizal inoculated plant *Gmelina arborea* was (13.9) compared then nonmycorrhizal plant (11.7). The root length of mycorrhizal inoculated plant *Diospyros melanoxylon* was (13.96) compared then nonmycorrhizal plant (6.96). The root length of mycorrhizal inoculated plant *Dulbergia Sissoo* was (11.94) compared then nonmycorrhizal plant (7.58). The root length of mycorrhizal inoculated plant *Delonix regia* was (14.44) compared then nonmycorrhizal plant (9.68). All the selected plant was found to have more root length than nonmycorrhizal plants.

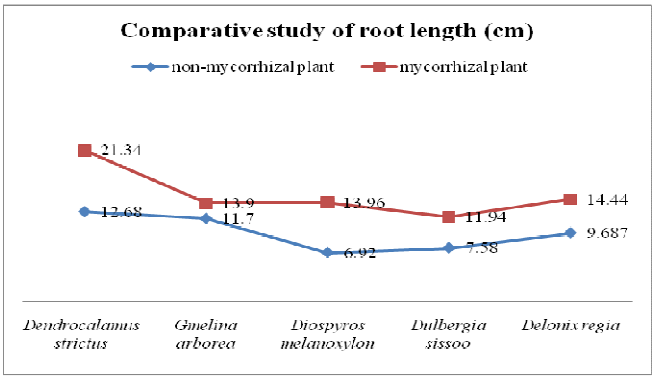

Fig 2. Comparative study of root length of the plants (cm)

Shoot length

Figure no. 03 shows comparative study of five mycorrhizal and nonmycorrhizal plants shoot length. The shoot length (cm) of mycorrhizal inoculated plant *Dendrocalamus strictus* was (27.74) compared then nonmycorrhizal plant (8.46). The shoot length of mycorrhizal inoculated plant *Gmelina arborea* was (14.98) compared then nonmycorrhizal plant (7.3). The shoot length of mycorrhizal inoculated plant *Diospyros melanoxylon* was (9.8) compared then nonmycorrhizal plant (6.44). The shoot length of mycorrhizal inoculated plant *Dulbergia Sissoo* was (13.66) compared then nonmycorrhizal plant (7.8). The shoot length of mycorrhizal inoculated plant *Delonix regia* was (12.08) compared then nonmycorrhizal plant (6.56). All the selected plant was found to have more root length than nonmycorrhizal plants. All the selected plant was found to have more shoot length than nonmycorrhizal plants.

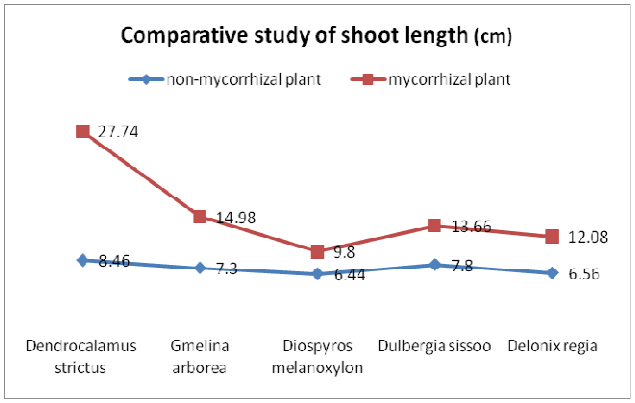

Fig 3. Comparative study of shoot length of plants (cm)

Fresh biomass

Figure no. 04 shows comparative study of five mycorrhizal and nonmycorrhizal plants fresh biomass. The biomass fresh weight (g / plant) of mycorrhizal inoculated plant *Dendrocalamus strictus* was (0.67) compared then nonmycorrhizal plant (0.237). The biomass

fresh weight of mycorrhizal inoculated plant *Gmelina arborea* was (3.347) compared then nonmycorrhizal plant (2.21). The biomass fresh weight of mycorrhizal inoculated plant *Diospyros melanoxylon* was (3.472) compared then nonmycorrhizal plant (2.06). The biomass fresh weight of mycorrhizal inoculated plant *Dulbergia Sissoo* was (3.82) compared then nonmycorrhizal plant (2.12). The biomass fresh weight of mycorrhizal inoculated plant *Delonix regia* was (3.39) compared then nonmycorrhizal plant (2.047). All the selected plant was found to have more root length than nonmycorrhizal plants. All the selected plant was found to have more biomass (fresh weight) than nonmycorrhizal plants.

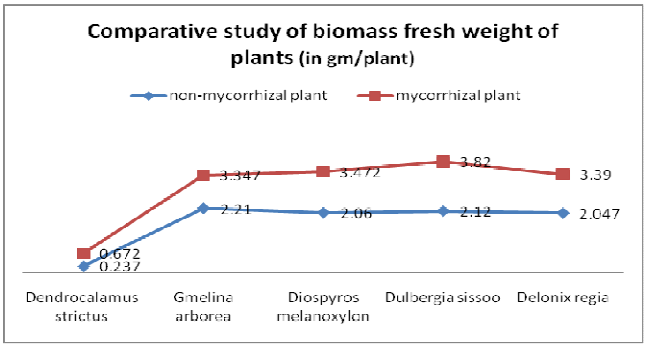

Fig 4. Comparative study of biomass fresh weight of plants

Dry biomass

Fig. no. 5 shows comparative study of five mycorrhizal and nonmycorrhizal plants dry biomass. The biomass dry weight (g / plant) of mycorrhizal inoculated plant *Dendrocalamus strictus* was (0.275) compared then nonmycorrhizal plant (0.098). The biomass dry weight of mycorrhizal inoculated plant *Gmelina arborea* was (2.015) compared then nonmycorrhizal plant (0.985). The biomass dry weight of mycorrhizal inoculated plant *Diospyros melanoxylon* was (1.9) compared then nonmycorrhizal plant (0.87). The biomass dry weight of mycorrhizal inoculated plant *Dulbergia Sissoo* was (2.01) compared then nonmycorrhizal plant (0.9). The biomass dry weight of mycorrhizal inoculated plant *Delonix regia* was (1.84) compared then nonmycorrhizal plant (0.895). All the selected plant was found to have more root length than nonmycorrhizal plants. All the selected plant was found to have more biomass (dry weight) than nonmycorrhizal plants. Results also attributed towards the suitability of this *Glomus sp*. This is the best inoculants for forest trees species being used as a revegetation and reclamation of destroyed land soils after mining.

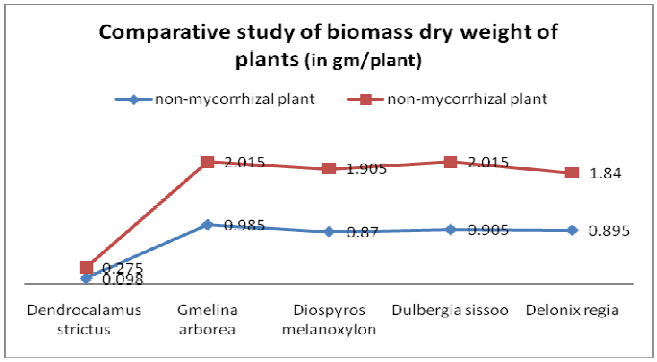

Fig 5. Comparative study of biomass dry weight of plants

Shoot-root and biomass ratio

Another parameter studied is shoot-root and biomass ratio in different tree species under non-mycorrhizal and mycorrhizal conditions. The ratio of root-shoot and biomass has been depicted in table no. 01

Table 1. Root shoot and biomass ratio of selected tree species.

S. no.	Name of plant	Non mycorrhizal (control)		Mycorrhizal	
		R/S length	F/D biomass	R/S length	F/D biomass
1	*Dendrocalamus strictus*	1.49	2.41	0.76	2.44
2	*Gmelina arborea*	1.6	2.242	0.92	1.66
3	*Diospyros melanoxylon*	1.074	2.367	1.42	1.822
4	*Dulbergia sissoo*	0.9715	2.34	0.87	1.895
5	*Delonix regia*	1.47	2.28	1.19	1.842

Though AM inoculations enhanced the growth of forest tree species, the differences in plant height and biomass among the control and inoculated plants were not very significant in *Dendrocalamus strictus* and *Delonix regia.* However the overall performance of *Gmelina arborea, Diospyros melanoxylon* and *Dulbergia sissoo* was encouraged through mycorrhization.

Comparative study of protein content

The comparative study of protein content in mycorrhizal and non mycorrhizal plant roots, more protein quantities extract were obtained from mycorrhizal roots as compared to non mycorrhizal roots. The concentrations of protein content have been given in figure 06 which shows more difference of protein content in *Gmelina arborea*, the mycorrhizal root gives 36.15 mg /g of root as compared to control of *Gmelina arborea* 27.73 mg / g of protein. The second comparative results show *Dulbergia sissoo* and *Diospyros melanoxylon* roots having less protein content.

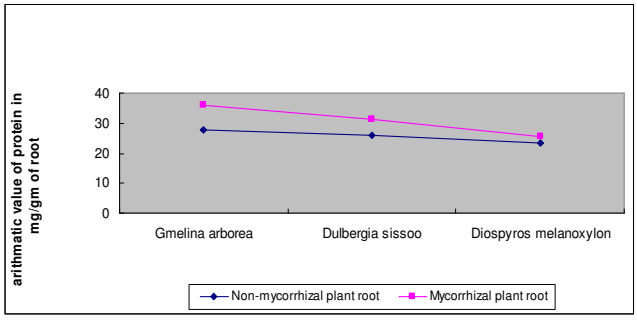

Fig 6.Comparative study of soluble protein content of mycorrhizal and non-mycorrhiza plants.

Cumulative mortality study

In cumulative mortality study of selected woody plants inoculated with native mycorrhizal and nonmycorrhizal fungi during the first months of plantation. The studies carried out for six months. Were studied at the Rajhara iron ore mines and the results depicted in figure 07. The rate of mortality of nonmycorrhizal fungi inoculated plants (control) of first month was zero, then second and third months was 20%, fourth and fifth months was 30% and in six months

the mortality rate reached in to 40%. The rate of mortality of mycorrhizal fungi inoculated plants (test) was zero up to four months from month of out planting. Last two month means fifth and six months few plants were died and their mortality rate was 10%. Overall the rate of mortality of nonmycorrhizal inoculated plants shows higher rate (40%) as compared then native mycorrhizal inoculated plants (10%). Inoculation with native AM fungi significantly decreased the planting mortality during the first months of the plantation. It is well known that the transplanting shock and the damage suffered by the transplanted plants could be critical to the success of plantation. As inoculated plants were stronger then the noninoculated ones, they could survive out planting by showing better resistance to environmental conditions.

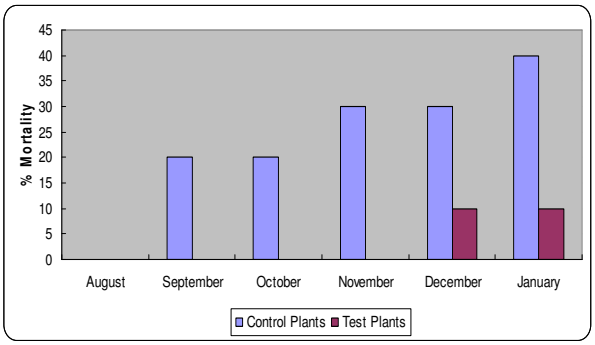

Fig 7. Cumulative mortality of plants

CONCLUSIONS

Following are the important experimental findings of the prevent investigation

- The genus Gigaspora is a common species that occurs widely in the soils of unmined zone of Rajhara iron ore mines.
- The genus Glomus is a common species that occurs widely in the soils of unmined zone of Ari-dongari iron ore mines.
- Unmined zone or untouched zone are having more microflora comparisons then mines dumped zone or mined zone.
- The excessive conductivity and total dissolve solid can also lower the efficiency of applied fertilizer for revegetation as well as mycorrhiza spore germination.
- Soil testing helps us to known the nutritional status of the soil to assess the profitability of applying a particular nutrient.
- If soil pH is very high there is no use of adding fertilizer for revegetation.
- Mycorrhiza shows diverse personality; in present study the root infection of mycorrhiza shows in all collected rhizosphere plants from mines area but percentage are different.
- Inoculation resulted in enhancement of plant height as compared to uninoculated (control). Similar differential responses due to AM inoculations were observed in plant root length. Fresh and dry biomass of plant enhanced to the maximum over to the control. All inoculated plants showed increased in fresh shoot length over the control. The maximum percentage of root infection was recorded in AM- infected plants.
- In the present molecular study of *Gmelina arborea, Diospyros melanoxylon* and *Dulbergia sissoo* plants showed higher soluble protein than non mycorrhizal ones.
- From the present study, the use of native AM fungi is a source of AM inoculum could be a great relevance to accelerate the process of revegetation in iron ore mines degraded land soils.

From the present studies, finally conclude the mycorrhizas are suitable for revegetation and help to balance of ecological disturbances of mining sites.

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