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Biofertiliser : Vam Fungi- A Future Prospect for Biological Reclamation of Mine Degraded Lands

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The mine-degraded lands initially lack viable mycorrhizal fungal population, and thus, the establishment of a vegetative community will be delayed. Mycorrhiza inoculation can enhance productivity of degraded land by increasing drought tolerance of plants and phosphorus availability, which are the main two limiting factors for plant establishment. A number of interacting factors affect the successful colonization of VAM fungi are pH, soil nutrients, organic matter, moisture, temperature, and age of disturbance sites. As mine spoils are devoid of natural mycorrhizal population, saplings should be inoculated in nursery itself. Bulk production of mycorrhiza inoculums, monitoring of plant growth response in pot and field trials are also discussed.

KEYWORD

Mine degraded land, Mycorrhiza, Edaphic factors, Innoculum, Growth response.

INTRODUCTION

The overburden (OB) materials vary widely in their physical, chemical and biological properties, than natural soil, which affect the plant establishment, survival and growth. To reclaim these OB dumps biologically, a long-term nutrient cycling between soil-plant has to be established. The long-term plant community stability on OB dumps relies upon the development of a functional soil microbial community. Soil microorganisms are responsible for decomposition of plant litter, mineralization of essential plant nutrients, nutrient cycling, accumulation of organic matter and beneficial changes of soil physical characteristics. One group of soil microorganisms important to the development of long-term plant community structure is mycorrhizal fungi. The absence of mycorrhiza may account for the poor survival of plants used for OB dump reclamation. To reclaim OB dumps biologically several types of biofertilizer are being used (Norland, 1993; Maiti, 1997; Rao and Tak, 2002; Juwarkar *et al.*, 2003). Biofertilizer (BF) is defined as 'fertilizer of biological origin' and broadly classified into 3 categories: (1) Nitrogen fixing BF- *Rhizobium,* blue green algae, *Azotobacter, Azolla*, (2) phosphorus mobilizing BF- phosphate solubiliser (*Bacillus, Pseudomonus, Aspergillus),* phosphate absorber (VAM fungi- *Glomus)* and (3) organic matter decomposer BF- cellulolytic (*Cellulomonus, Trichoderma)* and lignolytic (*Arthobacter, Agaricus)*.

THE MYCORRHIZAL ASSOCIATION- A PLANT ROOT/FUNGUS INTERACTION

The term mycorrhizae denote 'fungus roots'. It is a symbiotic association between host plants and certain group of fungi at the root system, in which the fungal partner is benefited by obtaining its carbon requirements from the photosynthates of the host and the host in turn is benefited by obtaining the much needed nutrients especially phosphorus, calcium, copper, etc., which are otherwise inaccessible to it, with the help of the fine absorbing hyphae of the fungus. Based on the type of association formed by the ubiquitous fungi two broad groups: Ectomycorrhizae and Endomycorrhizae have been recognized (Gerdemann, 1975).

Ectomycorrhiza

The diagnostic feature of ectomycorrhizae is the presence of hyphae between root cortical cells producing a netlike structure called the Hartig net, after Robert Hartig who is considered the father of forest biology. Many ectomycorrhizae also have a sheath, or mantle, of fungal tissue that may completely cover the absorbing root (usually the fine feeder roots). The mantle can vary widely in thickness, colour, and texture depending on the particular plant-fungus combination. The mantle increases the surface area of absorbing roots and often affects fine-root morphology, resulting in root bifurcation and clustering. Ectomycorrhizae are found on woody plants ranging from shrubs to forest trees. Many of the host plants belong to the families Pinaceae, Fagaceae, Betulaceae and Myrtaceae (Gerdemann, 1975). Over 4,000 fungal species, belonging primarily to the Basidiomycotina, and fewer to the Ascomycotina, are known to form ectomycorrhizae.

Endomycorrhiza

Endomycorrhizal fungi grow intra and intercellularly forming specific fungal structures in the cortical region of the host. These fungi are further divided into three subgroups. They are Ericoid, orchid and the ubiquitous and large group of vesicular arbuscular mycorrhizal (VAM) fungi. Ericoid mycorrhizal fungi have septate hyphae and colonize plants belonging to Ericaceae, whereas orchid mycorrhizal fungi are aseptate with clamp connections, and colonize orchidaceous plants. The mycorrhizal fungi having aseptate hyphae are grouped under vesicular arbuscular mycorrhizal fungi (VAMF). VAM fungi are ubiquitous in nature and colonize most of the plants except members of Chenopodiaceae, Cruciferaceae and Caryophyllaceae (Allsopp and Stock, 1994). All soil fungi that form arbuscule are placed under order Glomales. The order Glomales belongs to class Zygomycetes of the subdivision Zygomycotina. Of the six recognized genera, *Glomus, Sclerocystis, Enteroph-osphora* and *Acaulospora* form both vesicles and arbuscules. While *Gigaspora* and *Scutellospora* do not have the intraradical vesicles (Schenck and Perez, 1990). They are obligate symbionts, form vesicles and arbuscules, form morphologically distinct resting spores (chlamydospores), colonize the cortical region of feeder roots and form external mycelium. On root colonization the AM fungi produce two specialized structures known as vesicles and arbuscules in the cortex region of root. Arbuscules are complex structures similar to haustoria produced within the host cells. They serve as sites of nutrient exchange between host and the fungus. The vesicles are terminal, ovate to globose structures that contain drops of yellow oil. It is reported that vesicles are thin walled, act as temporary food storage organs, but when vesicles remain thick-walled they might function as resting spores.

Benefits of mycorrhizal association

The beneficial aspects of mycorrhizal association include the following (Paul and Kucey, 1981; Whipps, 2004).

Advantages to the plant	Advantages to the VAM-fungi
1. Increased nutrient uptake	1. A habitat free of competition- sometimes only habitat for growth
2. Access to organic forms of certain nutrients, for example nitrogen	2. A steady supply of photosynthate carbon
3. Increased rootlet size and longevity	
4. Protection from pathogen	

5. Improved water relations
6. Drought tolerance
7. Enhanced phytohormone activity
8. Enhanced phenol activity
9. Salt and heavy metal tolerance

Increased nutrient absorption - when soil having low nutrient: Nutrient absorption is increased by increasing the absorbing surface area of the root system, and the fungal hyphae serve as extension of the root system. Nutrient can, therefore, be transported beyond the narrow nutrient depletion zone. In mycorrhizal fungi exploiting greater soil volume, the fungal hyphae can often nutrients at lower solution concentration than the uninfected roots. There is clear evidence that some mycorrhiza plants can be connected via hyphae strands and this link provides a means of nutrient transfer from plant to plant. These mycorrhizal pathways facilitate nutrient conservation at the ecosystem level. Mycorrhizal association can also enable the plant host to access nutrients (particularly nitrogen) in an organic form that would otherwise be unavailable. Mycorrhizal structures effectively take up phosphorus from lower concentration at which normal plant roots fail (Howeler *et al.*, 1981). Absorbed phosphorus is converted to polyphosphate granules in the external hyphae and passed to the arbuscules for transfer to the host. The same mechanism also helps the uptake of potassium, zinc, iron, copper, magnesium and calcium. Legume-VAM interaction: *Rhizobia* and VA-mycorrhiza often synergistically resulting better root nodulation, nutrient uptake and plant yield (Smith and Pearson, 1988; Barea, 2005). This interaction is marked when soil has low level of P. This beneficial interaction has been shown in the following legumes: *Stylosanthus guyanensis, Centrosema pubescences, Medicago sativa, Phaseolus sp., Glycin max, Arachis hypogea, Vigna unguiculata, Pureria sp., Trifolium repens* and *Trifolium subterraneum.*

Enhancement of water transport under water stress condition: Mycorrhiza fungal hyphae may also enhance water transport to trees, although there is no direct evidence for this. According to Brown (1978) mycorrhizal tree seedling can often resist drought better than non-mycorrhizal seedlings. However, it has been proposed that VAM infection strongly increased water transport from soil through the roots. The mycorrhizae mediated drought tolerance are due to more hyphal entry point per unit of plant root length that allows more flow of water into the roots and hyphal length and linking of soil surface area with plant roots. It has been reported that (Tisdall, 1991) upto 50 m of hyphae length may be present/ g of grassland soil. It has been speculated that hyphal diameter of 5 μm and root diameter of 500 μm will lead to 1 m of hyphae having surface area equal to 1 cm of root length. On this basis it can be estimated that each gram of grassland soil may have hyphae equivalent to 50 cm of fine roots. Improvement of water relation to the host plant is also due to increased cytokinin production by mycorrhiza, which regulates the stomata movement. Sterols are also present at higher concentration in mycorrhizal roots and this could enhance plant growth under water stress (Smith and Pearson, 1988).

Other important benefits are :

1. Increase nutrient mobilization through biological weathering by breaking down complex minerals and organic substances;

2. Serve as a biological deterrent and physical barrier to root infection by soil pathogens;

3. Provide tolerance to heavy metal accu-

mulation by restricting the translocation of metals from roots to shoots, as the ions are absorbed on the cell wall of the hyphae in the root;

4. Evidence exists that mycorrhizae may provide the host plant with growth hormones, such as auxin, cytokinin and gibberellin (Allen and Allen, 1980). The mycorrhizal fungi benefit by utilizing photosynthates and derivatives from the host plant;

5. Increased production and activity of phenolic compounds due to AM colonization has been observed. Higher phenolic content increases the defence mechanism of the host plant and thereby imparts resistance to various diseases (Morandi, 1996).

Mycorrhizal status of plants is an important factor in revegetation of severely disturbed area (Mosse *et al.,* 1981). Thus in adverse environmental conditions, the impact of mycorrhiza is important to stabilize the dumps biologically.

IMPORTANCE OF VAM FUNGI IN COAL OVERBURDEN DUMP RECLAMATION

Many of the plants that grow on reclaimed or naturally colonized coalmine overburden (OB) dumps have VA-mycorrhiza, which may increase the growth and survival rate of these plants. Recovery of disturbed area in terms of mycorrhizal infection and spore population are controlled by a number of interacting factors, among them : (a) Initial spore count, (b) soil nutrients, (c) texture, (d) moisture, (e) host plant genotype, and (g) plant coverage of revegetated site. In coal OB dumps, the most limiting factors for plant establishment are water stress and nutrients particularly nitrogen and phosphorus. The mycorrhiza mediated drought tolerance to the plant has been discussed earlier. VAM infection was lowest in soil having high P content (Gruhu *et al.,* 1987). Plant growth was increased to a greater degree by mycorrhiza inoculation than P addition. The fertilization of coal spoil with phosphorous fertilizer inhibits mycorrhizal colonization and sporulation which suggested that reclamation and restoration involving P fertilization may reduce mycorrhizal colonization. After 2-3 year of disturbance, percentage of infection and spore count were increased upto 50%. They observed that percentage of infection and spore count was not correlated. However, plant density and percentage of cover is important in reclaimed sites. Mycorrhizal spore is usually influenced by wind and animal vector. Mott and Zuber (1987) reported that after 3 to 7 year of disturbance, the VAM infection was returned to pre-mining level. They also found no significant difference in number of spores in variously aged mine spoil.

FACTORS AFFECTING ESTABLISHMENT OF MYCORRHIZA FUNGI IN OB DUMPS

The establishment of mycorrhiza-plant association on OB dumps is influenced by the (a) essential elements, N and P, (b) pH, (c) organic matter, (d) trace elements, (e) moisture, (f) percentage cover of VAM plants and (g) age of vegetation since disturbance.

Essential element

Soil nutrients particularly nitrogen (N) and phosphorus (P) are of primary concern in the formation of mycorrhiza-plant association. High levels of N and P often suppressed the VAM development. The hyphal length was significantly greater at low P than at high P supply. High concentration of soil P has been shown to inhibit mycorrhizae formation (Kothari *et al.*, 1991). VAM may increase N concentration in plant shoots. Greenhouse and field studies have shown that VAM improve growth, nodulation and nitrogen fixation in legume-Rhizobium symbiosis (Smith *et al.,* 1986). Further VAM inoculation enhanced biological N fixation of legumes in a way, that is similar to P fertilisation. VAM hyphae can take up and translocate nitrate and ammonium, thereby increases the N uptake by the plants (Miller *et al.,* 1985). Studies have shown that calcium, potassium and zinc uptake is increased when plant have mycorrhizal association.

pH

Soil pH strongly control the mycorrhizal development, altering the bioavailability of nutrients and toxins, and many VAM developments is severely restricted in more acidic soils. Root infection by VAM fungi has been found to occur over a pH range of 4.2-7.0. *Glomus diaphanum* has been found in abandoned or partially reclaimed surface mine land characterized by acidic and high aluminum content. VAM have also been reported in alkaline soil conditions. Optimal pH for spore germination is slightly acidic (5.5-6.5). Porter (1987) found that decrease in pH to 4.3 decreased spore germination and hyphal growth of *Glomus* species. Pitter (1985) also reported that spores of *Glomus* species were found only in soil with a pH of 6.8, however, large numbers of *Aculospora laevis* spores were found in soils with a pH range of 4.5 to 4.9 and concludes that spoil pH is a major determinant of the distribution of VAM.

Organic matter

The number and type of organisms increase with application of organic matter; this can be attributed to the addition of an available energy source, oxidizable carbon. Mining activity typically results in the loss of soil organic matter and reductions in microbial populations. The development of a self-sustaining vegetative cover on OB dumps is dependent on establishment of decomposition and mineralization processes. VAM-plant association may be stimulated by substance produced by organic matter and the properties of organic matter (Allen and Allen, 1980).

Soil water

Soil moisture plays a key role in the formation of mycorrhiza. Excessively high soil moisture is inhibitory when it leads to an anaerobiosis, as all mycorrhizal fungi are obligate aerobes. Miller *et al.* (1985) found that soil moisture characteristics were significantly correlated to propagule level, while soil chemical characteristics were not. Mycorrhizal P supply are likely to be more advantageous for plant growth under arid conditions than under wet conditions since the diffusion coefficient of phosphate in soil is linearly related to soil moisture content (Pitter, 1985). Most aquatic plants and plants growing in wet areas are generally non-mycorrhizal. The lack of VAM formation under saturated conditions has been attributed to low availability of oxygen. Spore germination of VAM fungi is best at soil moisture contents between field capacity and soil saturation (Pitter, 1985). Root infection by VAM fungi is usually most rapid when soil water content is between field capacity and permanent wilting point.

Topsoil cover

The greatest amount of mycorrhizal infection was found on plots covered with 30 cm of topsoil. Stored topsoil of 1 to 3.5 year age is a poor source of VAM inoculation on reclaimed sites and only 5-10% infection was found (Gould and Liberta, 1981).

FORMATION OF MYCORRHIZAL ASSOCIATION

Four basic types of inocula are generally used for bioreclamation of OB dumps in mining areas. These are : (a) VAM colonised rhizospheric soil, (b) infected roots, (c) pure cultures of fungi, (d) VAM spores and (e) sometimes by addition of various organic amendments (topsoil) (Norland, 1993). Generally any of the five techniques could be used for mycorrhizal innoculation in OB dumps in mining areas. These are : (1) broadcast inoculation, (2) inoculum placement below the seeds at nursery field, (3) slurry dips of sapling in nursery, (4) pelletizing seed, and (5) mycorrhiza infected seedlings and roots.

1. **Broadcast inoculation** : A known quantity of inoculum is spread over a given area of spoil surface and the inoculum is mixed up to a depth of 10 to 20 cm before seeding.

2. **Nursery innoculation** : The soil-innoculum is placed below the seed in nursery that facilitates the concentration of inoculum near developing roots. This technique is very commonly used for the development of mycorrhizal association.

3. **Slurry dips** : The slurries of mycorrhiza inoculum are prepared by mixing the inoculum with water and a suitable carrier. Bare-roots or container-grown seedlings are inoculated by dipping them into the slurry prior to planting.

4. **Pelletizing seed** : Adhesive, such as methyl cellulose has been used to coat seeds with mycorrhiza.

5. **Mycorrhizal seedlings and roots** : Transplanting mycorrhizal seedlings is a successful inoculation method. Roots with abundant mycorrhizae could also be selected as a source of inoculum and that can be incorporated. The success rate of mycorrhizal inoculation depends on amount and weight of inoculum used. Field plots are inoculated by placing VAM-infected soil below each sapling. The soil inoculum rates ranged from 2 to 50 g of soil/sapling.

Bulk VAM-Inoculum production and use (soil culture method)

Inoculum of VAM fungi may consist of spores, mycelia and infected root pieces containing vesicles or chlamydospores (Powell, 1984). Most common method is soil culture method. In this method, inoculum is produced by growing suitable host plants (onion, clover, sorghum or maize) inoculated with sterilized or unsterilized spores (1 to 30/ plant) in open pots or large bins or sterilized soil or sand. Wet sieving or slurry of inoculum soil containing infected root segments; spores and hyphae are most commonly used. Chopped mycorrhizal roots have also been frequently employed. For pot trials, the inoculum is usually layered below seed at the rate of 0.5 to 10g inoculum / plant. In case of field plots, soil is inoculated with mycorrhizal soil on the surface or in the seed-bed at the rate of 0.5 to 2.0 kg/m^2.

Measurement of mycorrhizal growth response

1. Estimation of shoot dry weights is the most common measure of growth response to mycorrhizal inoculation although shoot fresh weight is occasionally used (Haas and Krikun, 2006).

2. Increase in leaf length and number of leaves also could be used to evaluate the mycorrhizal growth in case of trees and tiller number in case of cereals.

3. Root dry weights, fresh weights or length are often measured, and root/shoot ratio also calculated.

4. Increase in fruit production for orchids and timber yield for timber yielding plants should be considered for determination of mycorrhizal response.

5. The effect of mycorrhizal inoculation on nodulation and nitrogen fixation of legumes has been assessed visually by the removal and weighing of all nodules and by acetylene reduction.

6. The phosphorous concentration in roots and/or shoots is usually measured in inoculation trials along with other elements (Zn, Cu, N), where necessary.

Pot and field trial

Plants are grown normally at 1 to 10 per pot of sieved soil/sand mixture on glass house benches. Cores of undisturbed soil are collected from field of mycorrhizal soils. Soils are sterilized with formalin (4.8 L of 2% (w/v) solution/m^2 or, by autoclaving at 15 lb pressure, 121°C for 1 hr) (Mehrotra, 1991). Plot sizes for field trials are varied from 0.16 m^2 to 500 m^2 with 6 m x 2 m plots. There should be unplanted guard strip of 1 to 3 m between plots to prevent mycorrhizal spread and experiments should be laid out in randomized (square) block design.

CONCLUSION

1. Survey of the status of VAM association in coalmine overburden dumps should be based on vegetation cover, year of plantation, climatic conditions and physico-chemical and biological properties of overburden dumps.

2. Percentage of root infection of plants growing in overburden dumps and density of VAM spores in the rhizosphere of host plants should be assessed during selection of tree species for ecorestoration. Selection of suitable host plant for different geoclimatic and dump characteristics is essential.

3. The adaptability of selected VAM-fungi should be studied before application in the degraded site. The selected VAM-fungi must be adapted to a wide range of environmental and edaphic factors.

4. Measurement of mycorrhizal growth response should be conducted both in pot and field trials basis. The amount of inoculum to be applied and rate of root infection in the host plants should be correlated.

5. Study of the interaction between indigenous microbial population and applied VAM fungi should also be studied. The VAM association will be more successful where indigenous soil biota is low, like coalmine degraded land.

6. Possibilities of using a combination of symbiotic/asymbiotic nitrogen fixers, phosphate solubilising microbes and VAM together, will give maximum benefit to the plants growing under stress conditions.

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