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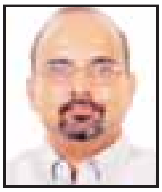
Mycorrhiza: A Friendly Association with Plants towards Sustainable Food Production



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Abstract

Arbuscular mycorrhizal fungi (AMF), a rhizospheric fungi from the phylum Glomeromycota are obligate symbionts of plants forming an association with a majority of them acting as extended root hairs and increasing the area of interaction with soil. They move nutrients such as phosphorus, nitrogen and other micronutrients through the roots into the plants thus enhancing plant growth. Spores from the fungi and mycelia from the infected roots can further colonize new host roots. This results in benefits for the host ranging from not only enhanced nutrient uptake but drought tolerance and soil improvement leading to higher plant productivity. Resistance to pathogens is also exhibited. These benefits imparted by the AMF has prompted its use in agricultural practices to enhance growth and consequently the yield. Mycorrhiza is the only known fungal system categorized as a biofertilizer and TERI's (The Energy and Resources Institute) endeavor is to produce the highest quality inoculum, constantly innovating technologies for newer applications and wider outreach.

Key words : Mycorrhiza, soil nutrients, drought tolerance, biofertilizer

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TERI's intervention

Mass production of arbuscular mycorrhizal fungi

As AMF is an obligate symbiont requiring plant roots for its survival the mass production became a demanding process and this acted as a constraint to the widespread usage of AMF in spite of its well known benefits. The conventional method that is being used for mass production of this fungus worldwide is the pot culture method. In this method of production host plants are grown with the desired AMF in pots or beds in green houses where the host gets colonized and are harvested after a period of three to five months. The roots along with the AMF spores and mycelia are left in the substrate and mixed well to act as inoculum and can be formulated to have a desired number of propagules. This soil based method though has its drawbacks as it does not have a long shelf life and can be easily contaminated with other microbes as well as the space requirement is a constraint to mass production.

TERI has developed an *in vitro* based mass production technology (Tiwari and Adholeya 2002) which successfully produces pure inoculum in large quantities and overcomes the limitations of the soil based system. This inoculum has a long shelf life and is very concentrated with a small quantity itself carrying a very large number of propagules. This facilitates the application of desired amounts as it can be easily transported and mixed with a substrate of choice and applied as required.

TERI uses the root organ culture system (Becard and Fortin 1988; Chabot et al 1992) with transformed roots of a host plant to develop the symbiosis on a specific medium *in vitro*. To have a large diversity of spores in the root organ culture system (ROC) a large collection of AMF isolates are being maintained as trap cultures and have been collected from different agro-climatic zones. These isolates are being characterized molecularly, biochemically as well as functionally and then developed as *in vitro* cultures for mass production. Rate limiting factors such as media composition, time of sub - culturing and optimal harvesting time is critical to achieve highest and viable propagules, and these have been optimized for maximal production. The whole production process has been efficiently structured such that the propagules produced have a high viability and efficacy. Specially designed equipment which is customized to the specific needs of AMF production has been fabricated and is a crucial part

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of the production chain. Benchmarks have been established and the quality of the inoculum is assessed at various points during the production process. Different bio - assays have been developed to ensure quality and include the viability test, the infectivity potential assay which scores the entry points for enumeration of propagules in the inoculum and root colonization assay which assesses the efficiency of colonization. As *in vitro* based culturing of AMF is a continuous process genetic stability is an important pre - requisite and this was established by lack of polymorphism studied in the fingerprints of spores produced both *in situ* and *in vitro* conditions over 4 generations (Gadkar et al 1997).

Regulation of Mycorrhizal biofertilizer

Regulation of biofertilizers is imperative as it controls the quality of the product being disseminated in the market. Initially the credibility of the biofertilizers was reduced as spurious material was distributed but which failed to produce the proclaimed results. The Ministry of Agriculture, Government of India had earlier recognized microorganisms such as *Azotobacter*, *Rhizobium*, *Azospirillum* and Phosphate solubilizing bacteria amending its Fertilizer Control Order (FCO) to include them as Biofertilizers. Technical specifications for the quality check of these biofertilizers have been laid out based on which the inspectors carry out their tests to verify the claims of the product. Recently in November 2010 the Fertilizer Control Order (FCO) was amended, this time to include AMF. Guidelines and specifications have been now laid out as to the quality parameters, procedure and protocol to be adhered to in the production and formulation of AMF.

TERI in its endeavor to include arbuscular mycorrhizal inoculum as a biofertilizer in the order of the Ministry of Agriculture assisted the regulatory agency, the National Centre of Organic Farming (NCOF) to lay out its technical specifications. TERI also carried out a training programme of two officials from NCOF to familiarize them with the protocols used for assessing the quality of the product.

Increasing the scope of AMF

To constantly address newer requirements and understand and increase the scope of AMF, TERI's endeavor has been to develop new technologies and avenues for its use. Synergistic formulations have been developed to tap the different benefits of consortia of AMF and which can be applied to crops as a single application. Clonal selection of hosts for arbuscular mycorrhizal fungi (AMF) is also being done as different hosts behave differently in the presence of a particular mycorrhizal fungus. This attribute can be utilized to optimize the production cycle and increase the productivity. Interactions between different other beneficial microbes such as plant growth promoting bacteria (PGPR) were studied with favourable result. The problems faced were the fall in viability of bacteria at high ambient environmental temperatures and a lack of cold chain transport system to maintain them. Moreover, the end - user would have to carry out two separate applications one for AMF and the other for PGPR. To overcome this hurdle a formulation has been prepared which contains the PGPR as powder to be resuspended and the suspension used for the application of AMF as seed coating. Real time shelf life tests have been carried out to determine the best carrier and storage conditions. This technology has helped in not only maintaining the cell viability but also reducing labour costs to the farmer.

In the quest to increase the scope of AMF TERI has also developed a broad spectrum inoculum which consists of both arbuscular mycorrhiza and ectomycorrhiza. This AM - EM - ROC inoculum has been developed *in vitro* and therefore has all the benefits of the AMF ROC along with the properties of ectomycorrhiza which is largely used for forestry. This too becomes a single inoculum having multifarious benefits with each application addressing a wide spectrum of hosts and growing systems and reclamation needs.

Application technology

As the inoculum is highly concentrated it needs to be diluted with a carrier. A specific application method is adapted to suit the needs of the host and the carrier selected should be inert and non - toxic. A large variety of formulations are being tried and tested in different forms such as tablets, powders of different particle sizes as well as spores in liquid. The different applications being optimized are for seed coating with fungicide, root dipping as well as irrigation compatible formulations. Formulation process has been optimized such that minimal damage is done to the inoculum especially during tablet formation. The formulation should be easily dispersible (30 seconds) for quick and maximal contact with roots, with germination of spores taking place in 24 - 72 h. Certain coating formulations have been prepared along with a binder which are showing promising results without damage to the spore or affecting their viability at the same time allowing rapid dispersion of spores.

Marching towards food security with mycorrhiza

Indiscriminate use of inorganic fertilizer and pesticides has deteriorated soil status with deficiency of macro and micronutrient becoming rampant, presence of residual contaminants in the environment which has led to the loss of diversity of soil species. (Rice - Wheat Consortium Report: ICAR Document). Research as reported by the Ministry of Agriculture, Fisheries and Foods and the Royal Society of Chemistry, UK shows that the mineral content of food has decreased by an average of 40 % from 1940 to 1991. Although the biofertilizer consumption has increased the use of mycorrhiza needed to be more widespread. One of the reasons for slow growth of mycorrhiza was lack of awareness among farmers. TERI set up multi - location field test trials all over the country on different crops. The objective was to assess efficacy of mycorrhiza

Table 1. The effects of arbuscular mycorrhizal (AM) fungal inoculation on dry shoot and dry root weight, root / shoot (R/S) ratio and percent AM fungal colonization

Host	Inoculation	Dry shoot weight (g)	Dry root weight (g)	R/S	Colonization (%)
	+AM	381.5 ± 11.5a	107.8 ± 2.1a	0.28	76.5 ± 1.1a
Z. mays	–AM	297.3 ± 11.0b	75.45 ± 1.2b	0.25	1.8 ± 0.5fg
	LSD	25.6	3.9		
M. sativa	+AM	122.6 ± 5.6a	28.5 ± 0.1a	0.23	70.9 ± 3.0b
	–AM	102.4 ± 0.8b	14.9 ± 0.4b	0.15	2.5 ± 0.2fg
	LSD	9.1	0.6		
T. alexandrinum	+AM	92.2 ± 9.4a	63.3 ± 1.0a	0.69	48.3 ± 0.6d
	–AM	25.8 ± 4.1b	13.6 ± 0.7b	0.53	4.1 ± 1.7fg
	LSD	16.5	1.9		
A. sativa	+AM	526.9 ± 20.0a	85.3 ± 1.2a	0.16	43.6 ± 0.8e
	–AM	348.6 ± 2.7 b	54.5 ± 1.0b	0.16	2.3 ± 0.2fg
	LSD	32.3	2.5		
S. bicolor	+AM	480.9 ± 2.4a	60.9 ± 0.4a	0.13	51.3 ± 0.4c
	–AM	453.4 ± 8.6b	34.9 ± 3.9b	0.07	3.6 ± 0.2fg
	LSD	14.3	6.3		2.06

For each host values followed by the same letter are not significantly different ($P \leq 0.05$) according to Duncan's multiple range test (DMRT). Values are means of least significant difference.

Table 2. Overall Input Reduction

Application time	% Reduction of fertilizer Dose
Basal dose	28.5 (already reduced)
Top dressing (1st irrigation)	50
Top dressing (2nd irrigation)	50

Table 3. Trials in Europe to demonstrate effect of mycorrhiza

Ireland	Winter Barley
Germany	Winter Rye, Barley, Maize
United Kingdom	Amenity Perennial Ryegrass *(Lolium perenne)* Smooth stalked meadow grass *(Poa pratensis)* Chewings fescue *(Festuca rubracommutata)* Slender creeping red fescue *(Festuca rubralitoralis)* Strong creeping red fescue *(Festuca rubrarubra)* Bentgrass *(Agrostis* spp*)*
Czech Rep	Perennial ryegrass *(Lolium perenne)* Smooth stalked meadow grass *(Poa pratensis)* Grass Mixture
Netherlands	Cucumber
Italy	Horticulture crops

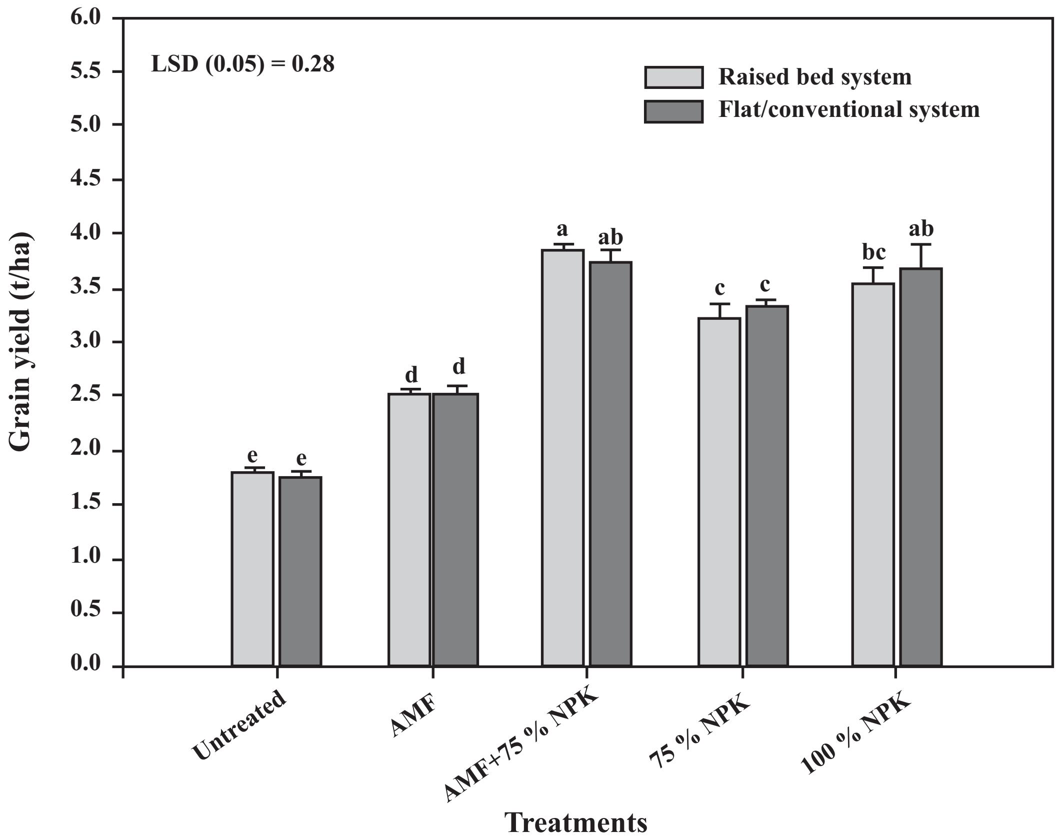

Fig. 1. Effect of arbuscular mycorrhizal fungi and fertilizers on grain yield (t ha^{-1}) of wheat grown under raised bed and conventional / flat system in a sandy loam Alfisol. The bars of treatment followed by same letter did not differ significantly by Duncan's multiple range test (DMRT; *P* ≤ 0.05)

through comparative analysis of mycorrhized crops with non - mycorrhized (control) crops in actual field conditions in different agro - climatic regions and states. Similar agricultural inputs like land area, seed variety, seed quantity irrigation etc. were maintained for a comparative analysis. In the control field full dose of chemical fertilizer (N, P, K) as applied by farmer as common practice was provided. In the mycorrhized fields half dose of chemical fertilizer (N, P, K) applied by farmers with respect to control field was added. The other agricultural inputs were same for both the fields. The parameters evaluated were soil condition, germination time, and height of plants, maturity time, yield / productivity, yield of the previous year, input cost and revenue generation. Trial variants were built through demonstrations in different agro climatic regions: Western Himalayan Region, Middle Gangetic Plains Region, Upper Gangetic Plains Region, Trans-Gangetic Plains Region, Eastern Plateau and Hills Region, Central Plateau and Hills Region, Western Dry Region. Different inputs that were added were: 100 % RDF, 75 % RDF, 50% RDF treatments with AMF and comparison with control plots of 100 % RDF with RDF being the State Agricultural University's recommended dose of chemical fertilizers. All the crops tested showed an increase in plant biomass (Table 1) (Gaur and Adholeya 2002) and yield (Fig. 1) (Sharma et al 2011). The overall input reduction is shown in Table 2. To increase the outreach of AMF and *in vitro* based mass produced inoculum trials were laid in Europe as well (Table 3).

Mycorrhiza also alters the soil property as was demonstrated at a hypersaline site selected for the purpose in Dukhan, Qatar using TERI's desert greening technology (Table 4). This is a mycorrhiza based technology and entails the greening of the selected sites without the use of any chemical fertilizers or introduction of good earth. The plant species chosen for the purpose were: perennial tree crops: casuarina, *Melia*, parkinsonia, paras peepal, babool, amla, *Albizia*, salvadora ; bio fuel crops: *Jatropha curcas*, *Jatropha dioica* ; ornamentals: bougainvillea, portulaca; medicinal/aromatic plants: vetiver, mint; fodder crops: alfalfa, barseem, rhodes grass, local forage grass; food crops: wheat, oats; vegetable crops: tomato, okra, brinjal, chillies, melon, water melon, coriander, amaranthus, squash, pumpkin, cucumber, mustard, beans, radish, broccoli, cauliflower, cabbage, onion, cow pea, snake gourd, bitter gourd etc. On assessing the inputs it was

Table 4. Changed soil properties at Dukhan Site: analysis done at Central Agricultural Laboratory, Ministry of Agriculture, Doha on 29.07.08

Parameter	Dukhan site before reclamation	Analysis 6 months after reclamation
Saturation (%)	27.21	25.95
pH	8.17	8.04
EC (M. MhoS/cm)	72.50	29.50
Anions (meq/l)		
Carbonate	0	0
Bicarbonate	1.30	1.32
Chloride	873.7	266.59
Sulphate	175.3	95.46
Cations (meq/l)		
Ca	80.94	60.55
Mg	154.37	64.88
Na	799	231.6
K	16	6.33
ESP (%)	8.45	2.08

seen that not only was the chemical fertilizer quantity reduced but the amount of water applied was also lowered. This emphasizes the point that mycorrhiza lowered the water usage by the plants. Application of mycorrhiza also improved the quality of wheat by increasing the nutrient contents such as phosphorous and nitrogen (Table 5) in the shoots.

Reclaiming precious land : Bioremediation

In spite of increased chemical inputs the productivity is going down. To meet the increasing food demands of a burgeoning population degraded and wastelands have to be made fertile. TERI has developed mycorrhiza based wasteland reclamation technologies for land degraded by various causes.

Table 5. Concentrations of mineral nutrients in the shoots of five fodder crop species inoculated or uninoculated with mixed consortia of indigenous AM fungi

Treatments		P (mg/g)			N (%)		
Host	Inoculation	30days	60days	90days	30days	60days	90days
Z. mays	+AM	2.8a	1.85b	1.68c	7.3a	2.07de	3.17c
	–AM	1.48e	1.49e	1.56d	4.25b	1.96e	2.13d
	LSD			0.073			0.14
M. sativa	+AM	2.88a	2.17b	2.14bc	9.93a	8.05c	6.18e
	–AM	2.13c	2.13c	2.16bc	8.63b	7.58d	5.50f
	LSD			0.03			0.25
T. alexandrinum	+AM	4.72a	4.71a	4.54a	9.7a	9.80a	8.67b
	–AM	3.82b	3.78b	3.45b	7.4c	7.47c	7.43c
	LSD			0.43			0.27
A. sativa	+AM	4.36a	3.52c	3.81b	4.7ab	4.80a	4.5b
	–AM	3.46d	3.45d	3.36e	3.53c	3.13d	3.2d
	LSD			0.04			0.21
S. bicolor	+AM	7.32a	5.04c	5.28b	8.21a	6.78c	6.26d
	–AM	4.14d	4.26d	5.25b	7.6b	5.89e	5.81e
	LSD			0.14			0.09

Data followed by different letters within a group of six values (for a given host for each parameter) are significantly different ($P \leq 0.05$) according to DMRT. Values are means of three replicates.

Reclamation of fly ash dumps. The barren fly ash dumps generated at the National Thermal Power Corporation (NTPC) sites at Korba and Badarpur suffered due to fugitive dust emission, ground water contamination, and high heavy metal concentration. All these challenges were overcome by greening these dumps with ornamental and woody plants by using mycorrhiza and no other chemical fertilizers or supplementing the fly ash with any good soil.

Reclamation of wastelands contaminated with chlor alkali sludge. The chlor alkali industry at TATA Chemicals, Mithapur, Gujarat was faced with high salinity, fugitive dust, non - suitable conditions for growing plants. Moreover, sea water seepage at site added to the problem. TERI was successful in reclaiming these sites by providing a green cover using mycorrhiza and no other chemical input.

Reclamation of distillery effluent loaded sites. The distillery effluent which is dumped into large tracts of land, degrades it in such a manner that it cannot then support any further growth of plants. TERI developed a technology whereby this sludge at Associated Alcohol and Breweries Ltd, Barwaha, Madhya Pradesh was converted into fertile land again. Mycorrhiza was utilized here as well to enhance the growth of selected plants in the shortest time.

The statistics for wasteland shows that 147 million ha of land is degraded in India and 6 million ha is affected by salinity and alkalinity (Indiastat 2007). Approximately 22 % of vegetated land (almost 500 million hectares) of Africa has been degraded and about 11% of total African land area (332 million hectares) has been affected by human - induced soil degradation (World Bank). Similarly, 2 million ha of agricultural land in Australia shows signs of salinity, with approximately 820,000 ha of land unable to be used for production (Australian Bureau of Statistics). Therefore, regions and countries which are dependent on agriculture, but land resource is getting scarce can adapt and implement some of TERI's technologies to sustain production without compromising on yield. These technologies also contribute to water saving without decreasing the area under cultivation or to substitute high yielding varieties with local ones. The soil condition and fertility improves as well as use of chemical inputs is reduced.

Conclusion

Concerted effort by TERI has resulted in generating an increased awareness as well as acceptance among users. As the quality and quantity both have now increased due to TERI's interventions the quantum of usage is also increasing. Finished product development is a challenge as it should retain efficacy, shelf life and ease of application. To this end sophisticated methods of seed coating are being explored. Use of mycorrhiza is highly relevant not only in India but also in Africa and Sub - Saharan region where productive land and fertility is low as well as paying capacity of the end user is low. The research carried out will positively impact sustainable agriculture, and the livelihood of farmers, enhancing the socio - economic benefits. TERI has demonstrated that AMF can be used for crop yield, soil reclamation, increasing water efficiency which effectively will lead to the empowerment of farmers. Considering all the aspects of *in vitro* based AMF, it can safely be said it is the biofertilizer of the future.

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