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# Integrated Effect of VAM and *Azolla* on Fluctuations in Fungal Population, Mycorrhization and Leaf Phosphates in Rice under Different Moisture Conditions.

**I. Ghosh, J. Chakrabarti, D. P. Kushari and N. C. Chatterjee***
DSA-III (UGC) Lab., Department of Botany,
University of Burdwan,
Burdwan-713104, West Bengal, India

Fluctuation in mycoflora population was studied under low(25%), medium(50%) and waterlogged moisture conditions by treating rice (IR-36) with VAM+*Azolla* combinations and singly with VAM and *Azolla*. Variations were recorded as number of CFU $gm^{-1}$ soil $x10^4$ using rhizosphere soil. Fungal population was found to be increased and well maintained both in medium moisture condition and in VAM+*Azolla* combined treatment. This condition was found to be congenial for both mycorrhizal colonization and leaf phosphate accumulation in rice.
**Key words :** VAM, *Azolla*, mycoflora, moisture,leaf phophate, mycorrhization, rice

## Introduction :

Microbe-microbe interactions are crucial to understand the dynamic processes characteristic of rhizosphere establishment and maintenance. Mycorrhizal fungi and certain other soil microorganisms are known to regulate mycorrhizal formation and function. Conversely, mycorrhizae affect the establishment of rhizosphere populations. Microbial population (root associated mycoflora) in soil concentrate around plant roots stimulated by root exudates supplied by the plants (Bowen, 1980; Curl and Truelove, 1986). The population dynamics of microorganisms determines the competitive interactions at the root-soil interface by the limited carbon resources released by the plants. On the other hand, added organic matter results in a flush of microorganisms (Russell, 1973 ). *Azolla* is a well established biofertilizer and is generally used in rice fields in the form of organic manure. Mycorrhizae and *Azolla* were both reported for their additive effect on increasing mobilization and accumulation of nutrients in plant parts. Rice is a semiaquatic plant and water is necessary for proper plant growth. But waterlogged condition may not be ideal for mycorrhization. So, the objective of this investigation is to study the integrated effect of VAM and *Azolla* on changing mycoflora population, mycorrhization and accumulation of leaf phosphates in rice under a set of suitable and stress conditions of moisture.

* Corresponding Author : Professor & Ex-Head. Department of Botany & corresponding author.

## Materials and Methods :

A soil culture of *Glomus fasciculatum* was propagated as endomycorrhizal species using sterile sand and soil in 1:1 ratio and *Zea mays* as suitable host in pot culture. The infected root fragments and rhizosphere soil of the pots were used as inoculum and also for the preparation of seedbed for getting prior infected seedlings. During preparation of seedbed, two treantments were applied , one with mycorrhizal inoculum and the other with only sterile soil. Rice seeds were germinated in growth chamber aseptically and spread on two seedbeds seperately.

After 20 days, the infected seedlings were subjected to transplantation in two separate non-sterile 6 inch pots, one with *Azolla* biofertilizer ( 15gm in three split doses ) and the other without any biofertilizer. The non infected seedlings likewise were subjected to transplantation in two separate, non-sterile 6 inch pots, one with *Azolla* and the other without *Azolla.* Pot with non-infected seedlings and without added biofertilizer was taken as control. These four treatments were again subjected to three moisture coditions i.e. waterlogged, medium moisture (50% approx.) and low moisture (25% approx.) conditions. Soil moisture percentage was measured following the method of Jackson(1971). Variation in fungal population was recorded as number of CFU $gm^{-1}$soil $\times 10^4$ using rhizosphere soil and standard plating technique using PDA medium at every 15 days interval. Percentage mycorrhization and leaf phosphate were measured at maximum growth stage. For estimation of mycorrhization standard technique was followed (Giovanetti and Mosse,1980). The root samples were stained following the method of Phillips and Haymann(1970).After treating with 10% KOH solution in hot water bath, root samples were washed with distilled water and 5% NaCl solution repeatedly upto reaching neutrality.Thereafter the root samples were stained with Trypan blue in lactophenol. Treated root samples were spread out evenly in petridishes that have gridlines marked on the bottom having 1cm squares. Vertical and horizontal gridlines were scanned and the absence or presence of colonization was recorded at each point where a root intersects a line. Such 100 gridlines were tallied. Leaf phosphate was measured following the method of Jackson (1971). For measurement of optical density values, Beckman DU-64 spectrophotometer was used. Leica DM-LP research microscope was used for estimation of mycorrhization.

## Results and Discussion

In medium moisture condition (50% moisture), there was an increase in microbial population (associated mycoflora ) in all the three treatments viz. in combination of VAM and *Azolla*, VAM and *Azolla* alone (Table-1). In case of treatment with VAM alone, there was an overall decrease in root associated mycoflora as is evident from total CFU $gm^{-1}$soil × $10^4$. It was also found after 15 days that there is maximum increase in CFU $gm^{-1}$soil x $10^4$ which tends to be maintained in rhizosphere soil treated with VAM + *Azolla* combination. On the contrary, the fungal colony gets slightly decreased in *Azolla* treatments, although an overall increase in their population was noticed. What actually happens is that some of the isolates of the total population either get increased or decreased.

If organic matter is added to the soil which is moderately wet and aerated, there happens usually to be a flush of microorganisms and microbial activity (Russell,1973). So increase in colony number under VAM+*Azolla* and *Azolla* alone treatments might be due to the addition of organic matter which is a source of necessary elements for microbial growth. It has been reported that several species of microorganisms increase in VAM rhizosphere and as such the species composition may be altered (Ames et. al, 1984 ). since VAM could alter the microbial equillibrium in rhizosphere, specific groups of microorganisms also has been reported to be altered (Meyer and Linderman, 1986 ) which corroborates the present findings. Mycorrhizae possess some nutrient concentrating mechanism around rhizosphere and thus altering microbial population (Harley and Smith, 1983 ) which might be the reason behind the increase in total CFU $gm^{-1}$soil x $10^4$ in VAM treated soil. Once association established, VA mycorrhizae can decrease root exudation rates (Graham et. al, 1981) which might be unfavorable for the establishment of fungal species as root exudation provides an important source of substrates to the microorganisms (Bowen, 1980 ). While exudation of specific compounds from the roots or mycelium may stimulate growth of microorganisms beneficial to the mycorrhizal symbiosis, extracellular metabolites may also have an antibiotic effect on certain microorganisms (Kope and Fortin, 1980) which is in line with also our findings. The extra nutrient supply through addition of organic matter in VAM+*Azolla* and *Azolla* alone treatments upholding the fungal population, was not added to VAM alone treatment, so as to maintain the higher fungal poulation . Medium moisture level permits aeration to the soil and also greater organic matter dicomposition for providing better nutrient supply (Russell,1973 ). In waterlogged condition , with each of the three treatments, there was an overall decrease in fungal population although a moderate increase in each case was noted on 15 days of experimental set up and that to having comparatively lesser in VAM treatment.

Addition of organic matter might be the reason behind the increase in fungal population both in VAM+*Azolla* and only in *Azolla* treatments. But in waterlogged condition, rhizosphere gradually becomes anaerobic. If the aeration becomes poor ,the rate of decomposition becomes slower and the proportion of soil population taking part in the decomposition becomes less (Russell,1973). It is also reported that in this anaerobic condition, the percentage mycorrhization is poor which might reduce the interaction with microorganisms or with soil nutrient accumulation.

Percentage mycorrhization was found to be maximum in VAM+*Azolla* combined treatment (Plate1a & b) under medium moisture condition followed by VAM alone treatment (Fig.2). Fungal colonization was found to be poor in waterlogged condition. As rice is a semi-aquatic plant, water is necessary for proper root growth and plant development (IRRI Ann.Rept.1966). On the other hand, mycorrhizae was reported to be well established in moisture stress condition ( Miller *et. al*. 1985 ). So in high moisture stress, VAM colonization was satisfactory both in VAM+*Azolla* and VAM treatments. Medium moisture was found to be most congenial condition for mycorrhization , root development and nutrient supply through proper decomposition of organic manure ( Emerson *et.al.* 1980; Russell 1973). So percentage colonization is evidently maximum in medium moisture condition and that too in VAM+*Azolla* combined treatment (Fig.2). Waterlogged condition was better in respect of root development but anaerobic environment in rhizosphere might have inhibited proper colonization by mycorrhizal species ( Miller *et. al*.,1985 ).

Treatment with VAM by *Azolla* showed maximum concentration of leaf phosphates followed by individual treatments with *Azolla* and VAM (Fig.1). All the three treatments showed better leaf phosphate accumulation over the control. When variable moisture availability is concerned, leaf phosphate was found to be increased in medium moisture condition. Better mycorrhization in high stress might help in mineralization and uptake of phosphate ( Hattingh *et.al*.,1973) but waterlogged soil has its own environment for percolation helping better maintenance of leaf phosphate (Russell,1973). Medium moisture condition provides most suitable environment for root growth, increased mycorrhization and associated uptake of phosphate as evidenced from the result. Mycorrhizal fungus specially *Glomus* sp. was well reported to secrete some acid phosphatases promoting phosphorus accumulation by plant parts (Dodd et. al.,1987) and medium moisture condition is also very suitable for green manure decomposition (Russell, 1973).

**Table 1 : Integrated effect of VAM and *Azolla* on changing mycoflora (CFU gm$^{-1}$ soil × 10$^{4}$) under various moisture conditions in rhizosphere soil of rice.**

| TREATMENT | DAYS | WATERLOGGED | MOISTURE 50% | MOISTURE 25% |
|---|---|---|---|---|
| VAM+*Azolla* | 0 | 17.0 | 16.0 | 15.6 |
| | 15 | 18.6 | 20.6 | 19.6 |
| | 30 | 13.6 | 20.6 | 18.6 |
| | 45 | 12.3 | 20.3 | 17.6 |
| VAM | 0 | 14.3 | 13.3 | 15.0 |
| | 15 | 16.0 | 17.0 | 18.0 |
| | 30 | 12.6 | 13.6 | 15.6 |
| | 45 | 10.3 | 11.3 | 15.3 |
| Azolla | 0 | 15.6 | 15.0 | 16.3 |
| | 15 | 17.3 | 19.6 | 20.6 |
| | 30 | 14.6 | 19.0 | 21.3 |
| | 45 | 11.6 | 18.6 | 19.3 |
| Control | 0 | 10.3 | 11.6 | 12.0 |
| | 15 | 11.3 | 12.3 | 12.6 |
| | 30 | 10.3 | 12.6 | 12.0 |
| | 45 | 8.6 | 11.0 | 10.4 |
| LSD (5%) | | 1.35 | 1.40 | NS |

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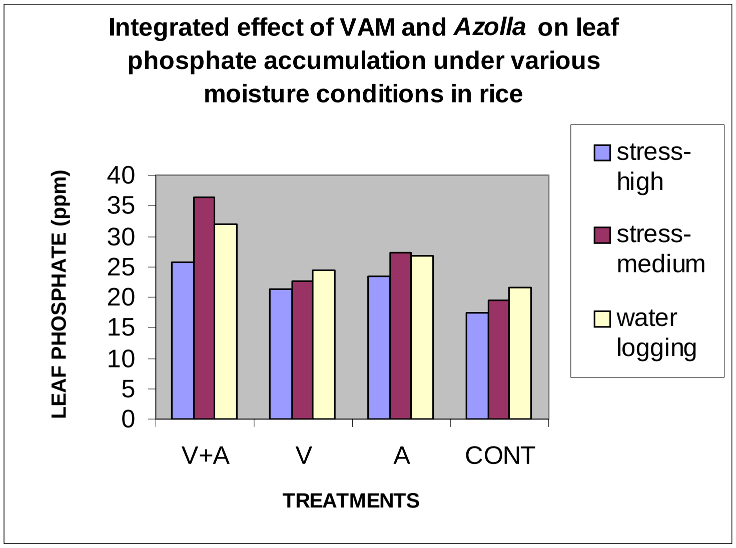


Fig 1

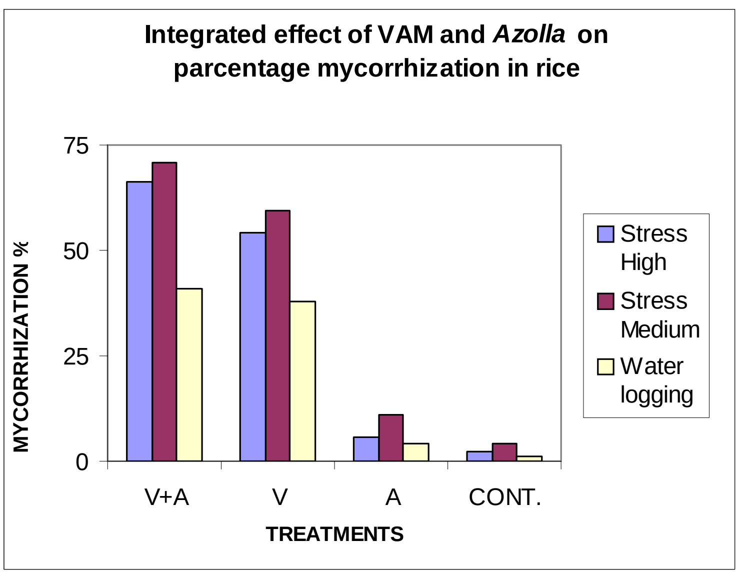


Fig 2

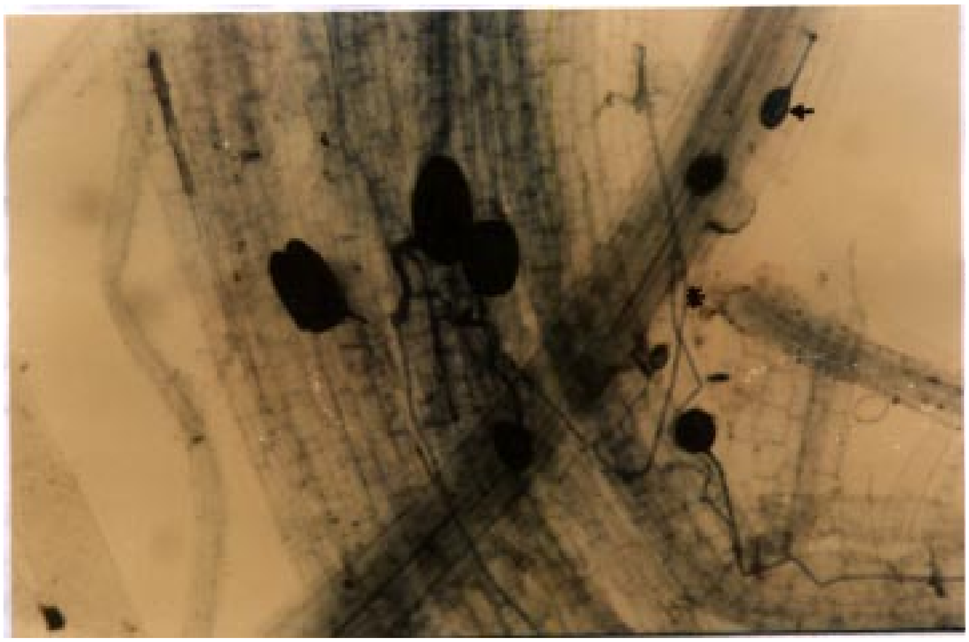

Plate I : Intercellular hyphal network (star) showing vesicles (small arrow) of *Glomus fasciculatum* in Rice (× 120)

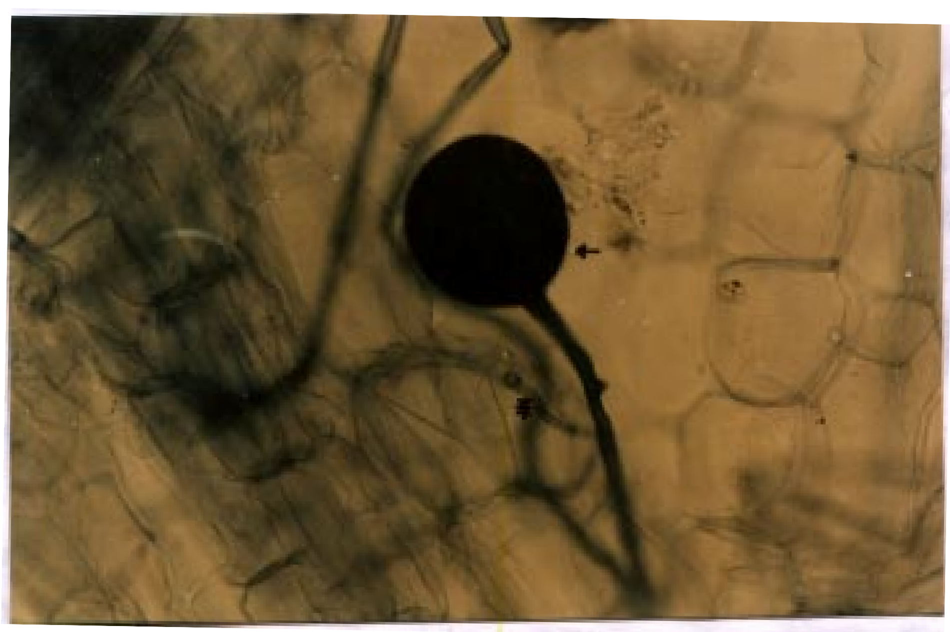

Plate II : *Glomus fasciculatum* with intercellular hyphae (star) showing vesicles (small arrow) in Rice (× 160)

## References :

Ames R.N., Reid C.P.P. and Ingham E.R. (1984) : Rhizosphere bacterial population response to root colonization by a VA mycorrhizal fungus *; New Phytol* **96,** 359-373.

Azcon-Aguilar. C., Barea. J.M. (1992) : Interactions between mycorrhizal fungi and other rhizosphere microorganisms; *Mycorrhizal Functioning* , Edt. Michael F. Allen.

Bagyaraj D.J. and Menje. J.A. (1978) : Interaction between a VA mycorrhizae and azotobacter and their effects on rhizosphere microflora and plant growth. *New Phytol*. **80,** 567-573 .

Bagyaraj D.J. (1991) : Ecology of vesicular-arbuscular mycorrhizae. In. *Handbbok of applied mycology Vol.1 Soil and Plants* (Eds.) D.K.Arora, B.Rai , K.G. Mukerjee and G. R. Kundson. Marreel Dekkor. New York. 1-34 .

Barea J.M. (1991) : Vesicular-arbuscular mycorrhizae as modifiers of soil fertility, *Adv. Soil Sci*., **15,** 1-40 .

Bowen G.D. (1980) : Misconceptions, concepts and approaches in rhizosphere Biology. In. *Contemporary Microbial Ecology*, 283-304. Academic Press, London.

Curl E.A., and Truelove B. (1986) : *The Rhizosphere*. Springer-Verlag, Berlin.

Dodd J.C., Burton C.C., Burns R.G. and Jeffries, P. (1987) : Phosphatase activity associated with the roots and the rhizosphere of plants infected with VAM fungi. *New Phytol*. **107,** 163-172.

Emmerson W.W., Foster R.C., and Oades J.M. (1986) : Organochemical complexas in relation to soil aggregation and stucture. In: Interactions of soil minerals with Natural organics and microbesw, 521-548. *Soil Science*. Society of America, Madison, WI.

Graham.J.H.(1988). Interaction of mycorrhizal fungi with soil borne plant pathogens and other organisms*:* An introduction. *Phytopathology* 72 : 95 .

Harley, J.L. and Smith S.E. (1983) : *Mycorrhizal Symbiosis*. Academic Press, London. Int. Rice. Res. Inst., Ann. Rept. 1966, 117.

Hattingh M.J., Gray L.E. and Gerdemann J.W. (1973) : Upake and translocation of 32p-labelled phosphate to onion roots by endomycorrhizal fungi. *Soil Science* **116,** 383-387.

Jackson K.H. *Soil chemical analysis* (1974)

Kennedy J.Z. and Rangarajan M. (2001) : Biomass production and root colonization of six VAM fungi in Papaya. *Indian Phytopath*. **54(1),** 72-77.

Kope, H.H. and fortin J.A. (1989) : Inhibition of phytopathogenic fungi *in vitro* by cell free culture media of mycorrhizal fungi. *New Phytol*. **113,** 57-63 .

Lumpkin T.A. and Plucknett D.L. (1980) : *Azolla*-Botany,Phsiology and use as a green mannure;*Econ. Bot*. **34,** 111-153.

Meyer, J.R. and Linderman, R.G. (1986) : Selective influence on rhizosphere bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil *Biology and Biochemistry* **18,** 191-196.

Miller R.M. (1985) : *Mycorrhizae*.Restoration and Management Notes **3,** 14-20.

Phillips, L.M. and Haymann, D.S. (1970) : Improved procedures for clearing roots and staining parasites and VA mycorrhizal fungi for rapid assessment of infection. *Trans. Brit. Mycol. Soc*., **55,** 158-160.

Russel. E.W. *Soil condition and plant growth* . Ed. 10 .

Singh A.K. and Mishra R.R. (1990) : VA mycorrhiza in rice under upland and lowland cultivation practices of north-east India . *Ind. Phytopath*.-Suppl.Issue, **44,** 14

Smith S.E., St. John B.J. and Smith F.A. (1986) : Effects of mycorrhizal infection on plant growth and phosphorus nutrition in *Allium cepa* L. *New phytol* **103,** 359-373.

Subramani S. and Kannaiyan S. (1987) : Effect of incorporation of *Azolla* biofertilizer on the grain yield of rice*; J. Agron. Crop*. **159,** 308-311.

Tholkappian P., Prakash M. and Sundaram M.D. (2001) : Effect of AM fungi on N and pod number of Soyabean under moisture stress*. Indian J. Plant Physiol*., Vol. 6 No.1 (N.S.) 98-99.

### *Commentary*

**Agriculture and Organic Farming:** India is a leading developing country, making economic progress through agricultural development. Farming is the major source of employment and livelihood in rural areas, where about 65-70% of the people are living. However, the agricultural production has not reached the expected stage because of lack of resources such as water, nutrients, good quality planting material, plant protection and post harvest management. Over 75% of the farmers hold less than 2 ha. land and about 72% of the area under agriculture is dependent on rainfall, whereby return on investment is not assured due to failure of rains and other calamities. These small and marginal farmers who spend over 85% of their income on food alone have no opportunity to make any savings. In the absence of cash reserves, the poor farmers are unable to procure necessary inputs for crop production. As against the world average of 172 kg/ha chemical fertilisers, Indian agriculture consumes only about 70 kg/ha. The average fertiliser consumption is even lower, if fertilisers applied for three important crops like paddy, wheat and sugarcane are not considered in the average. Thus a majority of the crops suffer from nutritional deficiency. Under such conditions, organic farming can be a boon, as the farmers can enrich their soil and manage their crops better, without depending on expensive external inputs. Organic farming can also help to avoid environmental pollution, while keeping the cost of food production under control. With the modernisation of agriculture in the developed countries, expensive agro-chemicals are being deployed for enhancing the crop yields.

**Blue-green Algae:** Blue-green algae belonging to genera *Nostoc* and *Anabaena*, fixes atmospheric nitrogen in symbiotic association with water-fern belonging to Azolla species. *Azolla-Anbaena* association can contribute over 100 kg nitrogen per ha per crop like paddy under lowland conditions. Hence algae is an excellent association to supply nitrogen in low lying areas.

**Vesicular-Arbuscular Mycorrhizae (VAM):** The symbiotic association between plant roots and fungal mycelia is termed as mycorrhiza and it belongs to the following orders :

| Order | Sub-order | Family | Genus |
|---|---|---|---|
| 1. Endogonales | | Endogonaceae | *Endogone* |
| 2. Glomales | 1. Glominae | i) Glomaceae | *GlomusSclerocystis* |
| | | ii) Acaulosporaceae | *EntrophosphoraAcaulospora* |
| | 2. Gigasporinae | i) Gigasporaceae | *Gigaspora* |
| | | | *Scutellispora* |

These fungi are found to be associated with a majority of agricultural crops. VAM occur over a broad ecological range from aquatic to desert environments. VAM have been associated with increased plant growth and enhanced accumulation of plant nutrients, mainly P, Zn, Cu and S through greater soil exploration by mycorrhizal hyphae. It has also been reported that VAM stimulate plant growth by physiological effects other than by enhancement of nutrient uptake or by reducing the severity of diseases caused by soil pathogens. The survival and performance of VAM fungi is affected by the host plant, soil fertility, cropping practices, biological and environmental factors. Maximum root colonization and sporulation occur in low fertility soils. The results of field trials conducted in India indicate that VAM inoculations increased yields significantly and the response varied with soil type, soil fertility and VAM cultures. The major constraint for field trials with VAM has been the inability to produce 'clean pure' inoculum on a large scale as the fungi are obligate symbionts and have to be maintained and multiplied on living host plants. In such a situation, until suitable methods are evolved to multiply on a large scale, it is better to concentrate on crops normally grown in nursery beds where they can be easily inoculated with selected strains and then transplanted.

Hegde, N.G. (1998): Organic Farming : A Boon for our Farmers. Published. Integrated Rural Development for Sustainable Livelihood. A BAIF Publication