SELECTION OF SUITABLE AM FUNGUS FOR WHEAT (*TRITICUM SATIVUM*) Lam. CASS. TO IMPROVE PLANT GROWTH BIOMASS YIELD AND N, P, UPTAKE.

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Abstract:

Screening of indigenous arbuscular mycorrhizal (AM) fungi was undertaken on *Triticum sativum* Lam. The results revealed that the magnitude of improvement varied with different fungal inoculant. *Rhizophagus fasciculatus* influence plants growth, chlorophyll content in leaves, leaf area, biomass yield percent root colonization, spore number, seed yield. It was followed by second AM fungi *Glomus macrocarpum* and thirdly *Glomus bagyarajii* as suitable strains for wheat. This kind of important was not observed in control or non inoculated wheat. Therefore, selection of a native AM fungal strains play role in uptake of N and P with soil conditions.

Keywords: indigenous arbuscular mycorrihzal. (AM) fungi, *Triticum Sativum*, seed yield, *Rhizophagus fasciculatus* and *Glomus macrocarpum*.

Introduction:

Living organisms in the biosphere exhibit a number interactions which either alter their environment and or the size and composition of each other populations. Perhaps the most striking relationship is 'symbiosis' in which both partners derive benefit from each other. Mutualistic symbiosis establish between photosynthetic plans and soil microbes play an important role in the ecosystem (Smith and Read, 1997). The well known example of such symbiosis between non pathogenic soil fungi and roots of flowering is “Mycorrhizae”. Arbuscular mycorrhizal fungi are geographically ubiquitous and occur over a broad ecological range. Both the partners are interdependent on each other for growth and survival. They are one of the few plant-fungal association with a fossil record and may even have facilitated the origin of land flora.

Mycorrhiza is the unique symbiotic association between roots of higher plants and fungi. This association has great economic significance in the growth of crop since it has been given to innovative technologies and sustainability. The improved plant growth is attributed in increased nutrient up-take especially phosphorus, tolerance to water stress, pathogens and adverse soil environmental, production of growth promoting substance and synergistic interaction with other beneficial soil microorganisms (Bagyaraj, 2006; Lakshman, 2009).

The conservation is soil covers many aspects of agricultural crop production and management programmers (Tilak *et. al*., 1999). The beneficial functions of AM fungi are concerned with the uptake, translocation, and transfer of nutrients, mainly phosphate from soil to host cells thereby improving nutrition and growth of host plants particularly the nutrients which are available at low concentration in soil (Lakshman, 2012).

Wheat is the world’s most widely cultivated crop plant. Every month of the year a crop of wheat is maturing somewhere in the world. Wheat and wheat products contribute substantially to the world’s food supply and constitute an important source of carbohydrates in human diet. Industrial use of wheat include the manufacture of starch,

gluten, distilled spirits, malt paste etc. Wheat starch is preferred by many laundries for its use in finishing clothes. Wheat bran is rich in 14-18% proteins and proteins and vitamins, and is and is a valued livestock feed. It is employed in the human diet not any for nutritional qualities but for its role as indigestible material, which stimulate intestinal peristalsis and adds bulk to the waste mass. However, no informational screening of AM fungi to Niger for its improve the biomass yield and nutrient uptake is rather scanty. Therefore, the present study is aimed to select the efficient arbuscular mycorrhizal (AM) fungus for its growth in green house conditions.

Materials and Methods:

The Green house, pot experiments were undertaken at postgraduate studies in Botany, Microbiology laboratory Karnatak University, Dharwad - 580003, India. Plastic bags measuring 20 cms × 25 cms (breadth × length) each bag is filled with 2.5 kg dy redloam soil: sand (3:1) ratio. Before filling the bags soil was fumigated in 750 gms methyl bromide m^{-3} soil) for 48 hours and was aerated for 4 days before inoculation. The used soil had P^H 6.8 and p 5.3 mg/kg soil, E.C. : 0.04 mmhos/cm^2 organic matter 0.19%, total nitrogen 0.16/kg soil, available potassium 110.6 ppm/kg soil.

Screening of Indigenous AM Fungi:

Field studies were undertaken, during September or October after summer monsoon rains are over 2013-2014. Soil and root samples were brought from eight districts; namely; Bellary (Be), Bagalkot (Ba) Bijapur (Bi), Dharwad (Dh), Haveri (Ha), Gadag (Ga), Koppal (Ko) and Raichur (Ra) to the laboratory in a labeled polythene bags. AM fnugi was screened and from these soil samples eight most dominated indigenous AM fungal strains from each districts have been selected and all the eight AM fungal spores were cultured in (sorghum) *Sorhghum vulgare* Pers as a host plant they were examined for the mass multiplication (Table 1). 15 g of mixed inoculum consists of 10 g. heavily AM fungus colorized - host (*Sorghum*) root bits and 5g of rhizosphere of host soil containing 230-245 AM fungal spores per 50 g soil. The selected AM fungal inoculums such as *Acaulosporic laevis, Gigaspora margirata, Glomus bagyrajii, G.leptotichum, G.macrocarpum, Rhizophagus fasciculatus, Scutellospora Sinuosa and S. verrucosa* were inoculated just 5 cm below the soil surface of each experimental pots, except control pots. Wheat seeds surface was sterilized in l% aqueous sodium hychlorite rinsed 3-5 times in distilled water and sowed 2-3 seeds per each experimental pots. To maintain the moisture, pots were watered in every alternate day, 10ml of Hoagland solution minus p was added to the pots once in a fifteen days. Plant growth parameters viz., plant height, root length, leaf area, short dry weight, total number of seeds per plant, total chlorophyll content, per cent root colorization, AM fungal spore number, P and N uptake in short was determined on 30, 60 and 90 days after sowing. However, only observations recorded on day 90 are presented in this paper. Plants were harvested on every 30 days interval. Shoot biomass was determined after drying the plant samples to a constant weight at 70°C in a hot air oven for 48 hours. The Phosphorus content of the plant was determined by employing vanadomslygdate phosphoric acid yellow colour method (Jackson, 1973). Nitrogen content was estimated following Microkjeldahl Method (Jackson, 1973). Soil sample 50g were collected from each polythene bag and subjected to isolation of AM fungal spores by wet-sieving and decanting technique following the procedure outlined by (Gerdemann and Nicolson, 1963). Fine terminal feeder roots were cut into 1 cm length and washed 2-3 times in tap water and boiled with 10% KOH. Neutralized in 2N HCl; and stained in 0,05% trypen blue in lactophenol as outlined by (Phillips and Hayman, 1970), and the per cent root colonization was estimated by adopting the gridline intersection method as per (Giovennetti and Mosse, 1980). The data obtained from the experiments were subjected to are way analysis of variance (ANOVA). All the treatment are means were tested for at least significant difference at 0.05% level

Results:

A fungi inoculants evaluated for wheat individually caused an improvement not only mycorrhizal colonization in root and spore number. But there was favourable enhancement of plant height, root length and spore number leaf area, dry weight of shoots. The total chlorophyll content in leaves number in wheat grain yield, N and P uptake in shoots and acid and alkaline phosphatase in root zones of mycorrhizal and non mycorrhizal plants. However, as excepted magnitude of improvement varied with the different fungal inoculants (Table 1-3). While, plants height, root length, leaf area, dry weight of shoot and total chlorophyll content in leaves (Table 2) had caused by the inoculation *of Rhizophagus fasciculatus* influenced per cent root colonization, spore and grain yield in each plant (Table 3). The second most influenced AM fungal inoculation was *Glamous macrocarpum* and it was followed by *Glomus bagarajiii* as suitable Strains for wheat. These results clearly demonstrated that indigenous AM fungal species influence the growth and biomass yield in wheat plants. All these three AM fungal species were screened from Gadag (*Rhizophagus fasciculatus*, *Glomus macrocarpum* from Haveri and *Glomus bagyarajii* from the soils of Bijapur districts respectively. In contrast to this observation, AM fungal species *Scutellospora verrucosa* (78.6%) and 706/50 g soil spore count and *Scutellospora sinuosa* (82.4 %) and 204/50g per soil spore count was determined, as these two AM fungal species were recovered from Raichiur and Koppal districts respectively. Other three AM fungal species *Acaulospora laevis* from Bellary district, *Gigaspora margirata* from Bagalkote districts and *Glamous leptonicum* Dharwad district had influenced 3-4 fold increase in plant growth, root length biomass yield, number of seeds per each wheat plants. All these treatments significantly higher over the control plants. Considering the chlorophyll content and biomass yield, to be an important parameter of crop performance especially in the wheat /grain yielding plants. Therefore, we can assume that the rate of growth, N and P uptake, acid and alkaline phosphates activities of plants was different depending on the indigenous AM fungal species inoculation. After, 30 days of different AM fungal inoculation Niger plants had an increase and reached significant growth at 60 days and optimum growth was recorded at 90 days harvest. Control or non inoculated wheat plants had mean percent root colourization and least AM fungal spores in their rhizosphere. However, there were far below compared to AM fungi inoculated plants. It was observed that sheet of P and N uptake are significantly correlated with dry weight of shoots in wheat plants.

Arbuscular maycorihzal (AM) of fungal species eight viz *Acaulospora laevis, Gigaspora margirata, Glomus baggyarajii, G.leptotichum, G.macrocarpum, Rhizophagus fasciculatus, Scatellospora sinosa* and *S. veroucosa* are indigenous to eight districts of Bellary Bagalkote, Bijapur, Dharwad, Haveri, Gadag, Koppal and Ranebennur. All the eight AM fungi was mass multiplied on (sorghum) *Sorghum vulgare* Pers. By using soilrite and observed optimum percent root colorization and spore number (Table l).

Discussion:

In all eight species of AM fungi recorded in the districts of North Karnataka found to be dominance. This association has great significance in the growth of wheat crops, since it has been given to innovative sustainability by mycorrhizal association (Smith and Read, 1997). Selected AM fungi is essential to characterize the native AM fungal population from the soil, had the realistic and effective, when the exact host plant is used (Bagyaraj, 2006; Lakshman, 2009). Species of AM fungi have differed to the extent by which, they increase wheat plant growth, biomass yield and N and P uptake. Therefore, the need for selecting efficient AM fungi that can be used for inoculating different mycotrophic, plants has been stressed (Abbott and Robson, 1982; Vinayak and Bagyaraj, 1990; Lakshmipathy *et. al.,* 2000). AM fungal inoculation enhanced the growth of (wheat) *Triticum Sativum* Lam. by increasing the concentration of N and P in the plants. Especially

Rhizophagus fasciculatus inoculation brought a signification biomass yield and N and P uptake. These results are on par with earlier studies of (Earanna 1999; Chiramel *et al.,* 2006.). In this context, the role of AM fungi as phosphorus supplier to the plaints appears to be of great relevance. Improved uptake of P is due exploration of external hyphae of the soil beyond root hair zone when phosphorus is depleted (Cooper and Tinker, 1978; Mcsonigle and Miller, 1993; Lakshman, 1996). Although, each plant received the some amount of (348 spores/ 50 g soil) inoculums, the efficiency of inoculums is most probably not the same. The quality of inoculum also is important. From besides some fungi have different colonization patterns and different effects on host plant growth (Ortas, 2002, 2008, 2009; Ortas and Varma, 2007).

In the present findings strongly support the observation made by (Ortas, 2009). Out of eight selected AM fungal species, *Rhizophagus fasciulatus* most excellent are influenced optimum plant growth biomass yield and it is followed by *Glomus macrocarpum* and *Glamus bagyarajii* second and third efficient species of AM fungi. It is often assumed that all species of AM fungi have the same function due to the ubiquity of the association and the fact that AM fungi occupy a similar plant/soil niche. However, there is increasing evidence that the mechanisms for establishing function mycorrhiza may differ among Species and genera (Boddington and Dodd, 1998, 1999; Dodd *et al.,* 2000). This present study indicated that, out of eight AM fungal species are three most effective stains and other five A fungal species viz; *Acaulospora laevis*, *Gigaspora margiratta, G.leptotichum,, Scutellospora sinuosa and S. siverrucosa* are better stains but not efficient strains for wheat. In conclusion the results indicate that the inoculation with different AM fungal species had the positive effects as growth and biomass yield on wheat crops. *Rhizophagus fasciculatus* inoculated plaints had higher yield, chlorophyll content, increase N and P concentration. Therefore, for wheat 1. *Rhizophagus fasciculatus, 2. Glomus macrocarpum, 3. Glomus baggarajii* tested were found to be the most excellent for the endorsement of favourable growth under green house conditions. And thus, we need more studies on different AM fungal inoculation under field conditions.

Table 1. Effect of soilrite for mass multiplication of AM fungi in (Sorghum) *Sorghum vulgare* pers. showing per cent root colonization and spore number for 50 g. soil at 60 days.

A M fungal culture	% root colonization	No of spores
Aculospora laevis Gerd and Trappe. (AL)	96.2 b	114.1 a
Gigaspora margirata Becker & Hall. (Gm)	94.4 c	109.7 a
Glomus bagyorajii Sinchari Greu & Eicher. (Gb)	100.0 a	208.4 bd
Glomus leptotichum Schenck & Smith. (GL)	98.3 d	103.6 c
Glomus macrocarpum Tul. & Tul. (Gma)	100.0 ab	214.2 a
Rhizophagus fasciculatus (Thaxb) Walker & Schubler. (Rf)	100.0 bd	278.4 b
Scutellospora sinuosa. Gerd & Bakshi (Ss)	98.6 d	157.3 f
Scutellospora verrucosa (Koske & Wal.) Walker & Sanders. (Sr)	88.76 c	146.5 g

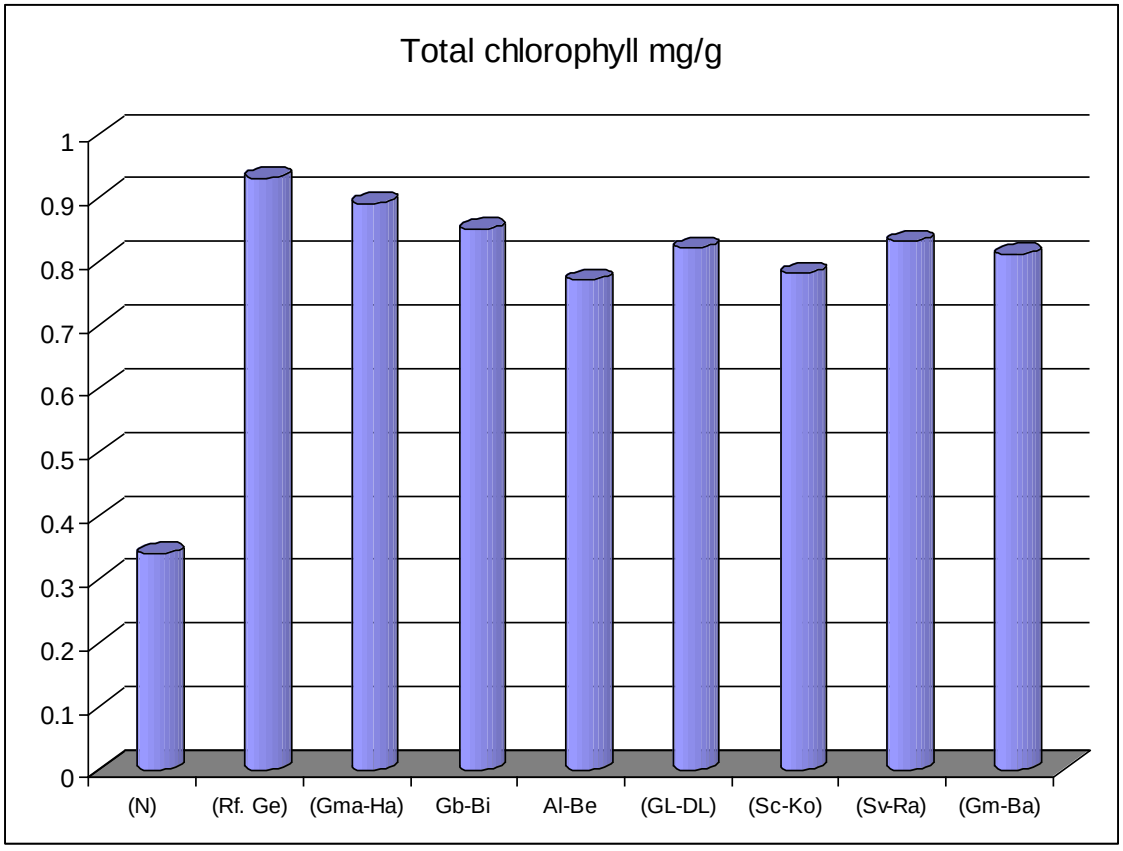
Mean values are followed by the same letter with in a column donot differ significantly at (P=0.05) according to Ducan's range test.

Table 2. Influence of AM fungi on plant height, root length, shoot dry weight and total chlorophyll of Niger for 90 days.

Treatment	Plant height (cm^{-1})	Root length (cm^{-1})	Leaf area (cm^2/plant)	Shoot dry weight g/plant	Total chlorophyll mg/g
Control (N)	31.5 a	16.7 c	8.5 d	6.4 c	0.34 a
Rhizophagus fasciculatus (Rf. G)	72.2d	33.3bc	14.2 cd	24.9 b	0.93 c
Glomus macrocarpum (Gma-Ha)	67.4ab	27.2ab	13.5b	22.8 a	0.89 b
Glomus bagyarajii (Gb-Bi)	48.1d	24.2c	11.8 a	20.5 ad	0.85 d
Acaulospora laevis (Al-)	46.3 b	22.2 cd	10.9 c	14.7 d	0.77 b
Glomus leptotichum (Gl-Db)	29.8 a	18.4 b	12.2 b	15.9 bc	0.82c
Scutellospora sinosa (Sc-Ko)	45.6 a	27.1d	11.7 ab	19.9 d	0.78 ab
Scutellospora verrucose (Sv-Ra)	36.3 c	20.0 ab	12.2 c	18.3 a	0.83 c
Gigaspora margrita (Gm-Ba)	109 bc	29.1 bc	11.4 d	17.8 b	0.81 cd

Acaulospora laevis-Bellary (Al-Be), *Gigaspora margarita*-Bagalkot (GM-Ba), *Glomus bagyarajii*-Bijapur, (Gb-Bi), *Glomus leptotichum*--Dharwad (Gl-Dh), *Glomus macrocarpum*-Haveri (Gma-Ha), *Rhizophagus fasciculatus*-Gadag (Rf-Ga), *Scutellospora Sinnosa*-Koppal (Ss-Ko), *Scutellospora verruucosa*-Ranebennur (Sv-Ra).
Mean values are followed by the same letter with in a column donot differ significantly at (P=0.05) according to Ducan's range test.

Fig. 1 Influence of AM fungi on total chlorophyll of Niger for 90 days.



Acaulospora laevis-Bellary (Al-Be), *Gigaspora margarita*-Bagalkot (GM-Ba), *Glomus bagyarajii*-Bijapur, *Glomus leptoricum*-Dharwad (Gl-Dh), *Glomus macrocarpum*-Haveri (Gma-Ha), *Rhizophagus fasciculatus*-Gadag (Rf-Ga), *Scutellospora Sinnosa*-Koppal (Ss-Ko), *Scutellospora verruucosa*-Ranebennur (Sv-Ra).

Table 3. Influence of AM fungi on number of seeds, Nitrogen, Phosphorus uptake in shoots, per cent root colonization and spore number of Niger for 90 days.

Treatment	grain number/ plant (g)	In shoot N content mg/ plant	P content mg/ plant	Percent root colonization	AM fungal spores/ 50 g soil
Control (NM)	0.341 a	124 b	11.1a	14.2a	27b
Rf-Ga	0.998 d	139 c	13.3 d	68.4 e	153 c
Gma-Ha	1.773 cb	147 d	13.8c	76.5b	167d
Gh-Bi	2.852a	I63ce	15.5 bd	88.3d	207 bd
Al-Be	1.819cd	151 bd	14.5 ge	79.4 a	198
Gl-Dh	1.152 b	173 e	17.1 a	93.5 c	213 g
Sc-Ko	2.895 bd	166 g	16.3 e	78.6 bd	179 ed
Sv-Ra	1.718 c	171 a	15.9d	82.4 e	206 a
Gm-Ba	1.304 b	155 b	14.2 c	86.3 g	204 b

References:

Abbott. L.K, and Robson. A.D., 1982, The role of vesicular arbuscular mycorrhizal and selections of fungi for inoculation, Aust J Agri Res, 33, 389- 408.

Boddington. C.L., and Dodd. J.C., 1998, A comparison of the development and metabolic activity of mycorrhizas formed by arbyscular mycorrhizal fungi from different genera on two tropical forage legumes Mycorrhiza, 8, 149-157.

Boddington. C.L., and Dodd. J.C., l999. Evidence that differences in phosphate metabolism in mycorrhizas formed by species of *Glomus* and *Gigaspora* might be related to their life cycle strategies, New Phytologist, 42, 531-538.

Bagyaraj. D.J., 2006, Arbuscular mycorrhizal fungi in sustainable agriculture, In: Techniques in mycorrhizae, (Eds. M.J.Bukhari and B.F. Rodrigues), pp. 1-8.

Chiramel. T., Bagyaraj, D.J., and Patil. C.S.P., 2006, Response of *Andrographis paniculata* to different arbuscular mycorrhizal fungi, Journal of Technology, 2(2), 221-228.

Cooper. M., and Tinker. P.B., 1978, Translocation and transfer of nutrients in vesicular arbuscular mycorrhizas II, Uptake and translocation of phosphorus, zinc and sulphur. New phytol, 81, 43 -52.

Dodd. J.C., Boddington. C.L., Rodriguez. A., Gonzalez. C., and Mansur. I., 2000, Mycellium of arbuscular mycorrhizal fungi (AMF) from different genera: Form, function and detection, Plant and Soil, 226,131-151.

Dodd. J.C., 2000, The role of arbuscular mycorrhizal fungi in agro and natural ecosystems, Outlook on Agriculture, 29, 55-62.

Earanna. N., Suresh. C.K., Bagyaraj. D.J., 1999. Effect of different VAM fungi on growth and yield of coleus barbatus. J Soil Biol Biochem, 37, 1460-1468.

Geredemann. J.W., Nicoloson. T.H., 1963, Spores of mycorrhizal and plant growth. Ann.Rev. Phytopathol., 6, 397-418.

Giovannetti. M., Mosse. B., 1980, An evaluation of technologies for measuring vesicular arbuscular mycorrhizal infection in roots, New Phytologist, 84, 489-500.

Jackson. M.L., Soil Chemical Analysis, Prenticed Hall (Private) Ltd, New Delhi, India, 446 pp.

Lakshman. H.C., 1996, Possible role of weeds in maintaining VA-mycorrhizal colonization of rice soils, J World weeds, 3, 53-56.

Lakshman. H.C., 2009. AM Fungi with soil influence on *Jatropha curcas* L. International Journal of Plant Science, 1(1), 120- 123.

Lakshman. H.C., and Channabasava. A., **2012,** AM fungi and mine spoil consortium: a microbial approach for enhancing Proso millet biomass and yield, Inter J of Pharma and Biosci, 3(4), 676-684.

Lakshmipathy. R.A.N., Balakrishna., Bagyaraj. D.J., and Kumar, D.P., 2000, Symbiotic response of cashew root stocks to different VA mycorrhizal fungi, The cashew, 14, 20-24.

McGonigle. T.P., and Miller. M.H., 1993, Response of mycorrhizac and shoot phosphorus of Maize to the frequency and timing of soil disturbance, Mycorhiza, 4(2), 63 -6

Ortas. I., Ortaki. D., Kaya. Z., and Cinara Onedge. N. 2002, Mycorrhizal depending of Sour orange in relation to Phosphorous and Zinc nutrition, J Plant Nutr, 25,1363-1279.

Ortas, I. Varma, A. 2007. Field trials of bionioculants. In Oelmuller, R., Varma, A. (Eds) Modern tools and Techniques. 11 Springer- Ver-lag Germany pp. 397 – 413. (Chapter 26.)

Ortas. I., 2008, Effects of selected mycorrhizal inoculation on phosphorus sustainability in sterile and non-sterile soils in the Hsarran Plain in South Anatolia, J Plant Nutr, 26,1-17.

Ortas. I., 2008, Field trials on mycorrhizal inoculation in the eastern Mediterranean horticultural region, In; feldmann, F., Kapulnik, Y., Baar. J. (Eds.), Mycorrhiza works, Hannover, Germany, pp. 56-77.

Ortas. I., 2009, Mycorrhizae application in horticultural production on plant growth, Healthy planets and healthy human, In. XVI International Plant; Nutrition Colloquium., Plant Nutrition for Sustainable Development and Global Health, August 26th - 30*2009, Sacramento, California USA, http:// repositories.cdlib.org/cgi/view content.cgi.article=1074&context=ipnc/xvi.

Smith. S.E., and Read. D.J., 1997, Mycorrhizal symbiosis, 2nd edition, Academic press, London.

Tilak. K.V.B.R., 1999, Associative effects of vesicular Arbuscular mycorrhiza with nitrogen fixers, Proceedings of the Indian national academy, Pp. 325-352.

Vinayak. K., and Bagyaraj. D.T., 1990, Vesicular arbuscular mycorrhizae Troyer citrange, Biological and Agricualture Horticulture, 6 , 303-311.