EFFECT OF AGRONOMIC TREATMENTS ON ARBUSCULAR MYCORRHIZAL FUNGI UNDER NURSERY CONDITIONS OF WARANGAL

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Influence of different agronomic practices on root nodulation, AM colonization and growth of forest tree species was studied in two agroforestry tree species *Acacia nilotica* and *Albizia lebbeck*. Neem cake and Farmyard manure and sewage water were analyzed for their effect on *A.nilotica and A.lebbeck*. These treatments along with different AM fungi were also analyzed. Farmyard manure was found to be effective with *Glomus fasiculatum* in *A. nilotica* and Neem cake was given best results in *A.l ebbeck* with *Glomus fasiculatum.*

Key words *Acacia nilotica,* agronomic practices, *Albizia lebbeck,* AM fungi.

Agronomic practice and climatic conditions alter the soil AM fungal population both qualitatively and quantitatively. The population of the virgin soil may depend to a large extent on soil chemical characteristics, especially the organic matter content. It is observed that abundant chemical fertilizers in the soil reduce AM Fungi but promote actinomycetes and bacterial population. Phosphorous and Organic matter together had an additive effect on AM colonization; growth and Phosphorous uptake (Pavan Kumar *et al.* 1999, Nagabushanam *et al.* 1999) have also recorded variation in the AM colonization with the different agronomic practices.

Studies have been made on modified potting media and fertilizer application (Lea Corkidi *et al.* 2004, Saito *et al.* 2002, Douds *et al.* 2010). Arbuscular mycorrhizal (AM) fungi form a symbiosis with a wide range of tree species (Hussain *et al.* 2013). Such mutualism offers various advantages to host plants and mainly significant in the advancement of seedlings developed in nurseries and organization of saplings planted in devastation areas. AM symbiosis results in increased growth of plants depending on the fungal strains (Pattison *et al.* 2004, Youpensuk *et al.* 2005). Resistance to drought increase in soil aggregation protection against plant root pathogens was well recorded (Dusky *et al.* 2002, Rilling *et al.* 2005, Wu,q. R. Xia and Z. Hu 2006). AM fungi can increase the nutrient absorption by hyphal uptake and translocation and this makes the AM fungi more advantageous (Koide and Mosse 2004). AM fungi are obligate symbionts, these can be propagated by co-culturing of living host plant in pot cultures. AM fungi, appropriate host plant and substrate media are essential for starter cultures (Setiadi 2000). Mycorrhizal inhabited soil, AM fungal spores and mycorrhiza infected roots, etc, can be used as inoculums and or seed treatment, saplings were grown in nursery conditions (Brundrett *et al.* 1996, Klironomos *et al.* 2002). The plant species and AM fungal interaction are important criteria for the success of plant restoration programs (Wubet *et al.* 2003).

The aim of the present study is to investigate the effect of AM fungal species with different concentrations of manures on plant growth and development in Phosphorus deficient soil medium under greenhouse conditions in two agroforestry tree species *A.lebbeck and A.nilotica*.

MATERIALS AND METHODS

Albizzia lebbeck and *Acacia nilotica* growing in nursery conditions of Warangal and were selected for these studies. Different agronomic treatments such as Farmyard manure (FYM), Neemcake and Sewage water were applied to the test plants. Brought the FYM and Neemcake from Organic farming unit of NGO for experimental studies. Pure cultures of *Glomus fasiculatum*, *Glomus aggregatum, Glomus mosseae, Gigaspora gigantea, and Gigaspora margarita* were employed as inoculums in different combinations of the test plants. The AMF cultures were maintained on Bajra as host plant roots in an earthen pot served as inoculum. AMF inoculums (125-150 spores/100 grams soil) were placed 3 cm below the seed sowing level. Suitable controls were maintained. Before seed sowing 200 grams of FYM + 100g of AM fungi had inoculated to each set of experiments.

i) For 30 days of plants (200g of FYM+100grams of AMF fungi)
ii) For 60 days of plants (400g of FYM+200g of AMF) and
iii) For 90 days of plants (600g of FYM+300 grams of AMF).

The soil samples were mixed and a unified sample was made. A similar set was maintained to Neem cake + AMF treated plants, at least five replicates were maintained. The mature and dried seeds of two test plants were surface sterilized with 0.01% mercuric chloride. Five seeds per pot were sown and watered regularly. After seed germination, they were thinned to a single seedling per pot for better growth. At the end of 30, 60 and 90 days of treatments the parameters were recorded, such as plant height, the number of nodules, fresh and dry weights of root and shoot were analyzed.

The samples were collected from different treated soils and analyzed for AM fungal spore population (Plate2). The degree of AM colonization was observed after clearing and staining the roots (Phillips, and Hayman, 1970) and the percentage of colonization was calculated (Giovannetti and Mosse,1980).

AM fungal spore population in the rhizosphere soil was determined by wet sieving and decanting method (Gerdmann and Nicolson 1963, Pacioni 1992). The AM fungal spores were observed under the stereo binocular microscope Nikon SMZ18 and identified based on morphological characteristics of spores by using the nomenclature method (Sturmer 2012). The physicochemical characteristics of the soils were analyzed according to the standard procedures (Warnck and Brown, 1998) (Table 3). Estimation of phosphorus content in oven-dried (shoot and root) samples by using the Molybdate-Vandate method (Jackson, 1973) Chlorophyll a and Chlorophyll b were also estimated by using method (Arnon 1949, Ikan 1969).

Multiplication of inoculum production and maintenance: AMF resting spores collected by stereo binocular microscope were surface sterilized with 200 ppm streptomycin for 15 min and washed in sterile distilled water for several times. The starter culture was prepared by soil funnel techniques (Nicolson, 1967). A glass funnel was filled (3/4th) with autoclaved soil and sand (1:1) and the neck was loosely plugged with cotton wool. The funnel was kept over a conical flask (filled with sterile water). Spores were spread near the neck and covered with a thin layer of soil. Seeds of Pennisetum glaucum (surface-sterilized) were evenly sown and watered (sterile). After 10-15 days roots can be seen sprouting from the neck. Meanwhile, they get infected by AMF spores at the neck. After 25 days, roots were examined for root colonization.

RESULTS AND DISCUSSION

The present treatments had a significant influence on AM colonization and growth of the plant. The marginal increase observed in AM colonization in FYM added plants. Root colonization increased with the progressive advancement of the age of *A.lebbeck*. Neem cake also recorded a stimulatory effect. It was responsible for the increased number of AM fungal spores in the rhizosphere soil. On the other hand, Sewage water adversely affected the AM colonization and AM fungal spore population which was almost the same for both the plants under investigation FYM had a comparatively more stimulatory effect than Neemcake for *A. nilotica.*

On the other hand, *A.lebbeck* was stimulated for its growth by Neem cake. Sewage water had a negative effect on the height of the plant. Root nodulation in both the plants was stimulated when they were treated with AM fungi. However, Neemcake was found to have a little stimulating effect on the nodulation and biomass yield; stimulation was comparatively more when the plants were treated with FYM. Chlorophyll content of both the plants

Table 1: Influence of agronomic treatments on AM colonization, nodulation, and growth of two forest tree species

Name of the treatment	**Age of the plant (in Days)**	**Percentage of colonization**	**No. of spores/100gm soil**	**Height of the plant (cm)**	**No. of Nodules**	**Biomass(g)**		**Chlorophyll(in O.D)**		**Phosphorous (mg/gms)**	
						Fresh weight in gms	**Dry Weight in gms**	**a**	**b**	**Shoot**	**root**
A.lebbeck											
	30	52.7±1.2	132±0.9	42.2±0.3	28±0.1	23.2±2.6	19.5±0.3	0.56±0.3	0.46±3.1	0.47±0.8	0.62±0.6
	60	58.2±0.9	143±1.1	48.3±0.5	36±1.2	36.2±3.5	27.5±0.8	0.59±0.2	0.27±0.2	0.52±0.5	0.76±0.2
Farmyard manure	90	63.2±1.4	152±1.6	56.2±0.8	43±3.0	42.3±0.9	33.4±1.3	0.65±1.4	0.23±2.1	0.64±0.7	0.82±1.2
Neemcake	30	48.2±0.3	138±2.0	38.2±3.2	24±2.6	25.5±1.2	19.2±1.2	0.81±0.2	0.52±3.2	0.29±1.3	0.67±2.5
	60	53.2±1.0	148±1.3	43.2±1.0	33±1.4	28.3±3.4	21.5±2.1	0.63±2.3	0.59±0.5	0.38±0.5	0.73±3.2
	90	59.2±2.3	156±0.7	59.5±1.2	38±1.2	32.2±0.3	27.8±3.1	0.75±3.7	0.65±0.9	0.45±1.5	0.78±1.0
Sewage water	30	32.5±0.4	72±2.5	30.2±2.5	12±1.5	16.5±0.8	9.5±2.4	0.21±0.5	0.13±1.2	0.18±2.3	0.27±1.2
	60	39.2±0.9	86±3.0	34.5±3.5	19±0.9	26.2±1.5	18.6±0.8	0.28±0.8	0.19±2.3	0.27±0.3	0.39±3.2
	90	42.2±2.7	105±2.9	39.2±1.3	24±1.0	32.5±2.6	24.2±2.7	0.32±0.7	0.24±0.7	0.34±1.5	0.46±5.2
Control	30	38.2±0.6	103±4.1	26.2±0.7	29±0.3	16.5±2.5	11.5±0.9	0.48±3.5	0.43±1.2	0.68±0.7	0.75±3.2
	60	43.2±0.3	110±2.0	29.2±0.1	32±0.9	19.8±0.8	13.6±0.3	0.34±2.6	0.19±1.8	0.48±0.8	0.56±0.5
	90	46.5±0.4	123±1.5	36.1±2.2	38±2.3	26.5±0.7	21.6±0.2	0.48±4.0	0.34±0.9	0.56±0.8	0.63±0.6
A.nilotica											
	30	48.2±1.0	148±2.3	18.5±0.2	19±0.2	16.2±3.2	12.1±0.3	0.48±2.5	0.32±0.5	0.36±0.3	0.56±0.6
	60	52.3±0.1	218±0.6	23.5±0.5	26±1.0	24.2±4.6	18.3 ±1.6	0.52±0.8	0.38±0.7	0.57±0.5	0.68±1.3
Farmyard manure	90	58.2±0.5	228±0.5	28.0±0.7	32±1.2	35.2±3.2	23.5±0.9	0.62±0.3	0.43±0.2	0.67±2.1	0.73±0.7
Neemcake	30	46.3±2.3	135±2.5	22.5±1.3	16±2.3	17.3±0.9	11.5±0.5	0.42±1.3	0.36±1.3	0.26±1.8	0.53±1.6
	60	56.2±0.8	146±3.7	27.2±2.2	21±0.5	19.6±2.1	19.6±0.6	0.59±0.9	0.47±0.5	0.69±1.3	0.72±1.8
	90	59.3±0.6	168±0.8	33.4±3.2	24±0.9	24.5±0.3	24.5±0.5	0.65±0.8	0.53±1.5	0.76±3.2	0.82±2.3
Sewage water	30	26.5±1.6	78±0.9	19.0±0.8	5±0.9	12.5±0.5	8.5±0.9	0.24±0.2	0.18±0.6	0.23±1.5	0.32±0.3
	60	32.5±2.3	83±2.5	22.2±0.7	8±1.6	14.2±1.2	10.2±0.3	0.39±0.8	0.27±0.3	0.29±0.5	0.36±0.7
	90	36.2±3.6	88±3.0	25.2±2.2	9±2.1	22.2±0.7	16.2±0.5	0.41±0.9	0.36±1.2	0.25±0.6	0.39±0.9
Control	30	25.2±1.3	93±0.9	33.5±1.2	8±0.9	25.2±1.2	21.2±1.5	0.28±1.6	0.17±0.5	0.33±0.8	0.46±0.5
	60	27.3±2.1	118±0.1	36.5±0.6	14±0.2	34.5±1.6	25.2±2.7	0.32±2.5	0.26±1.5	0.52±0.3	0.68±0.3
	90	37.2±2.7	124±0.5	43.2±0.9	19±0.4	38.2±0.8	27.2±0.9	0.39±0.5	0.29±2.3	0.59±0.7	0.73±1.3

Table 2: Effect of fertilizers on AM fungi and growth and development of two forest trees species under nursery conditions

Treatments	Percentage of colonization	No. of spores/ 100g soil	Height of the plant (cm)	No. of nodules plants	Biomass(g)		Chlorophyll(in O.D)		Phosphorus (mg/plant)	
					Fresh wt.	Dry wt.	a	b	Shoot	Root
Albizia lebbeck										
FYM +*G.fasiculatum*	62.4±0.9	158.1±2.5	52.3±0.6	41±1.2	9.5±0.3	4.2±0.5	0.152±0.1	0.168±0.3	0.138±0.3	0.186±0.8
FYM+*G.aggregatum*	58.2±0.3	152.3±4.0	45.2±1.3	57±2.6	7.6±0.5	3.8±0.3	0.162±0.2	0.178±0.5	0.148±0.5	152±0.2
FYM+*G.mosseae*	61.2±0.6	136.2±1.5	48.3±2.3	32±0.9	8.2±0.8	5.1±1.3	0.142±0.4	0.182±0.1	0.132±0.2	0.166±0.5
FYM+*Gig.gigantea*	58.8±0.6	143.6±1.5	50.2±1.5	48±0.7	8.9±1.4	5.6±2.3	0.159±0.1	0.173±0.7	0.143±0.3	0.172±0.8
FYM+Gig.margarita	53.4±0.7	138.1±3.6	41.2±0.9	42±1.6	7.2±1.4	5.3±0.8	0.169±0.6	0.148±0.4	0.133±0.8	0.148±0.5
FYM(Control)	48.3±1.3	122.7±2.6	36.3±0.3	28±1.5	5.3±0.7	3.8±0.9	0.128±0.8	0.136±0.2	0.158±0.7	0.187±0.2
Neemcake+ G.fasiculatum	64.8±0.6	148.3±2.6	64.2±0.2	44±2.4	10.2±2.8	6.5±3.1	0.164±0.2	0.152±0.1	0.148±0.7	0.174±0.3
Neemcake+ *G.aggregatum*	59.3±0.9	156.5±2.2	56.2±1.3	53±0.3	8.2±1.6	5.3±0.8	0.156±0.2	0.132±0.3	0.136±0.6	0.164±0.1
Neemcake+*G.mosseae*	62.4±1.6	139.1±3.0	54.3±2.0	37±0.2	7.8±0.9	4.8±0.4	0.136±0.4	0.123±0.1	0.146±0.2	0.168±0.2
Neemcake+*G.gigantea*	61.3±0.8	143.1±1.3	62.2±0.5	56±0.3	8.5±0.5	5.7±1.6	0.162±0.1	0.177±0.1	0.154±0.1	0.182±0.8
Neemcake+*Gig.margarita*	59.2±1.4	158.2±0.9	43.2±0.2	62±1.2	5.9±0.6	4.3±0.8	0.143±0.2	0.184±0.2	0.162±0.9	0.186±0.7
Neemcake(Control)	42.2±0.5	134.3±0.8	39.2±0.3	40±0.8	5.0±0.8	3.2±0.9	0.126±0.3	0.138±0.3	0.134±0.2	0.153±0.5
Acacia nilotica										
FYM +*G.fasiculatum*	68.3±1.0	162.7±1.2	54.2±0.8	42±3.2	12.5±0.8	8.5±0.3	0.158±0.3	0.138±0.4	0.132±0.1	0.149±0.3
FYM+*G.aggregatum*	64.2±0.6	158.5±0.5	62.4±0.9	36±0.2	10.6±0.3	7.2±0.2	0.162±0.5	0.142±0.2	0.152±0.5	0.163±0.5
FYM+*G.mosseae*	68.4±2.4	148.2±1.5	52.2±0.3	32±1.5	14.2±0.4	9.8±0.5	0.162±0.2	0.133±0.2	0.182±0.6	0.194±0.1
FYM+*G.gigantea*	59.2±0.7	153.2±2.0	54.2±0.5	28±0.6	12.3±0.2	8.7±1.2	0.158±0.4	0.142±0.6	0.154±0.7	0.182±0.5
FYM+*Gig.margarita*	61.2±1.3	142.1±2.0	52.8±0.6	36±0.8	11.2±0.7	8.3±2.2	0.132±0.6	0.126±0.1	0.142±0.1	0.168±0.1
FYM(Control)	53.2±0.9	134.2±1.8	49.1±0.1	26±1.5	10.0±1.6	7.8±1.3	0.168±0.2	0.152±0.2	0.123±0.2	0.128±0.2
Neemcake+ *G.fasiculatum*	69.3±1.4	174.3±2.3	56.4±1.3	36±1.3	11.5±2.1	8.6±2.5	0.148±0.3	0.156±0.4	0.138±0.3	0.163±0.6
Neemcake+ *G.aggregatum*	66.2±0.6	165.6±3.2	64.3±2.6	29±2.0	12.9±1.6	8.3±0.9	0.138±0.7	0.146±0.2	0.146±0.5	0.168±0.5
Neemcake+*G.mosseae*	60.8±1.2	159.5±1.3	61.2±1.5	43±0.6	10.6±2.3	7.3±0.7	0.123±0.9	0.152±0.1	0.159±0.1	0.183±0.2
Neemcake+*Gig.gigantea*	61.4±0.9	163.3±1.9	59.2±3.1	38±2.2	14.2±0.6	9.8±0.3	0.136±0.7	0.146±0.1	0.167±0.3	0.186±0.4
Neemcake+*Gig.margarita*	59.5±1.6	159.2±2.5	54.8±1.6	26±1.8	12.8±0.4	7.4±1.0	0.162±0.2	0.174±0.3	0.156±0.5	0.172±0.6
Neemcake(Control)	56.2±0.6	138.3±3.1	52.1±1.2	19±0.3	10.±0.9	6.2±0.6	0.124±0.6	0.133±0.5	0.132±0.9	0.146±0.1

increased under the influence of farmyard manure and Neem cake. Similarly, phosphorus content also increased in the presence of farmyard manure and Neem cake. Sewage water adversely affected both the tree species under investigation. (Table 1).

AMF inoculation along with farmyard manure and Neemcake resulted in the stimulation, of growth and development of both the tree species under investigation. *Glomus fasiculatum* along with farmyard manure stimulated the Percentage of colonization. Stimulation was comparatively more when Neemcake was added. *Gigaspora margarita* infection was comparatively low in root colonization in the presence of farmyard manure. A similar response was observed in *A. lebbeck* where AM fungal spore population was more in soil treated with farmyard manure and *Glomus fasiculatum* followed by Neem cake and G*lomus aggregatum. A.lebbeck* attained more height when treated with farmyard manure and *Glomus fasiculatum* as well as Neem cake and *Glomus fasiculatum*. Neem cake was found to be more favorable for the AM fungal spore population. Nodulation was promoted in the presence of AM fungi. Biomass increased when treated with Farmyard manure and *Glomus mosseae*. The chlorophyll content increased in the presence of Neemcake and Farmyard manure. Similar results were observed in *A. nilotica* treated with AM fungi and fertilizers. (Table 2).

Table. 3 reveals the soil Physico-chemical characteristics which were recorded in relation to two forest tree species. All treated soils are Loam sandy their pH varies slightly between 7.4 to 7.9. On the other hand treated soils of

Table 3: Physico-chemical characteristics of soils treated with agronomic treatments of two forest tree species

Soil characters	FYM	Neemcake	Sewage Water	Control
Acacia nilotica				
Soil texture	Loam sandy	Loam sandy	Loam sandy	Loam sandy
pH	7.9	7.6	7.5	7.4
EC mho/cm	0.22	0.18	0.12	0.2
Organic matter (%)	0.5	0.5	0.3	0.4
Available phosphorous($P_2 0_5$)	50.0	45.0	30.0	45.0
Available Potassium (kg/hect)	220	210	180	215
Albizia lebbeck				
Soil texture	Loam sandy	Loam sandy	Loam sandy	Loam sandy
pH	7.9	7.8	7.9	7.4
EC mho/cm	0.24	0.26	0.29	0.46
Organic matter (%)	0.15	0.28	0.52	0.49
Available phosphorous($P_2 0_5$)	216	282	268	222
Available Potassium (kg/hect)	260	272	242	316

Acacia nilotica showed lowest P, K, and Electric conductivity and Organic matter when compared to the treated soils of *Albizia lebbeck.*

***Description of AM fungal species and morphological identification of AM fungi isolated from the rhizosphere soils of study plants* (Plate 2):**

***1. Glomus aggregatum* (N. C. Schenck & G. S. Smith):** Pale yellow, Yellow-brown, orange-brown colored spores. Constricted with 1 wall layer, size (40 -85µm) spore inside spores formed by internal proliferation. Aggregates of interradial spores frequently formed. Irregular shaped globose cells formed in sporocarps. Few spores may have two laminated wall layers.

***2. Glomus constrictum* (Trappe)C.Walker, Vestberg & Schuessler):** Dark- brown, brown in color with 2 layers funnel-shaped. It consists of 2 layers and it size 150-330 µm**.** Just beyond the point of attachment, subtending hypha constricted and below the construction, the hypha inflated, the septum is often cup-shaped and presents some distance down the attached hypha.

***3.Glomus fasiculatum* (Thaxter) Walker &Koske):** Yellow or Brown colored cylindrical slightly flared **i**t has 3 wall layers and size (60-95 µm). Outer wall ornamented. Irregular-shaped spores frequently present in sporocarps.

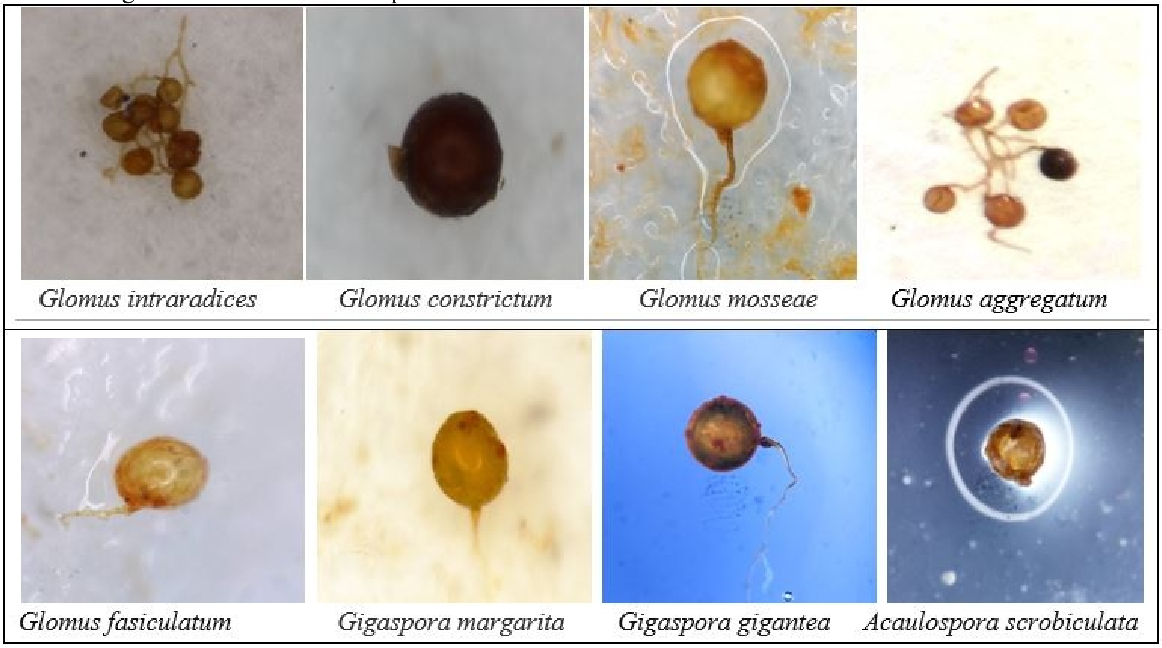
***4. Glomus mosseae* (Gredemann& Trappe):** Yellow or brown colored spores with 2 wall layers 68-290 µm in diameter, Flared to funnel-shaped spores subtending hypha, 20-50 µm wide.

***5. Glomus intraradices* (Schenck & Smith):** Found single in soil or in aggregates, brown, 93-131 µm diameter. Composed of 4 wall layers, yellow to grey-brown. Globular, yellow to light brown. Wall of the spore extending into the hyphal attachment forming an apparent tubeform flare at the juncture with the hyphal attachment. Hyphal attachment 9-33µm diam. constricted 2-3 µmat the base of the spore. Wall thickness 1.5-5.2µm at the base of the spore.

6. Gigaspora gigantea: Yellow colored spores with 1 spore wall layer,480 µm size.

Plate 1: Pot culturing of AM fungal pure cultures: **1.** *Glomus mosseae* **2.** *Glomus aggregatum* **3**. *Glomus fasiculatum* **4.** *Gigaspora margarita* **5.** *Gigaspora gigantea*

Plate 2: AM fungi isolated from the rhizosphere soil



7. Gigaspora margarita **(Becker & hall,):** White-colored spores with single spore wall layer 260µm in size. Arbuscles produce fine branches from a swollen basal hypha that is easiest to observe once the tip begins to degrade.

8. Acaulospora scorbiculata: Trappe Borne singly in soil, hyaline to light brown*, globose to* sub-globose, occasionally irregular, 100-240 µm in diam. Evenly pitted with depressions composed of 4 layers.

CONCLUSION

In the present study, effective results were observed when *Acacia nilotica* treated with Farmyard manure + *Glomus fasiculatum* and *Albizia lebbeck* with *Glomus fasiculatum* and Neem cake. These agronomic practices in nursery level showed the best results for the above tested two tree species. Future studies are to examine the best treatments of on-site field experiments in disturbed soils.

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