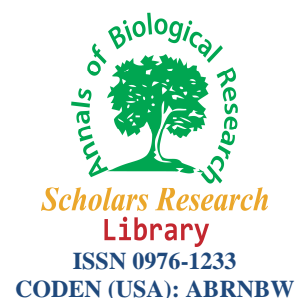




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Application of arbuscular mycorrhizal fungi to improve plant growth in *Solanum melongena* L.

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ABSTRACT

The present study was conducted to study the effect of indigenous *Glomus fasciculatum* as bio-inoculant in three different cultivars viz., Jyothi, Ankur and Manjirigutta of brinjal to evaluate the potential effect of AM fungi on the extent of root colonization, plant growth parameters such as shoot and root lengths, their fresh and dry weights, biomass and dry matter production and N, P, K status in pot experimental conditions. The three selected cultivars of brinjal showed positive response to AM fungal inoculation. The per cent AM fungal root colonization was maximum (48.6%) in Ankur variety. While, Jyothi and Manjirigutta showed 40.15 and 40.0% respectively. Plants inoculated with *G. fasciculatum* resulted in significant increase in plant height, shoot and root fresh and dry weights over non-mycorrhizal control plants. The cultivar Manjirigutta showed greater response to *G. fasciculatum* by recording maximum shoot fresh (63.7gm/plant) and dry weights (5.43gm/plant) followed by Jyothi and Ankur (61.83 and 56.30 gm/plant respectively) and (7.33, 7.03gm/plant respectively). Similar trend was observed with regard to root fresh and dry weight (8.88 and 1.87gm/plant) followed by Jyothi and Ankur (8.83, 0.75 and 8.38, 0.88gm/plant respectively). The analysis of variance for biomass and dry matter production in three selected cultivars showed statistically significant differences between groups, and between time intervals. Shoots and roots of AM inoculated plants recorded significantly ($p < 0.000$) higher N, P, K levels than control plants indicating that these three elements were efficiently transported in mycorrhizal inoculated plants. Phosphorus content increased more than two folds in mycorrhizal plants over non-mycorrhizal plants. The results of our experiment therefore proved that *Glomus fasciculatum* acted as a biofertilizer in three cultivars of brinjal in increasing the shoot and root biomass, dry matter production and nutrient uptake.

Key words: Biomass, dry matter, *Glomus fasciculatum*, nitrogen, phosphorus, potassium.

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi form symbiotic association between fungus and roots of plants and helps plants in increased absorption of nutrients and water uptake, through improved absorptive area, and translocation of elements to host tissues and their accumulation. Due to the unique ability of mycorrhiza to increase the uptake of phosphorus by plants, it is a potential substitute for phosphatic fertilizers. Brinjal (*Solanum melongena* L) is an easily cultivated plant. Its fruit and other parts of the plant are used in traditional medicine. Brinjal is one of the most common vegetables grown throughout the country. Brinjal is commonly considered as a vegetable, is cooked in various ways and used as a food item in every house. Apart from this, the fruit and other parts of the plant have good medicinal values. In traditional Chinese medicine, all parts of the plant are used to stop intestinal bleeding.

Brinjal occupies the third position amongst vegetable crops, according to market survey data of 2009-10 country wise, area wise, production (Economic Research Science, USDA, March 2012). China stood first with 55.17% of

the total world production. Second position is occupied by India contributing 30.09% to the total world production. The solution lies in developing and adopting hi-tech agriculture to improve the productivity in eco-friendly manner. Biofertilizers offer an economically attractive and ecologically sound means for improving the quality and quantity of crops.

Bio-fertilizers are less expensive, eco-friendly and sustainable. Mycorrhizal plants are often more competitive as they tolerate environmental stress better than the non-mycorrhizal plants. The beneficial effects of Arbuscular Mycorrhizae (AM) used as bio-fertilizers for plant growth, especially in soils of low fertility, are well documented. In the tropics, many crops are grown in infertile acid soils where, low levels of available phosphorus frequently limit their establishment. In such soils, an efficient mycorrhizal association can increase phosphorus uptake and crop yield. As a result, mycorrhizal plants frequently exhibit increased growth, yield and survival over non-mycorrhizal controls [1]. It is now recognized that AM fungi can be harnessed in order to improve productivity in agriculture, fruit culture, and forestry by reducing the input of fertilizers and/or by enhancing plant survival, thus offsetting ecological and environmental concerns. For this reason, studies on mycorrhizae gained importance due to its practical use as a low input technology for managing soil fertility and plant nutrition.

Therefore, present study is focused at improving our understanding on the effect of AM fungi as a bio-fertilizer. Hence, an attempt has been made to study the more commonly used vegetable crop brinjal and the effect of AM fungi on the extent of root colonization, plant growth and nutrient uptake using *Glomus fasciculatum* as bio-inoculant. Therefore, the objectives of the present study are focused to evaluate the potential effect of AM fungi on plant growth parameters such as shoot and root lengths, their fresh and dry weights, biomass and dry matter production and N, P, K status.

MATERIALS AND METHODS

Pot experiment was conducted under greenhouse conditions for a period of 60 days after transplantation to assess the effect of indigenous AM fungi *G. fasciculatum* on the growth of three different cultivars of brinjal using sterile alfisol soil in polythene bags. The three cultivars of brinjal viz., Jyothi, Ankur and Manjirigutta commonly grown, in and around Hyderabad were selected for the present study. They were surveyed for arbuscular mycorrhizal (AM) fungal association. The rhizosphere soil samples were collected from brinjal cultivated agricultural fields and AM fungal spores were extracted by wet sieving and decanting method [2]. Assessment of field soil samples of brinjal plants resulted in predominant occurrence of *Glomus fasciculatum*. These spores were collected and mass multiplied in pot cultures using sand and soil mixture in 1:1 ratio using sorghum as the host plant. As soon as 70 to 80% of infection is established, the root bits and soil sample mixture was harvested and used as inoculum. The inoculum consisted of spores, pieces of hyphae and infected root material. One gram of soil containing 20 spores and one gram of roots containing 70 to 80% root infection were the standard units used for inoculation of the pot experiments. [3] plant nutrient solution without phosphorus was added at regular intervals.

The soil used for pot experiments was analysed for soil texture, pH, water holding capacity and N, P and K status by following standard techniques. The plants received the following treatments: uninoculated control and *G. fasciculatum* (AMF) inoculated. Twenty two day old brinjal seedlings, grown in the nursery beds were transplanted into control and AMF inoculated polythene bags. Soil based inoculum of the bio-inoculant with root portions colonized by *Glomus fasciculatum* was placed 2cm below the roots of the seedlings so as to allow the roots to get in contact with the inoculum. Four seedlings were planted in each bag and three replicates were maintained for each treatment. The bags were watered as per the requirement. Hoagland nutrient solution was added at regular intervals of 15 days. Plants were maintained in green house chambers for a period of 60 days after transplantation.

Plants were uprooted on 30th and 60th days of growth period after transplantation. The following parameters were studied: per cent AM fungal root colonization, plant growth parameters such as shoot and root length (cm), their fresh and dry weights (gm/plant), biomass (g/day) and dry matter production (g/plant) and N, P, K status (mg/kg) were studied.

Percentage of mycorrhizal root colonization

On each sampling date the root samples of three selected test cultivars of brinjal were collected, washed thoroughly under tap water and suitably processed by clearing and staining technique [4]. The percentage of root colonization was calculated by morphometric technique [5].

Plant growth studies**Plant height**

On 30th, and 60th day of plant growth after transplantation, the height of the plants (shoot and root lengths) was recorded.

Plant fresh and dry weights

Shoot and root fresh and dry weights (shoot and root samples were oven dried at 70°C for 72 hours till constant weights were obtained) were recorded.

Dynamics of growth

The growth characteristics in respect of biomass increment and mean rate of dry matter production were calculated as:

Biomass increment

As an index of growth character, increase in biomass (W) was expressed in terms of dry weights [6].

Shoot and root biomass

The increase in shoot and root biomass was calculated using the following formula: $W = W_2 - W_1$ Where the subscripts 1 and 2 indicate the values of W on two occasions.

Rate of dry matter production (G) The mean rate of dry matter production is the mean growth rate G over an interval of time from D₁ to D₂ as given by:

$$G = \frac{W_1 - W_2 \text{ (grams per day)}}{D_1 - D_2}$$

Plant N, P, K status

Nitrogen (Kjeldahl method), phosphorus by colorimetric method (Fiske and Subbarow method) on spectroscopy (AnalytikaJena, Specord S600) and potassium by atomic absorption spectroscopic method (on Varian spectra AA220) were determined.

Statistical analysis

IBM SPSS version 19 was used for statistical analysis. The descriptive statistics like mean and SD were calculated for all parameters like shoot and root lengths, fresh and dry weights of shoot and root and biomass increase across groups, varieties and time points. Comparison of mean values across groups as well as time points were assessed with repeated measures ANOVA for given variety and experimental condition. T-test was also used for comparison of two mean values. Level of significance was considered as 0.05.

Results and Discussion

G. fasciculatum was observed to be predominantly occurring in the rhizosphere soils of brinjal cultivated in natural fields [7]. Indigenous AM fungi *G. fasciculatum* was grown in pot cultures and used as inoculum.

Physico-chemical factors of the Soil

The data of physico-chemical characteristics of the soil used for pot experiments is shown in Table 1. The soil pH was 7.2, moisture content 24%, phosphorus 3.3 mg/kg, potassium level 510mg/kg and nitrogen 710mg/kg.

Table 1. Physico-chemical factors of soil used for pot experiments

Moisture content (%)	pH	N (mg/kg)	P (mg/kg)	K (mg/kg)
24	7.2	710	3.3	510

Root Colonization

In the present study, the three selected cultivars of brinjal (Jyothi, Ankur and Manjirigutta) showed positive response to AM fungal inoculation. The data of our study clearly indicates mycorrhizal dependency of all the three brinjal cultivars studied. The per cent root colonization in AM inoculated plants of three selected cultivars ranged from 26.15 to 38% in 30 days and 40.1 to 48.6% in 60 days (Table 2) old crop. The per cent AM fungal root colonization was maximum (48.6%) in Ankur variety in 60 days crop. While, Jyothi and Manjirigutta showed 40.15 and 40.0% respectively. The uninoculated control plants of three cultivars did not show any AM fungal root colonization, as the mycorrhizal inoculum was not added to these pots. The per cent of root colonization in the three cultivars increased from 30 to 60 days period indicating increase in the percentage of AM root colonization with increase in age of the plant. The plants inoculated with AM fungi were colonized by abundant mycelium, vesicles and arbuscules (Fig. 1).

The increase in the percentage of root colonization with increase in age of the plant in all cultivars of brinjal with AM fungi inoculation is in agreement with the earlier reports [8, 9, 10, 11].

Table 2. Effect of *Glomus fasciculatum* on percentage of root colonization in Jyothi, Ankur and Manjirigutta cultivars

Treatment	Jyothi		Ankur		Manjirigutta	
	30 days	60 days	30 days	60 days	30 days	60 days
Control	-	-	-	-	-	-
AM	26.15	40.15	32.6	48.6	38.0	48.0

AM- *Glomus fasciculatum*



Fig. 1. Root colonization showing abundant mycelium, v- vesicles

Plant growth

Plant height

Glomus fasciculatum, the test inoculant was more effective in increasing the plant growth of three cultivars of brinjal (Jyothi, Ankur and Manjirigutta) in terms of plant height, fresh and dry weights. It is evident from the results that inoculation with *G. fasciculatum* resulted in significant increase in plant height over non-mycorrhizal control plants (Table 3 and 4). Among the three cultivars of brinjal, the variety Manjirigutta has shown maximum increase in shoot and root lengths followed by Ankur and minimum in Jyothi variety (Tables 3 and 4). Similar trend was observed with regard to root lengths. The analysis of variance for plant growth clearly indicates the significance between treatments, between intervals. The plant height increased with the age of the crop, similarly was percentage of AM colonization. These results agree with the earlier reports [12, 13, 14].

Table 3. Effect of *Glomus fasciculatum* on shoot and root lengths of three cultivars of brinjal – Jyothi, Ankur and Manjirigutta.

Treatments		Jyothi				Ankur				Manjirigutta			
		30 Days		60 Days		30 Days		60 Days		30 Days		60 Days	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	SL	10.43	0.287	12.00	0.141	12.02	0.160	14.27	0.175	10.03	0.186	10.88	0.640
	RL	4.00	0.141	4.75	0.123	5.98	0.256	5.97	0.103	3.28	0.256	7.07	0.197
AM	SL	15.00	0.179	12.48	0.172	13.40	0.141	14.85	0.235	13.50	0.894	16.07	0.207
	RL	4.48	0.172	6.82	0.232	5.50	0.141	6.33	0.320	3.78	0.109	7.10	0.200

AM- *Glomus fasciculatum*, SL- shoot length, RL- root length.

Fresh weights

The results of fresh weights of three cultivars of brinjal are presented in Tables 5 and 6. All the three selected varieties showed positive response to the test inoculants. There was a significant increase in the shoot and root fresh weights of mycorrhizal inoculated plants over uninoculated control plants. The cultivar Manjirigutta showed greater response to the test inoculant *G. fasciculatum* by recording maximum shoot fresh weights (63.7gm/plant) followed by Jyothi and Ankur (61.83 and 56.30 gm/plant respectively). Similar trend was observed with regard to root fresh weight.

Table 4. ANOVA showing effect of *Glomus fasciculatum* on shoot and root length (cm) of brinjal cultivars – Jyothi, Ankur and Manjirigutta

Treatments	Shoot length						Root length					
	Jyothi		Ankur		Manjirigutta		Jyothi		Ankur		Manjirigutta	
	'F' Ratio	p value	'F' Ratio	P value	'F' Ratio	p value	'F' Ratio	p value	'F' Ratio	p value	'F' Ratio	p value
Between Days	10826.27	0.000	5121.13	0.000	5626.32	0.000	1764.16	0.000	1131.49	0.000	3133.63	0.000
Between Treatment	17371.09	0.000	2886.13	0.000	3252.71	0.000	4378.39	0.000	27.41	0.000	100.04	0.000
Days & Treatment	2585.53	0.000	659.37	0.000	744.86	0.000	375.13	0.000	17.22	0.000	47.26	0.000

AM- *Glomus fasciculatum*, SL- shoot length, RL- root length.
p- level of significance at 0.05

Table 5. Effect of AM *Glomus fasciculatum* on shoot fresh weights (gm/plant) of brinjal cultivars – Jyothi, Ankur and Manjirigutta

Treatments	Jyothi		Ankur		Manjirigutta	
	30d	60d	30d	60d	30d	60d
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	18.80±.39	38.96±.05	16.18±.13	24.50±.06	21.95±.08	40.97±.05
AM	35.18±.31	61.83±.31	33.36±.23	56.30±.23	38.65±.32	63.7±.17
	Between Days	Between Treatments	Between Days	Between Treatments	Between Days	Between Treatments
'F' Ratio	136375.457	27166.828	2886.748	1965.888	33850.297	13841.42
p value	0.000	0.000	0.000	0.000	0.000	0.000

Table 6. Effect of AM *Glomus fasciculatum* on root fresh weights (gm/plant) of brinjal cultivars – Jyothi, Ankur and Manjirigutta.

Treatment	Jyothi		Ankur		Manjirigutta	
	30d	60d	30d	60d	30d	60d
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	3.17±.136	6.01±.117	2.93±.103	5.01±.133	4.76±.052	7.02±.117
AM	5.65±.055	8.83±.052	4.65±.054	8.38±.117	6.85±.055	8.88±.075
	Between Days	Between Treatments	Between Days	Between Treatments	Between Days	Between Treatments
'F' Ratio	6182.989	1479.705	9206.050	2979.799	10885.070	4504.267
p value	0.000	0.000	0.000	0.000	0.000	0.000

AM- *Glomus fasciculatum*, d- days

Dry weights

The mean and SD values of dry weights of shoot and root of the three different cultivars in brinjal by different days and groups are depicted in Tables 7 and 8. The three varieties across the days and groups are statistically significant. From, the tables 3 to 8, it is revealed that the data of plant growth in terms of shoot and root length, fresh and dry weights in different cultivars of brinjal showed significant increases in AM inoculated plants over controls. Further analysis showed progressive increase in plant growth from 30 to 60 days old crop. Our results are similar to the observations made earlier [15, 16, 8, 10, 11]. The results of our experiment therefore proved that *Glomus fasciculatum* acted as a biofertilizer in three cultivars of brinjal in increasing the shoot and root fresh and dry weights. The extent of root colonization varying with plant cultivar, also suggests a possible cause for difference in the amounts of benefits that the crop derives from such a symbiotic association. The increase in root colonization levels with increase in age of the crop can be attributed to the relationship between the degree to which a root system of a plant is colonized by AM fungi and the potential for the plant to benefit significantly from the symbiosis.

Table 7. Effect of AM *Glomus fasciculatum* on shoot dry weights (gm/plant) of brinjal cultivars – Jyothi, Ankur and Manjirigutta.

Treatments	Jyothi		Ankur		Manjirigutta	
	30 days	60 days	30 days	60 days	30 days	60 days
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	2.00±.089	4.48±.133	1.67±.046	3.02±.064	2.08±.075	2.08±.075
AM	4.25±.105	7.33±.081	4.03±.052	7.03±.082	5.43±.052	5.43±.052
	Between Days	Between Treatments	Between Days	Between Treatments	Between Days	Between Treatments
'F' Ratio	7631.296	2823.052	6769.225	5345.978	20761.068	6255.415
p Value	0.000	0.000	0.000	0.000	0.000	0.000

AM- *Glomus fasciculatum*, d- days

Biomass and dry matter production

G. fasciculatum inoculated plants resulted in better biomass and dry matter production than uninoculated control plants. It is evident from the tables 9 and 10, it is evident that *Glomus fasciculatum* favoured the growth of brinjal cultivars. The statistical analysis clearly indicates that the analysis of variance for biomass and dry matter production were statistically significant between groups, and between time intervals. Many researchers have established earlier

that the increase in biomass and dry matter production where mycorrhiza is involved [17, 18, 19]. Similarly, the biomass dry matter production also showed significant increase [20, 21] in *G. fasciculatum* inoculated plants.

Table 8. Effect of AM *Glomus fasciculatum* on root dry weights (gm/plant) of brinjal cultivars – Jyothi, Ankur and Manjirigutta

Treatments	Jyothi		Ankur		Manjirigutta	
	30 days	60 days	30 days	60 days	30 days	60 days
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD
Control	0.59±.007	1.05±.084	0.27±.007	0.70±.010	0.37±.005	0.81±.015
AM	0.31±.008	0.75±.005	0.51±.017	0.88±.007	0.87±.008	1.87±.052
	Between Days	Between Treatments	Between Days	Between Treatments	Between Days	Between Treatments
'F' Ratio	1906.214	361.249	19748.195	1991.835	9094.414	3891.535
p Value	0.000	0.000	0.000	0.000	0.000	0.000

AM- *Glomus fasciculatum*, d- days

Table 9. Effect of AM *Glomus fasciculatum* on shoot biomass and dry matter production in brinjal cultivars – Jyothi, Ankur and Manjirigutta

Treatments	Jyothi		Ankur		Manjirigutta	
	Biomass (g/plant)	Dry Matter (g/day)	Biomass (g/plant)	Dry Matter (g/day)	Biomass (g/plant)	Dry Matter (g/day)
Control	6.183	0.20	5.43	0.18	6.18	0.20
AM	6.71	0.22	6.71	0.22	6.71	0.22
Between treatments						
Frequency	1050.47	2550.00	44099.28	4752.27	542.61	1368.89
p-value	0.000	0.000	0.000	0.000	0.000	0.000

AM- *Glomus fasciculatum*, D₁, D₂ - 30 and 60days respectively; W₁, W₂ - Weight at 30 and 60 days respectively after transplantation

Table 10. Effect of AM *Glomus fasciculatum* on root biomass and dry matter production in brinjal cultivars – Jyothi, Ankur and Manjirigutta

Treatments	Jyothi		Ankur		Manjirigutta	
	Biomass (g/plant)	Dry Matter (g/day)	Biomass (g/plant)	Dry Matter (g/day)	Biomass (g/plant)	Dry Matter (g/day)
Control	0.52	0.16	0.52	0.01	0.53	0.01
AM	1.54	0.50	1.54	0.051	1.54	0.05
Between treatments						
Frequency	23506.48	2661.08	22943.96	2706.90	25331.35	2917.25
p-value	0.000	0.000	0.000	0.000	0.000	0.000

AM- *Glomus fasciculatum*, D₁, D₂ - 30 and 60days respectively; W₁, W₂ - weight at 30 and 60 days respectively

N, P, K status

N, P, K levels in mycorrhizal and non-mycorrhizal plants of the three different genotypes of brinjal are presented in Tables 11 and 12. Shoots and roots of AM inoculated plants recorded significantly higher N, P, K levels than control plants indicating that these three elements were efficiently transported in mycorrhizal inoculated plants. Phosphorus content increased more than two folds in mycorrhizal plants over non-mycorrhizal plants. It was also observed that there is an increase in N, P, K levels with an increase in plant age. High values of N, P, K in brinjal cultivars recorded at different intervals of crop growth clearly indicates the plant's enhanced capacity for absorption of nutrients by AM fungi. Our observations are in agreement with the earlier findings [8, 10, 11, 22, 23].

The analysis of variance showed that the values are highly significant. Thus, in the present investigation it has proved that the indigenous strain *Glomus fasciculatum* is a potential benefactor of all the three selected cultivars of brinjal. Similarly, significant increase in physical parameters and nutrient up-take was observed by earlier workers in mycorrhizal (AMF) inoculated plants of pigeon pea [24]; peanut [25], papaya [26], soybean [27, 28], tomato [29, 11] and sorghum [30, 17]. They observed that the AM fungi enhanced the plant height, fresh and dry weights, biomass and dry matter production over non-mycorrhizal plants and this is in agreement with our experimental data. In the present study, all the three selected cultivars of brinjal responded favourably to AM fungal inoculation showing positive responses to plant growth and nutrient uptake. From the data available from our study, we can say that AM fungi *Glomus fasciculatum* benefits the host plant brinjal under green house conditions by enhancing the plant growth in terms shoot and root lengths, their fresh and dry weights, biomass and dry weight production and N, P, K status.

Table 11. Effect of AM *Glomus fasciculatum* on NPK contents in shoot of brinjal cultivars – Jyothi, Ankur and Manjirigutta

Treatment	Nitrogen (mg/100 g)		Phosphorus (mg/100 g)		Potassium (mg/100 g)	
	Days after transplantation					
	30	60	30	60	30	60
JYOTHI						
Control	3.23	16.45	1.25	2.07	1.53	5.18
AM	15.22	36.53	1.73	3.32	1.83	7.03
Between groups						
Frequency	800390.666		1207.150		2827.533	
p-value	0.000		0.000		0.000	
ANKUR						
Control	3.72	18.55	1.23	2.33	1.62	5.24
AM	17.36	40.04	1.73	3.83	1.85	6.94
Between groups						
Frequency	1264440.071		450.000		204230.455	
p-value	0.000		0.000		0.000	
MANJIRIGUTTA						
Control	3.83	21.24	1.35	2.71	1.74	5.67
AM	18.71	41.72	1.85	4.18	1.87	7.11
Between groups						
Frequency	1232758.828		64413.991		35419.240	
p-value	0.000		0.000		0.000	

AM- *Glomus fasciculatum*Table 12. Effect of AM *Glomus fasciculatum* on NPK contents in root of brinjal cultivars – Jyothi, Ankur and Manjirigutta

Treatment	Nitrogen (mg/100 g)		Phosphorus (mg/100 g)		Potassium (mg/100 g)	
	Days after transplantation					
	30	60	30	60	30	60
JYOTHI						
Control	1.04	3.95	0.14	0.46	0.55	0.73
AM	3.83	10.11	0.74	1.85	0.79	2.08
Between groups						
Frequency	61345.365		53117.794		1071.889	
p-value	0.000		0.000		0.000	
ANKUR						
Control	1.08	4.12	0.15	0.61	0.57	0.82
AM	3.94	11.21	0.81	1.90	0.74	1.94
Between groups						
Frequency	1617870.727		84933.889		27267.936	
p-value	0.000		0.000		0.000	
MANJIRIGUTTA						
Control	1.14	4.31	0.81	0.78	0.52	0.76
AM	3.88	12.04	0.86	2.12	0.73	2.36
Between groups						
Frequency	2464900.000		27889.000		2421.235	
p-value	0.000		0.000		0.000	

AM- *Glomus fasciculatum***Acknowledgements**

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