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Research Article

COMPARATIVE STUDY OF LOCAL ARBUSCULAR MYCORRHIZAL FUNGAL SPORE CONTAINING SOIL ON THE YIELD OF MANGO GINGER

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ABSTRACT

Most of the terrestrial plant species are being infected by a large number of mycorrhizal fungi either ecto-mycorrhizae or endo-mycorrhizae. Different types of endo-mycorrhizae are present within the roots of herbaceous plants and arbuscular mycorrhizae (AM) are predominant within them. AM fungi play a vital role in symbiosis either in field condition or in experimental condition. In nature, rhizosphere soil of forest, degraded land, agricultural land and fallow land contain a good and diverse number of AM fungal spores throughout the year, though winter shows highest number of spore density. Application of chemical fertilizers and pesticides decrease the number of AM fungi in the soil hence deteriorates the quality of the soil as well as soil health. It is evident that soil inhabiting AM fungi enhance the growth and yield of plants and helps to combat with different pathogen infection and stresses. The rhizosphere soil with natural vegetation is a good repository of AM spores though agricultural land with huge application of chemical fertilizers and pesticides shows less number of AM sores. In this communication different forest soils, anthill soil, agricultural land soils and degraded land soil inhabiting AM spores were inoculated on Mango Ginger rhizome and the yield have been presented. Result showed anthill is a good repository of vivid AM spores in compare to other sites.

Keywords: AM fungi, forest soil, anthill soil, fungal bio-fertilizer.

1. INTRODUCTION

Vesicular Arbuscular (VA) mycorrhize play an important role to develop soil health and support plant growth in environmentally tuned condition. VA mycorrhizae play an essential role in nutrient transfer [1-4], soil aggregation [5], helps to obtain water, which is critical to plant survival and growth under dry condition [6], improvement of tolerance to drought and salinity [7-9] and plant protection against pathogen [10-15]. AM fungi play good role in soil health i.e. nematode control and significant nitrogen fixation by legumes via *Rhizobium* [16]. They also improve tolerance to heavy metals and soil salts [17, 18]. As a whole, mycorrhizae improve host plant photosynthesis and water status [19, 20], increasing adverse tolerance [21] and improving soil environment, fertility and soil quality [22].

VAM fungi acts as bio-fertilizer and have the unique ability to convert nutritionally important elements from unavailable to available form through biological processes [23]. Plants with VA-mycorrhizal association will have higher efficiency for nutrient absorption, such as nitrogen, phosphorus, potassium, calcium, magnesium, zinc and copper [24, 25]. At the same time VAM increases plant resistance to draught [26, 27]. Remembering these themes in mind the present study has been made to quantify the spore density in different soils and to know the yield of a plant under experimental condition. In this area rhizomatous plant *Curcuma amada* is important so it is included here to know the yield on green biomass basis after artificial inoculation directly with field soil in experimental condition.

2. STUDY AREA

The study site taken into account was *Sal* dominated dry deciduous forest of southwest Bengal. The altitude is 38.44m above mean sea level. The study area was situated in between 22° 01′ 55′′ N and 87° 10′ 41′′ E. The spots were Bhadutala, Lalgarh, Karnagarh, Nepura and Gopegarh. Temperature of the site is in between 12 to 38 degree centigrade and the average annual precipitation is 2120 mm.

3. MATERIALS AND METHODS

3.1. Sampling of rhizospheric soils

Available and easy to uprooting medicinal plants with their intact root inhabiting rhizosphere soil up to 10 cm depth was collected. Periodic survey was undertaken to study the seasonal variations of mycorrhizal fungi and status of the same. Based on the climate three seasons were taken namely monsoon (July, August, September and October), winter (November, December, January and February) and summer (March, April, May and June) for study of spore density in rhizosphere soil. Rhizospheric soil samples were collected in clean plastic carry bags with tag for each site. Each soil sample was spread on clean news paper and was allowed to dry in air under shade of net house. Pebbles and other unwanted matters were removed. Large lumps were broken with wooden roller. After grinding soil samples were sieved through sieves and fine soils were stored in clean plastic carry bag with tag for spore estimation. Collected field soil was directly used as substratum or culture soil for experimental design. Early summer soil was taken into account for experiment as well as for inoculums (Tables 1-7).

Table 1: Field 1-Soil of Bhadutala degraded land used as AM spore repository

Yield of *Curcuma amada* in experimental soil of Bhadutala degraded land, Paschim Medinipur							
Experimental pot no.	B1	B2	B3	B4	B5	B6	Cumulative weight of biomass
Initial rhizome biomass	12g	08g	07g	08g	09g	09g	53.0g
Yield of rhizome biomass	25.02g	16.11g	14.23g	15.15g	10.48g	8.73g	89.72g
Difference (Yield-initial biomass)							36.72g
% of yield on the basis of initial biomass							69.28%

Table 2: Field 2-Soil of Gopegarh forest used as AM spore repository

Yield of *Curcuma amada* in experimental soil of Gopegarh, Paschim Medinipur								
Experimental pot no.	G1	G2	G3	G4	G5	G6	G7	Cumulative weight of biomass
Initial rhizome biomass	08g	05g	07g	05g	07g	05g	30g	67g
Yield of rhizome biomass	39.42g	26.51g	31.04g	21.76g	27.97g	04.31g	51.89g	202.92g
Difference (Yield-initial biomass)								135.9g
% of yield on the basis of initial biomass								202.83%

Table 3: Field 3-Soil of Gopegarh anthill used as AM spore repository

Yield of *Curcuma amada* in experimental soil of Gopegarh anthill, Paschim Medinipur								
Experimental pot no.	GA1	GA2	GA3	GA4	GA5	G6	G7	Cumulative weight of biomass
Initial rhizome biomass	09g	08g	15g	06g	08g	-	-	46g
Yield of rhizome biomass	16.95g	19.66g	66.09g	33.97g	18.66g	-	-	153.33g
Difference (Yield-initial biomass)								109.33g
% of yield on the basis of initial biomass								237.67%

Table 4: Field 4-Soil of Lalgarh forest used as AM spore repository

Yield of *Curcuma amada* in experimental soil of Lalgarh forest, W.B.								
Experimental pot no.	L1	L2	L3	L4	L5	L6	L7	Cumulative weight of biomass
Initial rhizome biomass	05g	14g	04g	13g	19g	18g	12g	85g
Yield of rhizome biomass	30.31g	50.82g	18.05g	27.27g	49.14g	49.71g	15.68g	240.98g
Difference (Yield-initial biomass)								155.98g
% of yield on the basis of initial biomass								183.50%

Table 5: Field 5-Soil of Karnagarh Agricultural field (*Sesamum* field) used as AM spore repository

Yield of *Curcuma amada* in experimental soil of Karnagarh Agricultural field (*Sesamum* field)								
Experimental pot no.	KS1	KS2	KS3	KS4	KS5	KS6	KS7	Cumulative weight of biomass
Initial rhizome biomass	06g	08g	4.3g	3g	11g	13.5g	-	45.8g
Yield of rhizome biomass	2.46g	10.35g	17.30g	16.40g	27.87g	24.77g	-	99.15g
Difference (Yield-initial biomass)								53.35g
% of yield on the basis of initial biomass								117.64%

Table 6: Field 6-Soil of Karnagarh Agricultural field (Rice field) used as AM spore repository

Yield of *Curcuma amada* in experimental soil of Karnagarh Agricultural field (Rice field)								
Experimental pot no.	KR1	KR2	KR3	KR4	KR5	KR6	KR7	Cumulative weight of biomass
Initial rhizome biomass	04.5g	5.5g	6g	3.5g	1.5g	9.6g	-	30.6g
Yield of rhizome biomass	8.14g	8.07g	11.70g	8.02g	9.53g	7.17g	-	52.63g
Difference (Yield-initial biomass)								22.03g
% of yield on the basis of initial biomass								71.99%

Table 7: Field 7

Yield of *Curcuma amada* in experimental soil of Nepura Agricultural field (Home garden)								
Experimental pot no.	NH1	NH2	NH3	NH4	NH5	NH6	NH7	Cumulative weight of biomass
Initial rhizome biomass	2g	1.5g	1.5g	2g	1.3g	0.5g	-	8.8g
Yield of rhizome biomass	3.40g	6.72g	2.45g	5.28g	4.8g	0.15g	-	22.8g
Difference (Yield-initial biomass)								14.0g
% of yield on the basis of initial biomass								159%

3.2. Spore separation and spore density study

Separation of VA mycorrhizal spores from each soil sample was done by using wet sieving and decanting method. Prepared soil sample of 20g was taken and mixed with 500 millilitre tap water in a large beaker and stirred by glass rod until all the aggregates dispersed to leave a uniform suspension. After half an hour heavier particles would be settle down. Then the upper clear suspension was passed through stack of sieves, i.e. 710µm, 150µm, 75µm, 45µm and 32µm from top to bottom. Tap water was passed through the sieve stack several times for better result. The residues of respective sieves were collected in separate beaker. Then the aliquots were passed through filter paper placed in a glass funnel. To accumulate spores in a single circle clear water drops should be tickled through dropper. Now the filter papers were placed in wet Petri dishes and spores were counted and observed (Fig. : 8-9) through stereomicroscopes (×40) following Gerdemann and Nicolson [28]. Total spores were counted by adding the spore numbers of each respective filter paper spores. Spore density or spore number was calculated by counting the spores in the 20gm of soil. Spores were separated by fine wooden peg and mounted in lacto phenol for temporary work. Spores were mounted in Polyvinyl-alcohol-lacto-glycerol for permanent slide preparation and also for further work.

3.3. Identification of VA-mycorrhizal spore

Based upon hyphal attachment colour, size, shape, structure and compound microscopic character spores were identified. For identification and nomenclature INVAM's World Wide Web site at http:/invam.caf.edu was used. In this present study unexplored lateritic Sal dominated forest floor, degraded land, agricultural field and home garden were taken.

3.4. Experimental plant Mango Ginger

This plant is a popular medicinal pant in West Bengal. It used as vegetable and condiments widely in southwest Bengal. This plant has wide distribution in Indian sub-continent and in Southeast Asian countries. Botanically it is *Curcuma amada* under the family Zingiberaceae and usable part is rhizome. Herbaceous plants need minimum care for its cultivation. Rhizomes have aroma like green mango and popularly known as mango ginger. Medicinally it is important because it is a good appetizer, carminative and has properties like demulcent, aphrodisiac and laxative. Sprouting shoots are emerging out in the month of March. It may extend up to May. Maturity of yield takes place at the end of December.

3.5. Inoculation of plant by VA-mycorrhizal spore

Soil was collected from the field separately and stored in individual poly packs with tag. The 2 kg soil was used to fill 1/3rd of a poly pack. Sudan grass (*Sorghum* sp.) seeds and *Curcuma amada* rhizomes were surface sterilized with 0.5% NaOCl solution for 15 minutes. Surface sterilized *Curcuma amada* rhizome (Specific size with sprouting shoot) and sorghum seeds both were placed just over the soil substratum. It was covered by a thin film of dry soils. The pots were watered with sterile distilled water as and when required. The pot culture was maintained up to 120 days and intermediate samplings were done to confirm the root infection. Similarly spore density was recorded from the culture soil to know the spore population. After one month of culture shoots of sudan grass were chopped out. After 120 days the leaf portion of *C. amada* plant was discarded and entire root system was chopped off only rhizome portion was taken for study. Rhizome was cleaned with water and kept aside for air drying. After two days air dried rhizome was weighted. Initial weight of each rhizome was recorded and final weights of respective samples were recorded to calculate the % of yield.

3.6. Duncan's Multiple Range test

To analyze the data available from experiment, the Duncan's Multiple Range Test was used. Statistical software DSAASTAT was used to calculate the different significant levels at the probability level a, b, c, d and so on. The tabled value has been compared with estimated value.

4. RESULTS AND DISCUSSION

Present study showed different percentage of yield in term of green biomass of *Curcuma amada* rhizome (Fig.: 10) in experimental condition. Here, Bhadutala degraded land soil showed 69.28% yield whereas forest soil of Goopegarh and Lalgarh showed 202.83% and 183.50% yield respectively. Anthill soil of Gopegarh showed 237.67 % yield (Table 8). Agricultural land soils from Karnagarh *Sesamum* field and rice field showed 117.64 % and 71.99 % yield respectively. Nepura garden soil showed 172.89 % yield. From the present study it revealed that anthill soil showed better result and stood first among the 7 study site soil. Gopegarh and Lalgarh forest soils stood 2nd and 3rd position

whereas, Nepura garden soil stood 4th position in terms of % of yield of green biomass. Karnagarh *Sesamum* agricultural field soil and rice field soil stood 5th and 6th position in terms of yield respectively. Bhadutala degraded land soil showed least amount of yield among soils of above 7 study sites.

It is observed that spore density in experimental soil cum field soil showed highest spore density in case of Gopegarh forest soil (138.66 per 20g soil) followed by Lalgarh forest soil (125.2 per 20g soil) and anthill soil of Gopegarh (94 per 20g soil). Other sites like Nepura, Karnagarh (*Sesamum*) and Karnagarh (Rice) and Bhadutala (degraded) soils showed 69, 68.2, 60.4 and 57 numbers of spores per 20g rhizosphere soil (Table 8). From this study it is revealed that anthill soil showed highest yield but in terms of spore density it stood 3rd position among the 7 study soil samples (Fig. 1).

Table 8: Comparison of yield of *C. amada* after experimental maturation

Sr. No.	Comparative study on *Curcuma amada* yield in experimental condition from 7 different sites rhizospheric soil					
	Site Name	Initial wt. in g (W1)	Final wt. in g (W2)	Difference (W2-W1) g	% of yield	Spore density /2 kg soil
1.	Bhadutala (B)	53.8	89.72	36.72	69.28[f]	5700[g]
2.	Gopegarh (G)	67	202.9	135.9	202.83[b]	13866[a]
3.	Gopegarh anthill (GA)	46	153.33	109.33	237.67[a]	9400[c]
4.	Lalgarh (L)	85	240.98	155.98	183.50[c]	12520[b]
5.	Karnagarh S (KS)	48.5	99.15	53.53	117.64[e]	6820[e]
6.	Karnagarh R (KR)	30.6	52.63	22.03	71.99[f]	6040[f]
7.	Nepura (N)	8.8	22.8	14.0	159[d]	6900[d]

Note: Duncan's multiple range test for % of yield and for spore density/2 kg soil

MULTIPLE COMPARISON TEST

Procedure: Duncan's multiple range test (p= 0.05)

S.E.M.: 4.46206230346462; DF: 30

Critical range; 0; 12.895; 13.565; 13.922; 14.279; 14.502; 14.68

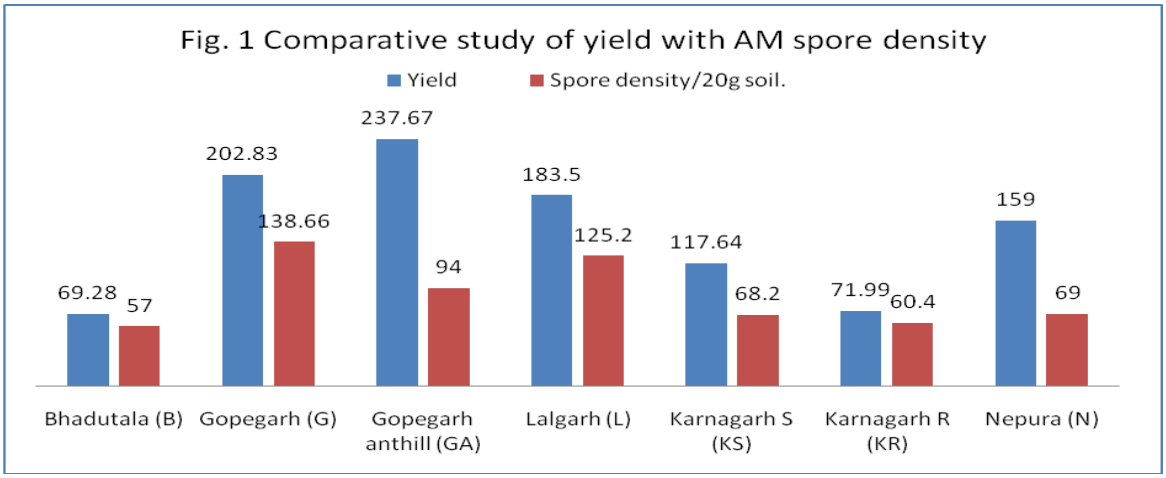

Fig. 1: Yield of *Curcuma amada* after inoculation with local AM fungi containing soil from different regions

The reason may be due to vivid AM spores (Fig. 2-7) which are very prone to infect the host rootlets. Home garden soil of Nepura is a good substratum for nutrients as it is an organic garden so the soil is rich in organic carbon and other nutrients which helped the rhizome production during the experimental period. So, it stood 4th position in terms of yield and very closely related to the soil activity of Gopegarh and Lalgarh soil. Two agriculture fields from Karnagarh sites are different in terms of yield and spore density. It may be due to rice field which is due to cultivation of high yielding rice variety that needs heavy application of chemical fertilizers and pesticides that affect the soil health and destroying the natural AM fungal community.

Fig. 2: ***Acaulospora laevis***

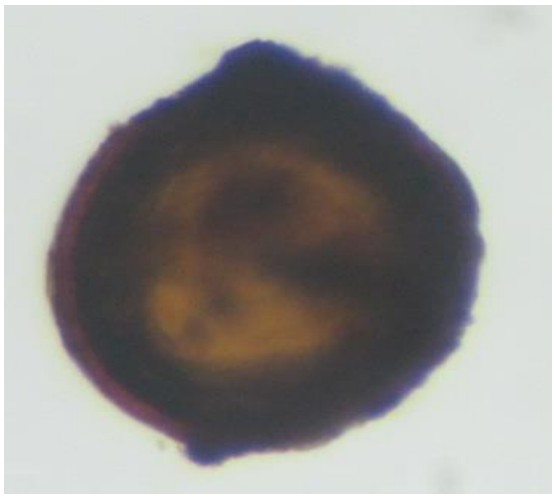

Fig. 5: ***Glomus*** **sp.**

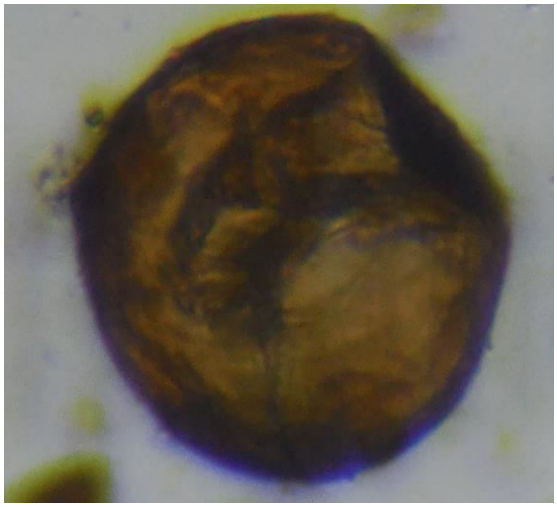

Fig. 3: ***Gigaspora*** **sp.**

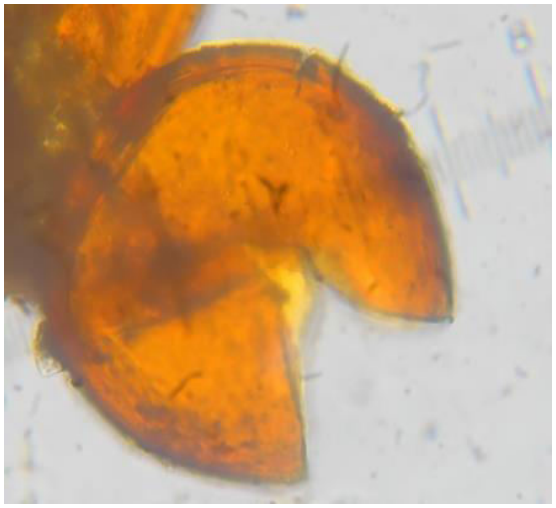

Fig. 6: ***Glomus*** **sp.**

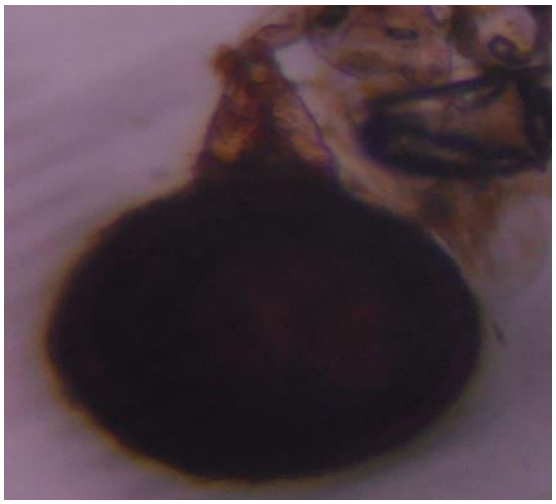

Fig. 4: ***Glomus*** **sp.**

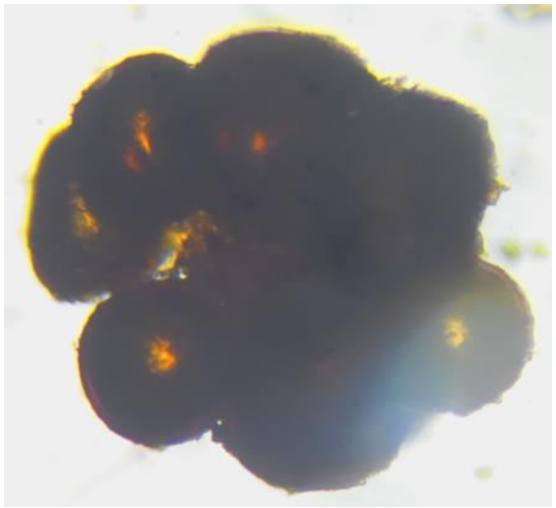

Fig. 7: Sporocarp of AM fungi

Fig. 8: Sieving technique adopted for AM study

Fig. 9: Final filtration for AM spore

Fig. 10: *Curcuma amada* rhizome in experiment

Agriculture *Sesamum* cultivation field soil needs less fertilizers and less pesticide in addition to organic manure or cow dung manure to grow it. Bhadutala degraded land is lateritic type and less water holding capacity with less number of weeds that support less number of AM spores to grow. In this result Bhadutala soil and Karnagarh rice field soils are more nearer in terms of yield and spore density. It may be stated that gradual increase in fertilizers and chemical pesticides may harm the soil and the cultivated land will be converted like degraded land in near future. The present work has been supported by Madawala [29] and argued that AMF bio-fertilizers have the greater potential to improve initial growth and establishment of tree seedlings in restoration projects.

5. CONCLUSION

Natural soils are good source of repository for AM spores. Anthill soils are good source of vivid AM spore for further culture and preparation of inoculua. Degraded soils and agricultural soils are with less number of AM spores due to scarcity of natural host plants and applications of chemical fertilizers as well as pesticides. AM culture may be used to artificial inoculation of degraded land and plantation programme that may be successfully raised after inoculation in nursery through local AM fungi. In agricultural practice, bio-fertilizers like AM fungi may be used with other bio-fertilizers as well as manures that can restore the soil health in near future rather than heavy and random chemical fertilizer use. AM fungal bio-fertilizer production is not costly and laborious but eco-friendly. Farmer and other plant growers can use and upgrade the application in field for better yield, though the lack of knowledge about AM bio-fertilizer production practice and application is the main hindrance behind it.

Conflicts of interest

Conflicts of interest is none

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