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Morphological Identification of Vesicular-Arbuscular Mycorrhiza as A Biofertilizer On Bulbous Plants

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Abstract

This study was conducted to investigate the morphological identification of vesicular-arbuscular mycorrhiza (VAM) on bulbous plant. Bulbous roots were taken from the rhizosphere of bulbous plant. The soils were analyzed for the number of VAM spores, chemicals and physical properties. In addition, the roots were examined for infection levels, and morphological identification of VAM spores made. In the recent past, the use of chemical fertilizer in agriculture has substantially increased throughout the world. But, because of over demand of fertilizers, increase in cost of energy, the cost of fertilizers, both in terms of currency and energy, has been rising tremendously and will continue to rise. Moreover, excessive use of fertilizers is leaving bad to worse impacts on the soil and water body environment. Therefore, the concept of using VAM fungi as a bio-fertilizer, in terms of cost effectiveness, energy saving and as environment friendly, is a promising perspective. Mycorrhizae are the root-symbionts which obtain their nutrients from the plant and provide mineral elements like N, P, K, Ca, S and Zn to the host plant. We also determined the capabilities of AMF to increase the productivity of cereal crops, fruits and vegetable crops and highlighted future research directions in mycorrhizal technology.

Keywords

Vesicular-Arbuscular Mycorrhiza(VAM), *Zea mays var. saccatrata*, Bulbous plant (*Alliumcepa*), Pot culture.

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INTRODUCTION

Arbuscular mycorrhizal(AM) fungi are ubiquitous obligate mycobionts forming symbiosis with the terrestrial plant communities. The mycosymbionts are widespread among both cultivated and wild plants. So far more than 170 species of AM fungi have been

recorded and described and many more still awaits discovery. The role of mycorrhizae in plant development pertains to mineral nutrition especially the uptake of phosphate. This effect has been attributed to an increase in the absorbing surface and exploitation of the larger soil volume by the extra radical mycelium, the small hyphal diameter leaded to an increased phosphate absorbing surface area and compared to non-mycorrhizal roots, higher phosphate influx rate per surface units, the formation of polyphosphate by mycorrhizal fungi and thus low internal phosphate concentrations, and the production of organic acids and phosphatases, which catalyze the release of phosphate from organic complexes. The symbiotic arbuscular mycorrhizal (AM) fungi develop on extensive hyphal network and provide water and nutrients to plants. Soil microorganism can influence the soil structure and play important role for AM fungi colonization in roots. The word Mycorrhiza is derived from classical Greek word for "mushroom" and "root". In a mycorrhizal association, the underground mycellium are in contact with plant roots, but without causing any harm to the plant. Fossil evidence [1] and DNA sequence analysis [2] suggest that this mutualism appeared 400-460 million years ago. Vesicular arbuscular mycorrhizal fungi belong to the class Zygomycetes, order Endogonales [3] and family Endogonaceae. Mycorrhizal fungi are responsible in improving growth of host plant species due to increased nutrient uptake, production of growth promoting substances, tolerance to drought, salinity and synergistic interactions with other beneficial microorganisms [4]. The soil conditions prevalent in sustainable agriculture are likely to be more favorable to AM fungi than are those under conventional agriculture [5]. The AM fungi are widely distributed in natural and agricultural environments and have been found associated with more than 80% of land plants, liverworts, ferns, woody gymnosperms and angiosperms and grasses [6].

Effect of plant growth in bio-fertilizer

The *zea mays var. saccharata* growth was tested by using different types of fertilizer applications in laboratory to evaluated the biofertilizer potential in plant growth.

1. Soil (control)
2. Chemical fertilizer (Di-Ammonium Phosphate)
3. VAM spore fertilizer

MATERIALS AND METHODS

Collection of sample:

Occurrence of Arbuscular Mycorrhizal (AM) fungi association was investigated on the experimental plant such as *Zea mays var.saccharata*. The soil samples were collected from the root region of *Azadirachtaindica*plant in the campus of Hindusthan collage of arts and science, Coimbatore.

Isolation and quantification of am fungal spores:

The AM fungal spores were separated from the soil by wet sieving and decanting technique. Fifty gram of rhizosperic soil sample was mixed in 200 ml of distilled water in a large beaker. After 1 hour the contents of the beaker were decanted through the sieves which were arranged in a descending order from 400 µm to 60µm size. The process was repeated for thrice. The procedure was repeated until the upper layer of soil suspension is transparent. The retained material on the sieve was decanted into a beaker with a stream of water and estimation of spores was carried out by modified method of Gaur and Adholeya [7]. A circular filter paper was taken a folded into four equal quadrants. The paper was reopened; two lines were drawn along the two folds to divide the filter paper into four equal quadrants. Vertical lines were drawn on one half of the filter paper so as to divide into approximately 20 columns about 0.5 cm apart. Each column was then numbered and the direction of counting was marked by an arrow. The filter paper was then folded in such a way that the marked portion becomes the receiving surface for the sample during filtration. This filter paper along with sample spores was spread in a bigger petri dish. The petri dish was observed under stereo binocular microscope. Two lines were focused in the field and moving the petri plate, the spores were counted in every space between the two lines and since the lines were numbered and the direction was set, it was easy to keep track of each spore on the filter paper.

For the identification of AM fungal spore, single spore or sporocarps were easily picked up from the filter paper with the help of syringe or fine point camel brush and mounted on a glass slide with a drop of polyvinyl lacto phenol (PVL) and a cover slip was placed. Subsequently, recovered spores were identified with the help of manual and different taxonomic keys proposed by different workers. The following characters are considered for identification sporocarps, spore morphology, size, shape, sporocarps

color, wall ornamentation, subtending hyphae and mode of attachment. Some of the important and selected spores were recorded and documented in form of photographs.

Assessment and quantification of AM fungi of *Zea mays var. saccharata*

Roots of *Zea mays saccharata* were first washed thoroughly in distilled water and then placed in 10 per cent KOH and heated to 90 °C for min. They were then washed in distilled water and immersed in alkaline 3 per cent H_2O_2 for 5-10 min. Then they were washed in distilled and acidified with 5 N HCl for 2-3 min. The roots were stained with 0.05 Per cent trypan blue in lacto phenol for 15-30 min and the excess stain was removed with clear lacto phenol [8].

Temporary mounts of root segments of test plants separately with gentle squashing on slides containing acetic acid: glycerol (1:1 v/v) solution were prepared and the coverslips were sealed with nail polish They then observed under a compound microscope using different magnification for AM fungal structures. The percentage of root colonization of test plants were estimated [9].

Estimation of AM fungi

Isolation and identification of AM fungal spores and sporocarps

Spore population of each soil sample of both test plants were separately estimated by a modified wet-sieving and decanting technique [10]. Hundred grams of composite soil sample suspended in 500 ml of water and the soil particles were allowed to settle down for 5 minutes. The suspension was passed through 180 and 38 um sieves. The residues from 38 um sieves were suspended in water, then loaded in a burette and left undisturbed for 5-10 minutes. The soil particles settled at the bottom were removed by opening the stopper for few seconds. The spores in the soil suspension were collected using filter paper. Repeated washing of the inner surface of burette wall with a wash bottle was done to collect the rest of the spores. The filter paper was spread over a glass plate and the spores were counted under an appropriate magnification (x 100) of a compound microscope. Variously coloured, 2 mm spaced grids drawn on filter paper facilitate easy counting. The spore population was expressed as the number individuals per gram of dry soil. Intact spores were mounted with PVA (Polyvinyl alcohol) and identified using the synoptic keys of Schenck and Perez [11].

The term frequency was used to assess the establishment and survivability of AM fungi in the rhizosphere of the host. Frequency denotes the number of samplings in which spores of a particular AM fungus present during the study period and expressed as percentage [12].

AM fungal inoculum preparation

Onion (Allium cepa plants were used as a host for AM fungal inoculum preparation. Two dominant indigenous AM fungi viz. *Glomus mosseae* and *Gigasporaalbida*, isolated from root zone soils were used for inoculum production isolated from root zone soils of *Zea mays var saccharata* were used for inoculum production separately (late Ivy. Glass funnels of 5 cm dia were filled with sterilized soil sand (1:1) mixture. Spores were surface disinfested with Chloramine-T (2%) and 50 200 spores each were layered on the soil sand mixture using funnel technique. Two onion bulbs were sown on each funnel; onion plants were transplanted from funnel to pots after 20 days of germination small pots of 18 cm dia x 15 cm height were filled with sterilized soil mixture. Onion plant from the funnels were transplanted to pots. The pots were kept in green house (30 °C) and watered regularly. The infectivity of onion roots by the AM fungi were checked interval of 15 days. After 3 months, the pot cultures were harvested by pruning onion plants to the soil level. The soil mass was removed from the pot and the mycorrhizal roots were chopped into small pieces.

Spore development in plant:

The VAM spore are mostly interact with the bulbous root. The bulbous plant *Allium cepa* was cultivated in the laboratory for the mass production of VAM spores. Using 2kg of soil 300 spores was placed in to the *Allium cepa* plant bag. After the mass multiplication of plant, the bulbous plant root was cutted and stored. Then the cutted roots was surface sterilization by 0.1% mercuric chloride. The *Zea mays var. saccharata* was chosen, where the isolated VAM spores were used as the bio fertilizer.

RESULT AND DISCUSSION

Spore separation from wet sieving method

Many natural and manufactured materials occur in a disperse form, which means that they consist of differently shaped and size particles. The particle size distribution the number of particles different size, is

responsible for important physical and chemical properties.

Spores were separated from the soil by wet sieving and decanting technique. Fifty gram of Rhizosphere soil sample was mixed in 200 ml of distilled water in a large beaker. After 1hours the contents of the beaker were decanted through the sieves which were arranged in a descending order from 400 µm to 60 µm size.

Microscopic identification and Spore morphology

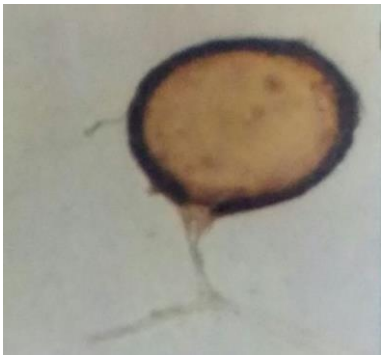


Fig: 1*Glomus mosseae*

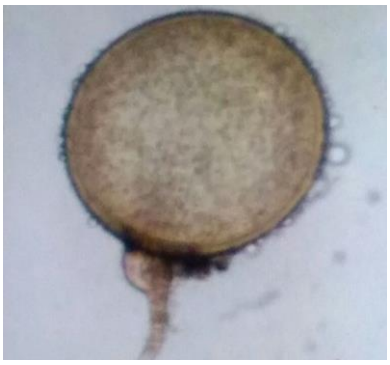


Fig: 2*Gigasporaalbida*

In Fig:1Thespore rarely filled with hyphae, sporocrop containing 1-10 spore diameter of sub tenting hypha at widest part 18-50µm, outer surface of inner wall is not ornamented; thin, hyaline outer line may not be obvious. Spore are 100µm in size, sub tenting hypha is generally funnel-shaped with cup shaped septum.

In Fig: 2 Acream pale green tint shape was globose to suglobose, size was 250µm. intraradical arbuscules and hyphae consistently stain darkly root treated with trrypan blue. Produse fine branches by swollen base hyphae easiest to see as tips degrade. Intraradical hyphae 3-8 µm in diameter, with inflated areas up to 10 µm and knob like projections distributed along length, usually coiled near entry points.

Based on the spore morphology the spores were identified as *Glomus mosseae, Gigasporaalbida.* *Glomus* was the dominate genus found in the root zone soils.

Mass inoculum Development

Glomus mosseae and *Gigasporaalbida* were isolated and identification they were used for inoculums production. The bioassay was conducted using onion as the test plant to study the infection, hence these two AM fungal strain were used for mass inoculums production.

Initially the *Alliumsepa* were grown using funnel culture technique and after 7 days there were transplanted to the pot soil which was inoculated with 300 spores for 2 kg of soil and it was allow to grow for 30 days and a control was maintained without AM fungi (Fig 4 and 5).

Fig: 3Funnel culture technique using *Allium cepa*

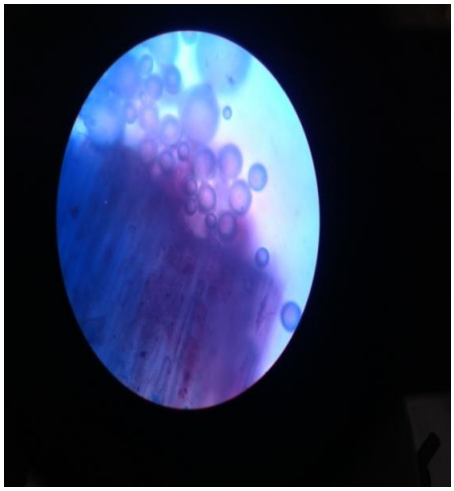
Fig: 4 Mass multiplication of bulbous plant in pot culture (*ALLIUM CEPA)*

Plant inoculated with AM fungi was significantly shows higher precent root colonization, extrametrical spore count, shoot and root length, shoot and bulb biomass than the control which is used without AM fungi which is shown in Fig:6 and Fig:7.

The infected plant was stained with Lacto phenol to check the efficiency and the mass multiplication of the AM fungi and it was found that this two organism shows the promising growth (Fig:8).

Fig: 5 *Allium cepa* with AM fungi

Fig: 6 *Allium cepa* with AM fungi



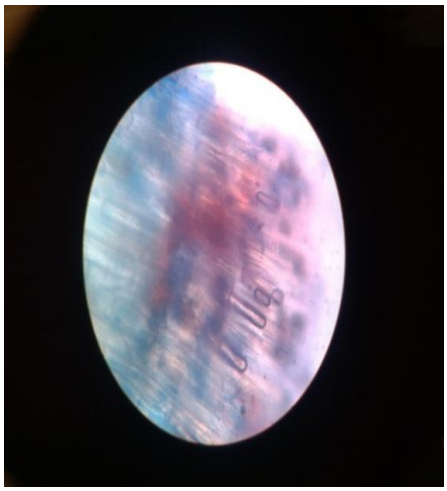


Fig: 7 AM spore colonization in A*llium cepa*root

Influence of growth using biological and chemical fertilizer in zea mays

A pot trial was conducted in the Department of Microbiology, Hindusthan College of Arts and Science, to study the influence of AM fungi on growth and biomass production of *Zea mays* separately in sand: soil 3:1 ratio with P_H 7.

Two AM fungal species isolated from rhizospore soil. *Glomus mosseae, Gigasporaalbida* was found to be colonized in the roots of *zea mays var. saccharata.* The extra metrical phase of the AM fungi differed greatly. The extrametrical mycelium with variable number of entry points were observed in the roots of *zea mays var. saccharata* which is shown in (Fig:8, 9 and 10).

Fig: 8 Influence of *AM on zea mays*

Fig:9 Influence of DAP on *zea mays Saccharata* (bio-fertilizer) *Saccharata* (chemical fertilizer)

Fig: 10 *Zea mays sachharata* Without fertilizer

Colonization of AM fungi in *Zea mays. Saccharata* roots

The roots of *Zea mays var. saccharata* with AM spore shows more colonization than the roots with chemical fertilizer and the control without fertilizer which is shown in fig:11 and table:1.

Fig:11. *Zea mays var. saccharata* roots with AM, chemical fertilizer and without fertilizer

Table:1. Root and Shoot length with AM, chemical fertilizer and without fertilizer

Plant length	VAM spore (Biological fertilizer)	Chemical fertilizer	Control
Root length	1 m	80 cm	32 cm
Shoot length	44 cm	36 cm	25 cm

Hence the mycorrhizal inoculation resulted in significant increase in shoot and root compared to uninoculated control plant and plant with chemical fertilizer.

We also determined the capabilities of AMF to increase the productivity of cereal crops, fruits and vegetable crops and highlighted future research directions in mycorrhizal technology [13].

CONCLUTION

In recent year due to over exploitation of natural resources, bio-fertilizer are emerged as an important component of integrated plant nutrient supply synthesis hold a promise for reducing the cost, improve the crop yield, quality nutrient supplies and sustaining the productivity over a longer period. Hence the present study was undertaken to isolate and identify the AM fungi using wet sieving and decanting technique and they were multiplied in the roots of onion (*Allium cepa*) by funnel culture and pot culture method. A pot trial was conducted to study the effect of AM fungi in zea mays var. saccharata. This study result would become a great boon to agriculturists as it can minimize the requirement of expensive phosphate fertilizer.

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