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Isolation and Identification of vesicular Arbascular Mycorrhizae (Vam) using it as a biofertilizer

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Abstract
Bio-fertilizers are the organic substances which make use of microorganisms to increase the fertility of soil. These fertilizers are not harmful to crops or other plants like the chemical fertilizers. They are actually taken from the animal wastes along with the microbial mixtures. Microorganisms are used to increase the level of nutrients in the plants. They let the plants grow in a healthy environment. Mycorrhizal plants exhibit greater uptake of phosphorus and trace elements when these nutrients are sparingly soluble in soils (Powell and Bagyaraj, 1984; Abbot *et al*., 1992). They can also show improved resistance to drought, environmental stress and also fight against the root borne pathogens (Allen and Boosalis, 1983).
Our isolates shown good growth rate compare to the control, by this experiment we can show our cultures are good biofertilizers. Arbascular Mycorrhizal fungi are ubiquitous in soil habitats and form beneficial symbiosis with the roots of angiosperms and other plants.

Keywords: Non-formal education, illicit drugs, antidote

Introduction

The main sources of bio- fertilizers are bacteria, fungi, cyanobacteria, etc. Such bio-fertilizers are cultured and are used for inoculating seed or soil or both under ideal conditions to increase the availability of plant nutrients. Among this mycorrhizae is an important one in agriculture field for the cultivation of many crops.
Mycorrhizae are highly evolved and have non-pathogenic symbiotic association between roots of most vascular plants and cortical tissues of roots during periods of active plant growth both in natural environment and in cultivation.In the tropics, many crops are grown in infertile acid soils, where low level of available phosphorus frequently limits their establishment. In such soils, an efficient mycorrhizal association can increase phosphorus uptake and crop yield. Mycorrhizal plants exhibit greater uptake of phosphorus and trace elements when these nutrients are sparingly soluble in soils (Bagyaraj, 1984 [7]; Abbot *et al*., 1992). They can also show improved resistance to drought, environmental stress and also fight against the root borne pathogens (Allen and Boosalis, 1983) [2].
It is now recognized that VAM 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.

- Since a biofertilizer technically contains a living organism, it can symbiotically associate with plant roots.
- Microorganisms can readily and safely convert complex organic material into simple compounds, so that they are easily taken up by the plants.
- Microorganisms function in long duration, causing improvement of the soil fertility.
- It maintains the natural habitat of the soil.

It increases crop yield by 20%–30%, replacing chemical N and P, thereby stimulating plant growth. It can also provide protection against drought and some soil-borne diseases.

Role of VAM fungi in Soil Fertility

The most significant threats to soil function at the global level are soil erosion, soil organic carbon, excessive use of input, and nutrient imbalance. The depletion of soil fertility in the world has increased due to unsustainable land management practices, such as overgrazing, bush burning, continuous crop cultivations, and tillage practices. However, inoculation with Arbuscular Mycorrhizae Fungi (AMFs) has been identified as an eco-friendly approach to improve soil fertility. AMF is the most widespread soil microorganisms that form a symbiotic relationship with more than 80% of plants, except for a few plant families,

Materials and methods

1. Soil sampling

Soil samples were collected from the college premises Samples were withdrawn at a depth of 10–15 cm below the surface, and collected into sterile polyethene bags then stored at 4°C.

2. Isolation of VAM spores

Collected soil sample from college is mixed in lot and weigh 20gm of soil. Transfer the sample into a blender. Blend it at high speed for 1 to 2 minute so that the Spores attach to the soil particles or roots of Spores carp may become free. Filter the content through a fine sieve and wash with strong steam of water.Pour 10ml of 20% sucrose into a centrifuge tube followed by the same amount of 40% and 60% of Sucrose solution into the bottom of the tube. Then 10-15 ml of blended sieve and add onto the surface of 20% Sucrose layer. Centrifuge the contents for 3 minutes at 3000 rpm there after remove the debris which accumulates at the interfaces of the 20-40% of Sucrose 40-60% of solution. Gently wash the Spores present on the fine sieve

with a strong steam of water so that the Sucrose should be removed. Collect the Spores and observe under microscope.

3. Screening of VAM Spores
The isolated VAM Spores are stained with Lactophenol Cotton Blue Stain and observed under microscope.

Mass Cultivation of VAM Spores
The Arbascular Mycorrhizae Fungi are not host specific, any plant species can be infected by an Arbascular Mycorrhizae Funagal species but the degree of Arbascular Mycorrhizae infection and its effect can differ with different host endophyte combinations.Cultures of Arbascular Mycorrhizae Fungi on plants growing in disinfected soil have been frequently used technique to increase propagule numbers. A highly susceptible host plant should be used. It should produce abundant roots quickly and tolerate the high light conditions required for the fungus to reproduce rapidly. Plants with mycorrhizal associations predominate in most natural eco-systems, so inoculums of mycorrhizal fungi is present in most soils. The quantity of inoculum of Arbascular Mycorrhizae fungi were compatible with a host plant in soils can be measured by bioassay experiments.

VAM Spore
A mycorrhiza is a symbiotic association between a fungus and the roots of a vascular plant. In this association, the fungus colonizes the host plant's roots, either intracellular as in arbuscular mycorrhizal fungi or extracellularly as in ectomycorrhizal fungi. They are an important component of soil life and soil chemistry.In the symbiotic associations of plant and fungi, arbuscular mycorrhizae, which is formed between plants and Glomeromycota fungi, has the widest distribution in the nature. VAM fungi inhabit a variety of ecosystems including agricultural lands, forests, grasslands and many stressed environments, and colonize the roots of most plants, including bryophyte, pteridophyte, gymnosperms and angiosperms. such as Amaranthaceae, Brassicaceae, Cruciferae, Chenopodiaceae, Caryophyllaceae, Juncaceae, Cyperaceae, and Polygonaceae, which do not exhibit any association (Bartlett, 1959) [4]. They can be found in various ecosystems worldwide. AMF is a key component of soil microorganisms and belongs to the glomeromycota phylum. Several studies have reported that they play a crucial role in Plant resistance against biotic and abiotic stresses. This review aims to summarize knowledge about AMF symbiosis, in particular, the beneficial effects on soil (Figure 1). First, the role of AMF in the physical, chemical, and biological properties of the soil is considered. The contribution of AMF in soil aggregation, nutrient availability, and boosting beneficial soil microorganisms is discussed. Finally, the role diversity of interactions between AMF and other soil microorganisms is examined.

Results & discussions
1. Collection of soil sample
Soil samples were collected from college premises. Samples were withdrawn at a depth of 10–15 cm below the surface, and collected into sterile polyethene bags, then stored at 4°C.

2. Isolation of VAM SPORES:
Floatation method was performed and VAM Spores are observed under microscope.

3. Morphological Characterization Study
The isolated VAM Spores were observed under microscope by performing staining technique, and the spores are Round and Irregular shapes.

4. Identification of VAM Spore isolates
Based on the morphological and microscopic observation, it was tentatively identified as VAM Spore.

5. Mass Cultivation of VAM Spores
The isolated VAM Spores are used as an inoculation separately in the broth (250ml) and inoculated until the number of cells or sufficient. And incubated for 2-3 days at room temperature.

Result and Discussion
VAM spores proven to be an effective biofertilizer as it has many importance towards the plant growth. It also increases the soil fertility by degrading pesticides, hazardous chemicals and organic material these degradation helps in humus production which alternatively increases soil fertility and water holding capacity of the soil Use of biofertilizer also maintains the ecological balance of the soil microorganisms.

		MOONG DAL		CHICKPEA		GROUNDNUT	
DATE	DAY	CONTROL	COATED	CONTROL	COATED	CONTROL	COATED
10/09	1	----	---	----	----	-----	-----
11/09	2	2cm	4cm	1cm	3cm	---	----
12/09	3	3.5cm	6cm	3cm	4.5cm	---	----
13/09	4	5cm	8cm	4.6cm	5.8cm	---	---
14/09	5	7.5cm	10.5cm	5.7cm	6.5cm	----	Seed germinated
15/09	6	10.5cm	14.5cm	8cm	9cm	Seed germinated	1cm
16/09	7	13.5cm	16cm	10cm	12cm	1cm	2.5cm
17/09	8	15cm	18cm	13cm	15cm	2.5cm	3.5cm
18/09	9	17cm	20cm	14cm	16cm	4cm	6cm
19/09	10	18.5cm	22cm	16cm	18cm	7cm	8.5cm

Observation Fig-1

Fig 4.5: Seed growth on 5th day

Fig 4.6: Seed growth on 7th day

Fig 4.7: Seed growth on 10th day

Moong Dal

Mass cultivation of VAM was done and mixed with sterilized soil. Then the seed was coated with the soil containing VAM culture. Then the coated seeds are sowed in pot containing moistured soil. Two pots are taken one for control and the other for coated. The control pot contains only those seeds which are not coated. Whereas, coated pot Contains seeds coated with VAM. Sowing was done on 10/09/2022. After 24 hours, seedlings were observed in both the pots and the coated seeds were slightly longer compare to control pot. Day to day measurements of both the pot were recorded for 10 days.

At last graphical representation was done by comparing both control pot seedlings and coated pot seedlings.

Fig 4.8: Seed growth on 5th day

Fig 4.9: Seed growth on 7th day

Fig 4.10: Seed growth on 10th day

Chick pea

Mass cultivation of VAM was done and mixed with sterilized soil. Then the seed was coated with the soil containing VAM culture. Then the coated seeds are sowed in pot containing moistured soil. Two pots are taken one for control and the other for coated. The control pot contains only those seeds which are not coated. Whereas, coated pot contains seeds coated with VAM. Sowing.

After 24 hours, seedlings were observed in both the pots and the coated seeds were slightly longer compare to control pot. Day to day measurements of both the pot were recorded for 10 days. At last graphical representation was done by comparing both control pot seedlings and coated pot seedlings.

Summary & conclusion

A mycorrhiza is a symbiotic association between a fungus and the roots of a vascular plant.In this association, the fungi colonizes the host plant's roots, either intracellular as in arbuscular mycorrhizal fungi or extracellularly as iectomycorrhizal fungi.Biofertilizers have an important role in improving the nutrient supplies and their crop availability in upland crop production.

These inoculums may help in increasing crop productivity by the way of increased biological uptake of nutrients, degradation of pesticides etc. Some of the seeds are coated with the biofertilizer produced by our isolates (using mass production)and they are studied under in-*vitro* conditions.

Our isolates shown good growth rate compare to the control, by this experiment we can show our cultures are good biofertilizers. Arbascular Mycorrhizal fungi are ubiquitous in soil habitats and form beneficial symbiosis with the roots of angiosperms and other plants. Most terrestrial plants associate with root colonizing mycorrhizal fungi, which improve the fitness of both the fungal and plant associates. Ubiquitous occurrence and importance of Arbascular Mycorrhizae fungi for plant growth is now a well established fact.

The primary goal of AMF inoculation is to increase and enhance the yield and production plants. The main benefits of AMF are enhancing plant the acquisition of mineral nutrients and increasing the ability of host plants to withstand or reduce acquisition of toxic elements to growth. AMF provide a greater effective root surface area to explore greater volumes of soil and to overcome water and nutrient depletion zones around active root surfaces.Mycorrhizal plant roots have increased weight, length, number and layer diameters than the non-mycorrhizal one.

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