TOJSR
The Oxford Journal of Science and Research

ISSN No.: 2581-9380
TOJSR, 2019, 2(1) 65-71

Potential Vesicular Arbuscular Mycorrhiza Isolates from Western Ghats and their Biofertilizer Assay

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Key words: *VAM, Biofertilizer, Vermicompost, protein content, chlorophyll*

Abstract: Root samples from wild variety of crop plants in Western Ghats were subjected to serial dilution (up to 10^{-6} and 10^{-7}) and plated on nutrient agar and screened for isolation of potential biofertilizer agent. The isolates were subjected to morphological and biochemical tests and was identified to be Azospirillum species. The biofertilizer property of the isolate was studied on a leguminous plant in a flowering pot and the assay was based on percentage of seed germination, primary root length, number of secondary roots, shoot length, number of leaves and average leaf length, plant water content, protein and sugar content estimation and chlorophyll content respectively. Azotobacter and Azospirullum was used as standard control and uninoculated pot was considered normal control. The study was done in triplicates and the results showed that the isolate had higher influence in plant growth and further mass cultivation and field study was required.

Introduction

Biofertilizers refers to living organisms which augments plant nutrient supply, either by fixing atmospheric nitrogen or by enhancing the solubility of soil nutrients. These are capable of mobilizing nutritive elements from non-usable form to usable form through biological processes. Biofertilizer production also helps in reducing the input of chemical fertilizer to an extent of 25% for obtaining the same yield. They are less expensive and eco-friendly.

A symbiotic association between the hyphae of a soil-borne fungus and roots of a plants is called mycorrhiza. Among the three types of mycorrhiza the vesicular arbuscular mycorrhiza (VAM) is the penetrating hypae of the fungus in the cortical cells may be coiled or finely branched (arbuscules) haustorial branches[1]. Spores of VAM fungi from soil are isolated by various techniques and in the present study the sieving method was followed. They help plant growth but are influenced by human acivities, soil replishnment of VAM is essential [3].

The inoculum or the microbial preparation has greater significance towards agricultural and horticultural crops. They play a significant role as biological nitrogen fixers, phosphate solubilizers and phosphate scavengers. Selection of microbial strain is very important for mass production as the field performance of the biofertilizer depends on their effectiveness, competence and survival in soil. Rapid urbanization has lead to environmental pollution and further usage of chemical fertilizers may add up to the present problems of environmental degradation [16,17].

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AM fungi are abundant in rhizosphere soil and they ability to colonize plant roots depends various abiotic/ environmental factors [7]. AM fungi preferably colonize better when the source of inoculum and the inoculated plant species are one and the same [4, 10,13]. Colonization on plant roots is essential for proliferation of AM fungi. AM fungi are thus recognized as obligate symbiotic fungi [5].

Rapid urbanization has lead to environmental pollution and further usage of chemical fertilizers may add up to the present problems of environmental degradation [16, 17].

Materials and Method:

Isolation of VAM

Spores of VAM fungi from soil are isolated by various techniques: floatation method, floatation- bubbling method, sucrose centrifugation, airstream fractionation, separation of pores by gelatin column and various others[1]. In the present study the sieving method was used for isolation of VAM spores. Spores of AM fungi in soil can be collected by the wet sieving method[5]. Spores can be separated from soil based on the gravity differences of fine soil and the fungal spores, the spores are much finer than the soil particles and are lower density, the employment of fine mesh could help in separation can concentrate the spores from soil that are globular or sub–globular in 50–500 µm in diameter, the spores were put in watch-glass recognized under a dissecting microscope by their colour and shape[5].

Mycotrophic plants (leguminous plant *Trifolium*spp and grass species *Paspalum notatum)*were used for culturing AM fungi and present study the AM fungi colonizing roots, mycorrhizal plants collected from field can be transplanted to potting medium as Plant Trap Culture [6].

Biofertilizer assay

Garden soil and sand were mixed in the ratio 3:1 and sterilized and dispensed into small pots. The mehti plant seeds were treated with different combination of biofertilizer (*Azospirillum* and *Azotobacter*sp.) along with liquid potash and were sown. (Care was taken to water the pots regularly). The seeds untreated with Biofertilizers were used as control. These were grown for a period of about 45 days. Following growth, various morphological and biochemical parameters were carried out with the control and treated plants and the results were tabulated.

Total Protein assay

The plant extract was taken onto centrifuge tube and spun for 10 min at 5000 rpm. One gram of culture sample was taken & homogenized with 10ml of distilled water. This homogenate is used as a source of protein. Estimation of the protein was performed by lowry method whereby a stock solution of 100µg/ml of tyrosine was prepared by dissolving 10mg of tyrosine in 100ml of distilled water in a standard volumetric flask. Using this stock solution, dilution ranging from 10-100µg/ml was prepared. To 1ml of each of the dilution, 5ml of the alkaline reagent was added & the test tube was incubated at 40°C for 15 min. To the incubated tubes, 0.5ml of Folin's reagent was added & tubes were further incubated at 40°C for 15 min for the reaction to be completed. The OD was then taken at 640nm. Using a blank solution, the colorimeter was adjusted. The OD of the protein sample was plotted on the standard graph & extrapolated to get the concentration of protein.

Total sugar estimation

The plant extract was collected for sugar estimation. Each of the broth was diluted to obtain three concentration of 1/10, 1/25 and 1/50 dilution respectively. To 1ml of starch solution, 0.5ml of the above dilutions were added and incubated at room temperature for 5 min. The reaction was stopped using 2ml of 1% DNS reagent. The tubes were boiled in the boiling water bath for 5 min, cooled immediately and 5ml of water was added to it. They were then mixed thoroughly and absorbance was read of 540nm. A stock solution of 1mg/ml was prepared by dissolving 100mg of glucose in 100ml of water using a volumetric flask. The stock was further diluted with water to obtain a final sugar concentration of 100µg/ml to 1000 µg/ml. the above mentioned method was followed and the concentration of glucose was plotted o the X axis against the absorbance (OD) on Y axis and a straight line was derived from the point of origin using standard values.

Chlorophyll content

The plant photosynthesis is essentially dependent on chlorophylls and their concentration decides the plants efficiency in growth and development. This parameter helps directly to evaluate the plant health and robustness and their shortfall could lead to poorly developed plants and indicates plant diseases. Chlorophyll content can be easily analyzed by simple extraction protocol wherein they are found to be loosely bound and solvents like ether and/or acetone can help in extraction.

Though the chlorophyll type varies among prokaryotic and eukaryotic autotrophs the extraction mechanism is almost similar wherein they are characterized by porphyrin nucleus with a chelated magnesium atom. One gram of plant material would be fine for analysis, it has cut finely and macerated with organic solvent, 80% of acetone was used, 20 ml of solvent was used to grind 1g of plant material then centrifuged followed by collection of supernatant and absorbance was taken at 645, 663 and 652nm against the solvent as blank [12].

Results and Discussion

The VAM spores were isolated by sieving method and was observed under microscopes and latter identified.The positive influence of the potential isolate was tested on 45 days old leguminous plant *Trifolium*spp and grass species *Paspalum notatum*along with controls. The isolate inoculated pot showed better seed germination and was comparatively higher than the standard VAM fungi and vermicompost based pots.(**Fig 1**). But higher germination was observed in pots with a combination of biofertilizer and vermicompost.

The primary root development was monitored during 30th day and 45th day of plant growth (**Fig. 3**). The combination of vermicompost and the potential isolate in the pot led to better proliferation of primary roots and the fact was clearly visible during the measurement of the length of the root. Similarly the secondary root development was found to be impacted by the presence the AM fungi which was significantly higher than the un-inoculated control pot.

Mycorrhiza increase root surface area for water and nutrients uptake. The use of mycorrhizal biofertilizer helps to improve higher branching of plant roots, and the mycorrhizal hyphae grow from the root to soil enabling the plant roots to contact with wider area of soil surface, hence, increasing

the absorbing area for water and nutrients absorption of the plant root system [5].

Figure 1: Total number of plants per pot

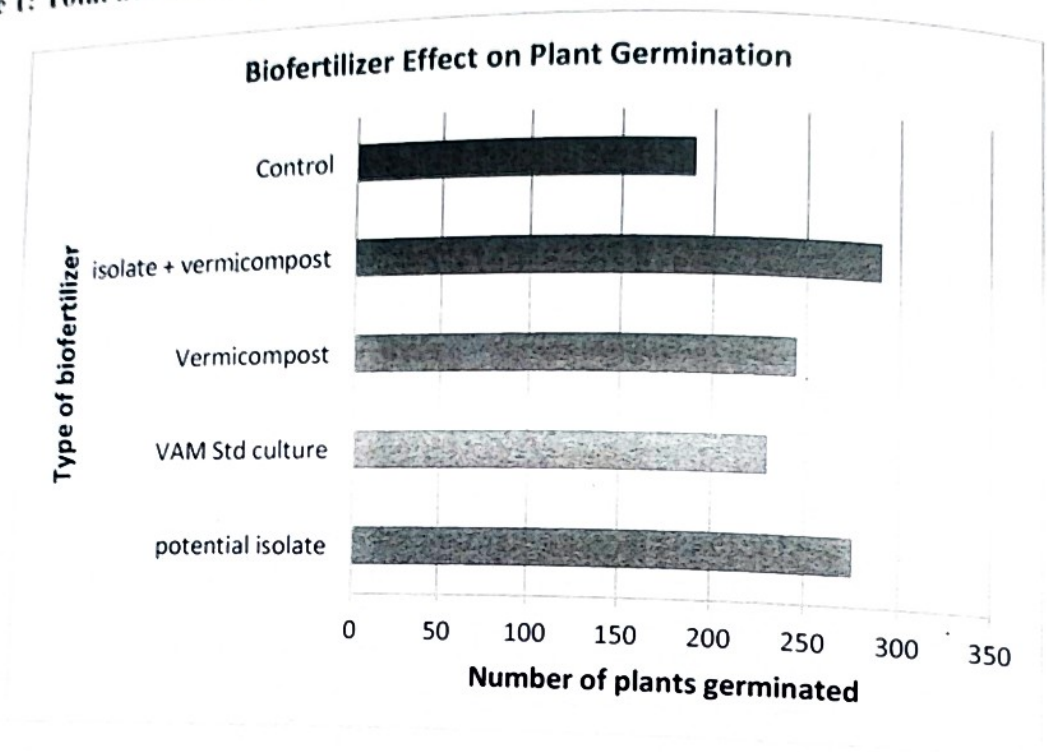
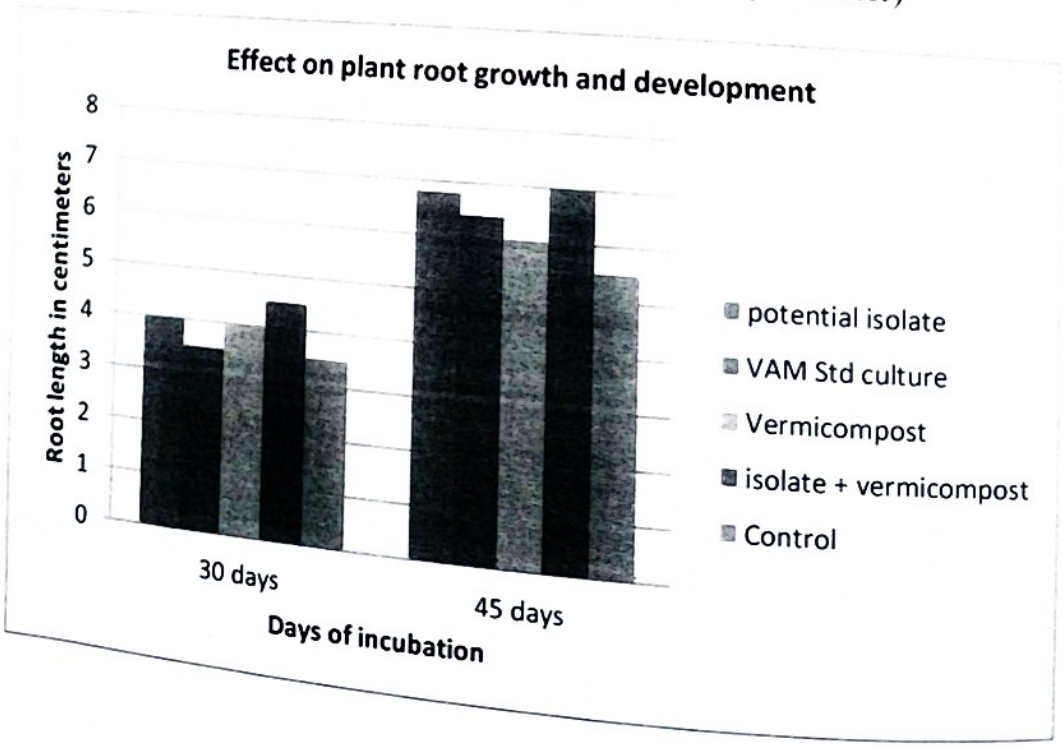


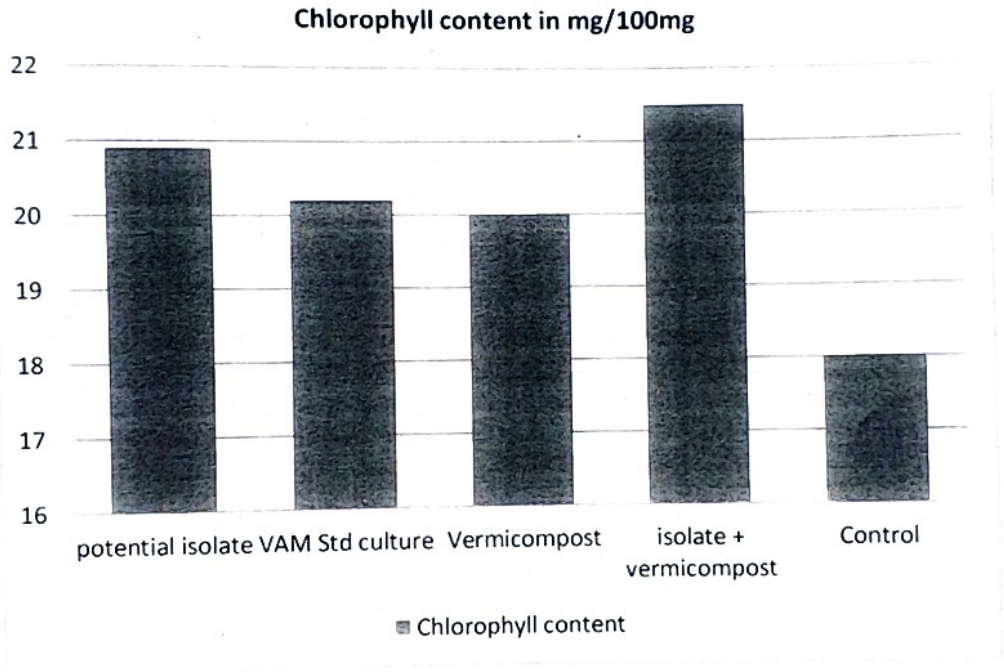
Figure 2: Plant primary root length measurement (Morphological parameter)



Nutrient rich soil could help in plant growth but indiscriminate use of chemical fertilizers have changed the balance of soil composition that have further deteriorated the soil quality and only beneficial microorganisms could help in changing the scenario by fixing essential nutrients to plants and reduced use of chemical fertilizers. There is considerable interaction between AM fungi and other soil organisms that leads to enhanced crop productivity. The vermicompost and VAM combination yield maximum and protein content and sugar content in test plants indicating and the combination has been reported by [18,2,19,8,9,11 and 15].

The average chlorophyll content was comparatively more in the isolate but still the combination of biofertilizers (**Fig: 3**) which yielded more chlorophyll content than the individual inoculants. And all the biofertilizers showed better result than the control.

Figure 3: Chlorophyll content of the various biofertilizers agents



Arbuscular mycorrhizal fungal structure in roots is usually not observed without appropriate staining. Freshly collected root samples should be washed gently and be free from soil particles. Ultrasonic treatment is effective to disperse soil particles closely adhered to roots[5]. The protein content and reducing sugar content was found to be higher in potential isolate treated pots (Table 2).

Table 2: Chlorophyll content of the various biofertilizers agents

Sl. No.	Biofertilizers	Protein content mg/g^{-1}	Reducing sugar content mg/g^{-1}
1	Potential isolate	6.1	18.1
2	VAM Std culture	5.3	17.5
3	Vermicompost	5.1	17.2
4	Isolate + Vermicompost	6.4	19
5	Un-inoculated Control	4.8	16.1

Mycorrhiza plays a very important role on enhancing the plant growth and yield due to an increase supply of phosphorus to the host plant. Mycorrhizal plants can absorb and accumulate several times more phosphate from the soil or solution than non–mycorrhizal plants. Since it is laborious and cost-consuming for production of AM fungal inocula because of their obligate biotrophic nature, the ways to increase the function of the indigenous AM fungi in soil have also been developed [5]. In this regard the potential isolate based biofertilizers may pave way for further enhancing the use and yield of crop plants.

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