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Effect of Inoculums with Different Carrier based Application of Alginate Entrapped Vesicular Arbuscular Mycorrhiza

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Abstract

An attempt was made to analyze the correlation between the physical and chemical factors of soil with the distribution of VAM fungi under different Agro ecological condition. Two fields from Chandur Bazar Tehsil of Amravati district were selected for the present investigation because the edaphic factors of the particular district show highly variable soil geography. The study revealed that all the physical and chemical factors viz; soil moisture, soil texture, soil pH and EC, nitrogen, Phosphorus, Potassium and Carbon are positively correlated with the distribution of VAM fungi. This is the first comprehensive study to describing the many beneficial roles of vesicular-arbuscular mycorrhizal (VAM) fungal structures. The present experiment was conducted to evaluate the possibility of immobilizing sand: soil inoculum of a VA-mycorrhizal fungus along with different carrier materials and to determine the infectivity and influence on plant growth and nutrition of onion. Sand: soil mixture containing chlamydospores and infected root segments (chopped) of Plectranthus ambinicus infected with *Glomus aggregatum* trapped grown for 90 days served as the mycorrhizal inculum. The different carrier based alginate entrapped vesicular – arbuscular mycorrhizal inocula were prepared. The various carrier materials used in me study were perlite, soilrite, talc, vermiculite, kaolinite and bentonite. Wet and dry beads were examined for the number of propagules they harbour. Perlite based alginate entrapped VAM inoculum contained higher numbers of propagules. A pot culture experiment was conducted to know the infectivity of alginate-entrapped inocula and their effect on growth and P nutrition of onion. The biomass, and P content of onion inoculated with different carrier based alginate entrapped VAM incula was equal to plants inoculated with sand : soil inoculam and significantly higher than the alginate beads are non-toxid in nature, biodegradable by soil microorganism and cause no ecological pollution. Further, me alginate beads could be used for the introduction of VAM fungi along with other beneficial soil microorganism which promote plant growth.

Keywords

VAM fungi,
physical and chemical factors,
carrier materials,
P nutrition.
plant growth.

Introduction

Mycorrhizae are a fungus which is associated with the root system of the higher plants. Spores of the Mycorrhaizae fungus are either very small and are non-randomly dispersed in soil, hence it is easy to recover the spores from the soil. In order to isolate Mycorrhaizae fungus from the soil, isolation of single spore of the fungus is essential.

There are three major groups of Mycorrhaizae based on infection, morphology endophyte taxonomy. The ecto-mycorrhaizae is generally associated with higher plants, wood plants, and the fungus from intercellulose mycelium with the root cortex. Ecto mycorrhaizae are most important in forest eco-system. They are characterized by fungal penetration of host cells. Those are several types, Ecto mycorrhaizae with

septate hyphae occurs in the Orchideceae and Ericaceae family. Ecto mycorrhaizae with aseptate hyphae are the Phycomycetous or Vesicular Arbuscular Mycorrhaizae. Seven genera are engaged in Mycorrhaizael interaction-Acaulospora, Gigaspore, Glomus, Sclerocystis, Midicella, Endogone and Enterophosphora. Very little is known of the life cycle of those organisms mainly because pure culture methods are available, hence development and standardization of monosporal cultures become prime importance for culturing the organisms in invitro system or invitro to characterize there beneficial physiological effect on host plant.

Most land plants form associations with mycorrhizal fungi. Mycorrhizas are mutualistic associations between fungi and plant roots. They are described as symbiotic because the fungus receives photosynthetically derived carbon compounds and the plant has increased access to mineral nutrients and sometimes water. The two most common associations are the arbuscular endomycorrhizas (AM) formed by Zygomycete fungi, and the ectomycorrhizas (EM) formed by Basidiomycetes, Ascomycetes, and a few Zygomycetes. Other mycorrhizal associations include the orchid, ericoid, arbutoid, monotropoid and ectendo- mycorrhizas. Mycorrhizal associations predominate in most natural terrestrial ecosystems. Whereas the AM fungi are widespread geographically and have a very extensive host range, the EM fungi are more restricted, forming associations predominantly with genera of important woody plants. Nevertheless, EM fungi are dominant components of the ground-dwelling macro-fungi in ecosystems where members of the following plant families abound: Betulaceae, Dipterocarpaceae, Fagaceae, Myrtaceae, Pinaceae, Ulmaceae, Salicaceae. EM fungi are common in tropical forests of Asia but are uncommon in many forests in Africa and South America. In Asia, the number of host species tends to increase with altitude and at higher latitudes.

Arbuscular mycorrhiza fungi (AMF) are geographically ubiquitous and occur over a broad ecological range. They are commonly found in association with numerous plant species including agricultural crops. It is estimated that AM fungi occur over 90% of earth's plant species. Vesicular – arbuscular mycorrhizas are though widely distributed but, there is limited knowledge of occurrence of individual or dominant species in relation to soil and climate. Several biotic and abiotic factors influence the VAM in various ways. Arbuscular mycorrhizal fungi are most frequent in plants growing on mineral soils. The population of AM fungi is greatest in plant communities with high diversity where they have many potential host plants and can take advantage of their ability to colonize a broad host range. There is a lower incidence of mycorrhizal colonization in very arid or nutrient rich soils. Mycorrhizas have been observed in aquatic habitats; however, water logged soils have been shown to decrease colonization in some species (Smith *et al*., 1983). Mycorrhiza is undoubtedly of extra ordinary importance in plant production, plant and soil ecology plays a key role in sustainable agriculture (Moawad, 1979; Gianianazzi *et al.,* 1994).

The term 'Mycorrhizae' was coined by Frank (1885), for the mutualistic symbiosis between the roots of vascular plants and certain fungi. Mycorrhiza literally means 'fungus root'. Of the different kinds of mycorrhizae, vesicular-arbuscular mycorrhiza (VAM) is the most prevalent type. The term VAM denotes the formation of special structures namely, vesicles and arbuscules by the colonizing fungi inside the host plant tissue, especially in the inner cortex of the root. The VAM are non-septate, belonging to the families of Glomaceae, Gigasporaceae and Acaulosporaceae of the order Glomales in the class Zygomycetes (Morton *et al.,* 1990). The VAM association is formed by a great variety of plants of different taxonomic groups. It is also geographically ubiquitous, occurring in plants from artic to Antarctic regions, over a broad ecological range from aquatic, terrestrial and to desert environments (Mosse 1981).

Mycorrhizae are of great interest to mycologist, plant pathologists, foresters, agriculture scientists and horticulturists. The plant roots provide an ecological niche for many of the microorganisms that abound in soil. Most live in an organic materials released by roots but a few actively penetrate the roots. Some of which form disease complexes while a few others may form harmless and even beneficial associations. The importance of microorganisms in the root-zone and their relationship with plant life has been clearly understood through several studies (Bagyaraj, 1990). Symbiotic mycorrhizal association between the roots of higher plants is a common feature and it is gaining increasing importance due to the fact that mycorrhizae help in plant production.

The role of VAM fungi in plant growth and nutrient uptake is well documented (Jeffries, 1987). The major part of the beneficial effects of VAM is attributed to their role in the uptake and translocation of immobile elements like P, Zn and Cu and also more mobile elements such as S, K, Mg, Ca, Fe and Mn (Tinker, 1984). Enhanced water and nutrient uptake, tolerance to drought, salinity and decreased severity of root diseases are the chief benefits of VAM association too most plants. VAM fungi are not only structurally efficient for extraction of nutrients from exchange sites in soil but also produce enzymes such as phosphatases, phytases and nitrogenous compounds (Selvaraj *et al.,* 1995).

Isolation and multiplication of native efficient straints of VAM is important for mass production and tailoring roots of crop plants suitable for their cultivation in non cultivated soils. Numerous techniques are available for the mass inoculum production of VAM in an almost sterile environment. However, the conveninent method of producing large quantities of inoculum is by the traditional 'Pot Culture' technique developed. Several host plants including sudan grass, bahia grass, guinea grass, cenchrus grass, sorghum, maize and onion have been studied for their suitability in producing VAM inoculum. Most studies have confined so far, for selecting suitable host plants mainly grasses for mass production of VAM fungi but there is meager information on the selection of suitable vegetable host plants for mass production of native strains of VAM fungi. The inability to

grow the VA mycorrhizal fungi in chemically defined media, in the absence of living roots has been a major impediment to the development of technology for the large scale commercial application of these fungi.

The benefit of AM fungi in agriculture is widely known. Increases in P uptake and the resulting biomass growth response of plants are well documented (Vinayak *et al.*, 1990). However, application of AM fungi is minimal. One reason for this is the difficulty in large scale inoculums production. This is because the fungi are obligate symbionts and cannot be cultured on nutrient or synthetic laboratory media (Jeffries, 1987). Pot cultures, hydroponics and geoponics are the methods employed to mass produce AM fungi. The VAM inoculum produced from the above methods are usually formulated to concentrate the inoculum and to facilitate efficient transport, distribution and placement of the inoculum in fields (Sylvia *et al.*, 1992).

The medium which is used to formulate AM inoculum should maintain the viability of AM propagagules and even after a relatively long period of storage should be able to colonize host plants and improve their growth. Immobillization by entrapment in alginate has been successfully used to formulative various microbial inoculants like Rhizobium (Hegde *et al.*, 1992), Azospirillium, Pseudomonas (Bashan, 1986) or ectomycorrhizal fungi (Cudin *et al.*, 1992), and potential biocontrol agents (Fravel *et al.*, 1985). Immobilization procedures can preserve the physiological properetiesx of mycorrhizal roots and promotes regeneration of intraradical forms of mycorrhizal fungi. Strullu *et al.*, (1991) entrapped the intraradical forms and the roots fragments colonized by AM fungi in calcium alignate beads. They observed the successful colonization of leek plantlets by such alginate entrapped AM beads.

Methods to produce AM root inoculum through nutrient film technique or circulating hydrophonic culture system have not reached commercialization because of many limitations. So the large scale inoculum production of AM inoculum is feasible using pot culture technique where the desired fungus is grown in the roots of a desired host raised on sand: soil mix or other substrate (Sreenivasa *et al.*, 1988).

The present experiment was conducted to evaluate the possibility of immobilizing sand: soil inoculums of an AM fungus along with different carrier materials and to determine their infectivity and influence on onion plant growth.

Materials and Methods

For the present investigation, the Chandur Bazar Tehsil of Amravati district was selected because of its distinct soil geography conditions. The area represents plains with black clayey soil. Hence, two different sites were selected for collection (Site 1 and Site 2).

$$\text{Moisture \%} = \frac{(W_1 - W_2)}{100} \times 100$$

Where, W_1 = Weight of soil before oven drying

W_2 = Weight of soil after oven drying.

1. Physicochemical analysis of soil:

The soil mycorrhizal population in terms of percentage of root colonization and the number of resting spores produced was greatly affected by edaphic factors as well as soil nutrient conditions (Daniels Hetrick, 1984). Soil pH, type, moisture influenced the VA mycorrhizal population in natural ecosystem (Mohankumar *et al.*, 1999). Therefore, it is necessary to understand the VAM fungal dynamics, their qualitative and quantitative association and impact of soil physicochemical factors on VAM distribution. The following standard methodology was adopted to analyze the physical and chemical status of the soil samples.

A. Physical parameters:

a. Soil moisture:

Moisture content of the soil samples were calculated immediately by oven drying method (Jackson, 1967). 10 g of composite soil sample was kept in hot air oven for 24 hrs, at 105°C. Dry weight of the sample was taken till it showed its constant weight. The percent moisture was expressed as follows.

b. Soil texture:

Relative proportion of different size soil particles is an important physical parameter to determine soil texture. The percentage of the soil particles were determined by Robinson's pipette method (Piper, 1964). 20g air dried soil sample was mixed with 50 ml distilled water. The mixture was heated for 5 minutes to break the large sized soil particles.10 ml hydrogen peroxide was added to the mixture. The solution was filtered through ordinary filter paper. The soil residue of filter paper was then treated with 250 ml of 5 N HCl and left it for overnight. The solution was filtered again and soil residue dried in air. The dried soil was passed through 0.2 mm pore sized scientific sieve. The coarse sand left on the sieve was weighed (W1). The sieved soil was transferred into a flask and sufficient amount of 1 N sodium hydroxide (NaOH) was added to make the contents alkaline. After shaking the flask for six hours on the mechanical shaker, the content was transferred to 500ml measuring jar and the volume was made up to 500ml by distilled water. It was stoppered and was allowed to settle after shaking. The solution was pipetted out with Robinson's pipette into a dish. The required quantity was pipetted and evaporated in a dish and weighed which gave the quantity of clay content (W4). Silt (W3) was calculated by deducing clay from silt. The supernatant was poured into a separate beaker and soil was washed again and again till

the suspension was clear. This fraction was transferred to another tarred dish, heated and weighted, which was the reading of fine sand (W2).

Reading:

W1-Coarse sand, W2- Fine sand, W3 - Silt, W4 – Clay sand.

c. Determination of soil pH:

pH value as a measure of the hydrogen ion activity of the soil water system and expresses the acidity and alkalinity of the soil. It is a very important property of soil as it determines the availability of nutrients, microbial activity and physical condition of soil. The pH of soil water suspension was determined using Equiptronics pH meter as described by Jackson (1967). 20 g soil sample was mixed with 40 ml distilled water in 1: 2 ratios. The suspension was stirred intermittently with glass rod for 30 minutes and left for one hour. The electrode was inserted into supernatant and pH was recorded .The electrode was washed with distilled water every time to record the other new reading of the soil sample.

d. Determination of electrical conductivity of soil:

Electrical conductivity (EC) expresses ion contents of solution. Conductivity as the measure of current carrying capacity, gives a clear idea of the soluble salts present in the soil. The electrical conductivity of a soil samples was determined on an Equiptronic's digital electrical conductivity bridge. In a 20g soil, 40 ml of distilled water was added. The suspension was stirred intermittently for half an hour and kept it for 30 minutes without any disturbances. Conductivity cell was inserted in solution and EC was recorded.

B. Chemical parameters:

a. Estimation of available nitrogen (N):

Nitrogen of soil mainly present in organic form together with small quantities of ammonium and nitrate forms. The nitrogen supplying ability of the soil was determined by distilling soil with alkaline potassium permagnate solution. During the distillation easily utilizable and amino- N hydrolyzed nitrogen liberated as ammonia is measured. This serves as an index of nitrogen status of soil. Alkaline potassium permagnate method was followed to estimate available N of soil samples.

In 1000 ml round bottom distillation flask (Kjeldahl flask), 20g soil was taken. To this 20ml distilled water was added. Then 100ml each of 0.32 % potassium permanganate and 100 ml 25% NaOH solution were mixed and immediately connected it to keelhaul assembly. The froth during boiling was prevented by adding liquid paraffin (1ml) and bumping by adding a few glass beads. The contents were distilled in a kjeldahl at a steady rate and liberated ammonia collected in an Erlenmeyer flask (250 ml), containing 20 ml of 2 % boric acid solution with methyl red and bromocresol green indicator. With the absorption of ammonia, the pinkish colour turns to green. After 30 minutes it was titrated with 0.02 N H2SO4 till the colour changed from green to original shade (pink), Blank (without soil) was run simultaneously.

Available nitrogen was calculated from the following formula,

$$\% \text{ Available N} = (A-B) \times (\text{N. of acid}) \times 0.014 \times \frac{100}{\text{Wt. of soil (g)}}$$

$$\text{Available nitrogen (Kg/he)} = \% \text{ N} \times \frac{2240000}{100}$$

Where,

1. Wt. of soil sample - Wt.
2. Volume of std. acid required for soil - A ml
3. Volume of std. acid required for blank - B ml.
4. Normality of Sulfuric acid. – N

b. Estimation of available phosphorus (P):

Soil available phosphorus found as orthophosphate in several forms and combinations, but only a small fraction of it may be available to plants. Available phosphorus was estimated by Olsen's method (Olsen, *et al*., 1954) modified by Watanbe (1965). The reagent for Olsen's P was 0.5 M $NaHCO_3$ (pH 8.5) prepared by dissolving 42 g NaHCO3 in distilled water and made up to 1 lit. The pH was adjusted at 8.5 with 20 % NaOH solution. 2.5 g of air dried soil was weighed into 150 ml Erlenmeyer flask, 50 ml of Olsen's reagent (0.5 M $NaHCO_3$ Solution , pH 8.5) and one teaspoonful of activate charcoal were added. The flasks were shaken for 30 minutes on the electrical shaker and contents filtered immediately through Whatman filter paper (No. 41). 5 ml of the filtrate was pipetted out into 25 ml of volumetric flask and was neutralized with 1: 4 H_2SO_4 using paranitrophenol as indicator. The volume was made up by adding distilled water. Colour developed when few crystals of stannous oxalate were added. The solution was shaken well and intensity of blue colour was read in photoelectric calorimeter within 10 min. at wavelength of 730 to 840 µm. A blank was run without soil.

Standard curve:

Analytical grade potassium dihydrogen orthophosphate (KH_2PO_4) was dried in hot air oven at 60 C for 1 hr and allowed to cool. Exactly 0.439 g of KH_2PO_4 was dissolved in 500 ml of distilled water.25 ml of 7N H_2SO_4 was added and made up to 1 lit with distilled water. This gives 100 ppm standard stock solution of KH_2PO_4. From this diluting it 5 times and made a 2 ppm P solution. For the preparation of standard curve different concentrations of P 0,2,4,6,8 and 10 ml of 2 ppm P solution were taken in 25 ml volumetric

flask separately, which responds to 0, 0.16, 0.32, 0.48, 0.64 and 0.80 ppm P respectively. To these 5ml of the extracting reagent 0.5 ($NaHCO_3$) was added to each flask and pH was adjusted as above. The content was diluted with 20 ml water and 4ml reagent.

(Dickman and Brays reagent) Volume was made up and intensity of blue color was read in photoelectric calorimeter using 730-840 m filter or using red filter (660nm). Graph was constructed by plotting reading on X-axis and on centrations of P on Yaxis.

Calculations:

The amount of phosphorus was estimated by using formula,

$$\text{Factor (F)} = \frac{\text{Concentration of P}}{\text{Corresponding reading}} = \frac{0.32}{30} = 0.01$$

Of above concentration
= 1 Calorimeter reading = 0.01 ppm (P) phosphorus.

Total volume of extract 1

$$P(\text{ppm in soil}) = \text{ppm P in aliquot} \times \frac{}{\text{Aliquot taken (ml)}} \times \frac{}{\text{Wt. of soil (g)}} (RxF)$$

(kg) / ha = ppm P in soil x 2.24

P_2O_s (Kg/ha) =P (Kg / ha) x 2.29

Conversion factors=P x 2.29 x P_2O_s
P=P_2O_s x 0.437

c. Determination of available potassium (K):

Only small fraction of total K is held in exchangeable form, while the rest remains in fixed or non-exchangeable form. When e crop exhausts the supply of exchangeable K, more K is released from the fixed reserve. Exchangeable K, is therefore, also referred to as 'available K'. The flame photometric method (Jackson, 1958) was employed to estimate available K of samples. 5g of air dried sample was taken in 150 ml Erlenmeyer flask and 25 ml of 1 N ammonium acetate was added to the flask. The contents were shaken for 5 minutes on a mechanical shaker and filtered immediately through a dry filter paper (Whatman No.1). The filtrate was collected in a beaker. 5 ml of filtrate diluted with 25 ml with distilled water, Atomized the above diluted extract to flame photometer to note the reading. The amount of potassium was estimated by formula:

Calculations:

2.24×10^6

$$\text{Available (Kg/ha)} = (RxF) \times \text{Vol. of extract} \times DF \times \frac{}{\text{Soil wt} \times 10^6}$$

Available K_2O (kg/ha) = Available K (Kg/ha) x 1.20

Where,

R = reading
F = Con. of K/corresponding reading
DF = Dilution factor

d. Determination of organic carbon (C):

Organic matter plays an important role in supplying nutrients and water and provides good physical conditions to the plants. The quantity of organic carbon of the soil was estimated by the method described by Jackson (1967). 1g finely ground soil sample passed through 0.5 mm sieve without loss was taken into 500 ml conical flask, to which 10ml of 1 N potassium dichromate and 20 ml Conc. H_2SO_4 were added with measuring cylinder. The content was shaken for a minute and allowed to set aside for exactly half an hour. Then 200 ml distilled water 10 ml orthophosphoric acid and 1 ml diphenylamine indicator was added. The solution was titrated against std. ferrous ammonium sulphate (FAS) or ferrous sulphate, till colour flashes from blue violet to brilliant green. The blank titration was carried at the beginning without soil.

The observation was:

1. Weight of soil taken = Kg
2. Vol. of 1 N Potassium Dichromate added= 10 ml
3. Vol. of 0.5 N FAS required neutralizing 10ml of 1 N Pot. Dichromate solution (blank without soil) = B ml
4. Vol. of 0.5 N FAS required for all = T ml
5. Vol. of 1 N $H_2Cr_2O_7$ solution used for the oxidation of organic carbon present in the sample = 10(B-T)

The organic carbon content (in %) of the soil was calculated as follows

$$\text{Organic Carbon \%} = \frac{10\ (B-T)}{B} \times 0.003 \times \frac{600}{\text{wt. of Soil (g)}}$$

Inoculum Host Plant used

Glomus aggregatum mass inoculum production of VAM experiment was done using Plectrathus amboinicus. For growth experiment involving VAM studies using onion (*Allium cepa* L) plants.

Mass inoculum production of Glomus aggregatum

24 cm dia earthen-ware pots were used to carryout inoculums production of VAM experiment. Pots were filled with 5kg of sterilized (by autoclaving twice for 2hr. on two consecutive days) Sandy loam: sand mixture (1:1v/v) deficient in P (5mg/kg soil extracted with NH_4 F and HCl (Olson *et al.*, 1965) with a pH of 7.4. After sterilization of the soil, nitrogen as urea (35 mg/kg soil) was applied as bascal application of soil contained in the earthern-ware pots irrespective of the treatments. Sandy loam soil filled in the pots irrigated with Ruakura nutrient solution added at the rate of 50 ml per 2.5L substrate once in 8 days (Smith *et*,

al., 1983) was most suitable for VAM mass inoculum production (Sreenivasa *et al.*, 1988).

Production of alginate entrapped VAM inoculum.

Sand: soil mixture containing chlamydospores and infected root segments (chopped) of Plectranthus amboinicus infected with Glomus aggregatum trapped grown for 90 days served as the mychorrhizal inoculum. The inoculum was air dried and passed through 425 µm sieve. To an aqueous suspension of sodium alginate 2% (2 gm in 100ml of distilled water boil at 80ºc), 10% (10 gm) of the sieved sand: soil inoculum of the VAM fungus plus 2% (2gm) of the carrier material (perlite, soilrite, talc, vermiculite, kaolinite and bentonite) were added separately and mixed using a magnetic stirrer. This mixture was taking in a syringe and added dropwise in 0.1M sterile calcium chloride solution to form beads (Plate-2). After 30 minutes the beaded inoculum was rinsed with tap water. Aportion of the alginate entrapped wet VAM inoculum was dried to surface dryness and stored at 4ºc. This formed the carrier based alginate entrapped wet VAM inoculum. A portion of the beads were air dried for 5 days to form dry VAM alginate inoculum packed in polythene bags, and stored at room temperature (32+/-5ºc).

The pH of the carrier materials used in the study was estimated by using a digital pH meter (substrate; water ratio=1:10 w/v). The moisture content of the wet VAM beads was determined after drying to a constant weight.

The number of propagules in the different carrier based alginate entrapped VAM inoculum was determined by the MPN method using four-fold dilutions. The alginate beads were solubilized in 0.2 M sodium citrate solution (pH adjusted to 7.2) prior to carrying out the MPN test. The propagule numbers in the dry VAM alginate beads was computed from the propagule numbers of the wet VAM alginate beads and its moisture content.

A pot culture experiment was also conducted to know the effect of alginate entrapped VAM inoculum on the colonization of roots and growth of onion (Allium cepa) host plant as the (plate-3). The soil used for this study was a sandy loam soil of pH 7.2, with 2.4 mg available p/g (NH4+HCl extractable) and an indigenous VAM population of 0.31 propagules/g of soil. Plastic pots 12cm dia were filled with 1L of soil and inoculated with alginate entrapped wet and dry VAM inoculum (with pertile, soilrite, talc and vermiculite as carriers) and sand: soil inoculum of Glomus aggregatum at the rate of 500 propagules per pot. Two of three germinated onion seeds were planted per pot. Each treatment had five replicates. The pots were arranged in a green-house (temperature 29+/-2ºc) in randomized complete block design and watered whenever necessary. 50ml of Ruakura nutrient solution was added thrice (First application with P, 20 days after planting and the other two later applications without P, on 40 and 60 days after planting).

Observations on plant height and number of leaves were recorded on days 30, 60 and 90 after planting. The plants were harvested 90 days after planting. Plant samples were oven dried at 60ºc to a constant weight to get shoot samples were determined by the vanadomolybdate phosphoric acid method (Jackson; 1973). Mycorrhizal colonization of the root was determined by the Gridline-intersect method after staining the roots with trypan blue.

Trypan blue method

The 1cm long root segments were first washed thoroughly in distilled water and then placed IN 10% KOH and heated to 90ºc for 15-30 minutes. They were then washed in distilled water and then immersed in alkaline H_2O_2 for 10-15 minutes. Then they were washed in distilled water and acidified with 5N Hcl for 2-5 minutes. Then the root bits were stained in 0.05% trypan blue in lactophenol for 15-30 minutes and the excess of stain was removed with clear lactophenol and observed.

The data obtained from the pot experiment was subjected to analysis of variance by randomized complete block design and treatment means were separated by Duncan's multiple ranges (DMR) test (Little *et al.*, 1978).

Results and Discussion

Physico-chemical analysis of soil:

Physical Parameters:

Soil moisture:

If water content becomes too low, a plant becomes stressed. The amount of soil water available to plant is governed by depth of soil that roots and AM fungi can explore. The average percent soil moisture of both the sites were recorded in between 7.886 to 8.85%, which clearly indicates that soil moisture percentage in site 1 is higher than site 2 as shown in the The soil moisture is found to be correlated with spore density. The increase in spore population may be due to increase in soil moisture. Similarly reports were reported by Mohankumar *et al.*, (1999) and Bakshi (1974). Khan (1974) reported low spore density in the month of May. It indicates that spore density is correlated with moisture content.

Soil pH and EC:

pH is a measure of the hydrogen ion concentration i.e. acidity or alkalinity of the soil. pH can affect the availability of nutrients and activity of many essential micro-organisms. The pH of a soil may influence crops grown in the field and the types of soil microbiota. The pH of all soil samples were found to be ranged in between 7.28 to 8.02 which indicated the slight alkalinity of soils (**Table-1**). The unit of electrical conductivity is dsm m-1. Electrical conductivity of two composite soil samples ranging between 0.41 to 1.75 as shown in (**Table-1**) there was no specific correlation found

between EC and spore density. The response of VAM fungi is variable according to the strains present in that soil. VA mycorrhiza is positively correlated with high pH value.

Chemical Parameters:

Available Nitrogen:

Chemical analysis was carried out for the elements like N, P, K and C. It is presented in (**Table-1A**). In site 1, it was greater i.e. 179.2 and in site 2 comparatively less i.e. 153.0 mg/kg. Nitrogen content of soil directly affects soil pH which may be responsible for variation in spore density. Some other reports states that high nitrogen did not leads significant reduction in AMF colonization (Hartwig *et al*., 2001).

Available Phosphorus:

Phosphorus is one of the key macronutrient required for plant growth and metabolism. Inorganic phosphate supplied to the soil as a fertilizer is rapidly converted into unavailable form. Soluble P converted into insoluble phosphate involves microorganisms.

Mycorrhizal plants can take up more phosphorus than non mycorrhizal plants. Comparatively higher P- availability is recorded in site 1, 51.80 and in site 2 it is 43.90 mg/kg as shown in the (**Table-1**) Percent colonization and spore density is greater in the soil having low P- values. This view is supported by Janaki Rani *et al.*, (1994).

Available Potassium:

Estimated K was recorded in the (**Table-1**) the values were ranging between 123.2 to 3365.6mg/kg. The positive correlation was found by Joshi *et al.*, (1995).

Available Carbon:

The value of organic carbon was tabulated in (**Table-1**) ranging between 0.54 - 0.29 mg/kg. Higher C percentage was record in fertile soil due to addition of higher inputs. Kliromonos *et al.*, (1998) observed that *Glomus* Sp. production were significant higher in response to high CO_2 elevation.

Table -1. Physico characteristics of soil

Site	Soil Type	Soil pH	EC (dsm/m)	Soil Moisture%
S1	Clayed	8.02	0.41	8.85
S2	Clayed	7.28	1.75	7.886

Table -1A. Chemical characteristics of soil

Site	Available nutrients (mg/Kg)			
	N	**P**	**K**	**C**
S1	179.2	51.80	123.2	0.54
S2	153.0	43.90	3365.6	0.29

Table-2. pH of the different carrier material and material and moisture content of the wet VAM alginate beats.

Carrier material	pH	Moisture content Of wet VAM alginate Beads (%)
Perlite	7.4	82.20
Soilrite	7.2	84.00
Talc	7.8	81.83
Vermiculate	8.2	86.62
Kaolinite	5.62	82.40
Bentonite	5.46	82.24

The pH of the different carrier materials varied from an acidic to alkaline range. The moisture content of wet beads was around 81% **(Table 2).** Pelite based wet VAM alginate beads had highest number of propagules of VAM per g of the inoculum followed by soilrite based wet alginate entrapped VAM inoculum. Among the dry VAM alginate beads, the beads with perlite as carrier ranked first with 20.18 propagules of VAM per g of the inoculum; while Kaolinite based alginate entrapped VAM beads had only 5.05 propagules if VAM per g of beads **(Table 3).**

Table-3. VAM fungal (*Glomus aggregatum*) infective propagules (I, B) number in the different carrier based alginate entrapped VAM inoculate.

Carrier material	Wet beads * I.P.g^{1}	Dry beads. P.g^{1}
Perlite	3.82	20.18
Soilrite	2.82	17.14
Talc	2.24	12.68
Vermiculite	1.35	7.65
Kaolinite	0.98	5.05
Bentonite	0.95	5.14
Sand:soil inoculum	49.92	--

Inoculation with the VAM fungus *Glomus aggregatum* either as sand: soil inoculum or as alginate entrapped inoculum produced plants with greater height And leaf number, compared to plants grown in soils having only the indigenous VAM fungi. Inoculation of soil with soilrite based dry VAM alginate beads resulted in plants with maximum height and leaf number. The highest level of VAM root colonization occurred in plants grown in soil inoculated with the sand: Soil inoculums, and this was statistically equal to the root colonization level observed in plants inoculated, with wet perllite and dry vermiculate based alginate entrapped VAM inoculums **(Table 4).**

Table-4. Effect of inoculation with different carrier based alginate entrapped VAM inoculam on height, number of leaves and mycorrhizal root colonization of onion (*Allium cepa).*

Carrier material	Plant height (cm)	Leaves (No.Plant^{1})	Mycorrhizal Colonization (%)
Wet beads			
Perlite	42.45	7.2	72.2
Soilrite	40.10	7.4	68.4
Talc	34.45	7.0	69.2
Vermiculite	39.48	6.5	62.5
Dry heads			
Perlite	56.42	8.4	68.4
Soilrite	58.12	9.2	87.5
Talc	48.15	7.2	64.5
Vermiculite	52.46	7.5	70.0
Sand:Soil inoculum	54.15	7.2	72.5
	50.45	7.0	60.4

* Means in the same super script do not differ significantly at P=0.05 level by Dm R test.

Table-5. Effect of inoculation with different carrier based alginate entrapped VAM inoculam on height, number of leaves and mycorrhizal root colonization of onion (*Allium cepa*).

Carrier material	Biomass of plant	P control mg plant
Wet beads		
Perlite	3.25	2.20
Soilrite	4.10	3.76
Talc	2.19	3.42
Vermiculite	1.85	3.27
Dry heads		
Perlite	4.24	4.07
Soilrite	4.32	4.24
Talc	3.24	4.06
Vermiculite	3.50	4.12
Sand:Soil inoculum	4.10	4.30
Control	3.35	2.98
SEM+	0.42	0.31
CD at 5%	1.02	0.92

Dry biomass of plants inoculated with sand:soil inoculum and either dry alginate entrapped inoculum (with carriers perlite, soilrite, talc and vermiculite) or wet alginate entrapped inoculum (with carriers perlite and soilrite) were statistically equal with each other. There was a significant increase in plant P content because of inoculation with alginate entrapped VAM inoculum of Glomus aggregatum compared to non-inoculate of plants **(Table 5).**

The root inoculum of the VAM fungus *Glomus aggregatum* was immobilized in alginate beads by Strullu *et al.,* (1991). They reported that inoculation of leek plantlets with VAM fungal entrapped alginate beads stored at 40^{o}C for a period of one month induced colonization of root by fungus. The present study gives further information on the number of VAM propagules in the alginate entrapped inoculum and its influence on the growth and P nutrition of onion used as a host.

Perlite, Kaolinite and bentonite have been tried earlier as carrier materials for immonilization. Soilrite, talc and vermiculite have been used for the first time in this study. Among the carrier based wet alginate beads, perlite-alginate beads had the highest propagules of VAM fungi followed by soilrite based alginate entrapped VAM inoculum. Perlite is a relatively inert material, light in weight with a neutral pH and it has been used earlier as a carrier material in alginate beads for Rhizobium (Hedge *et al,*. 1992) and ectomycorrhizal inoculum (Kropacek *et al.,* 1989; Cudlin *et al.,* 1992) and has been reported to support the growth of microbial inoculant. Alginate is also an inert material and offers a mild condition for entrapment (Brodelius *et al,*. 1987). The favourable plH required for the spore germination and effective infection of Glomus aggregatum is around 7 (Gerdemann *et al,*. 1974) which is the pH of perlite too and this could be the reason for the survival of highest numbers of VAM propagules in the perlite VAM alginate beads.

Kaolinite and bentonite, when used as carriers in alginate beads did not support high propagules of VAM fungus. Kaolinite (Jung *et al.,* 1982) and montomorillonite when added to improve the survival of Rhizobium in alginate beads, it was observed that it protected some rhizobial strains only. Further, it was also observed that a part of the VAM fungi propagules might have been lost during the entrapment process or during the solubilization process which was done prior to conducting the MPN method as the carrier based alginate entrapped VAM inoculum even though 10% if sand: soil inoculum was added to sodium alginate and carrier mixture prior to entrapment process.

Inoculation of alginate entrapped VAM inoculums to onion resulted in good colonization. The extent of VAM root colonization was lesser when plants received alginate entrapped VAM inoculums when compared to plants inoculated with sand: soil inoculums. This is probably because the propagules are released at a slower rate from alginate beads. This upholds the view expressed by Hedge *et al.,* (1992) working with Azospirillium and Rhizobium respectively that bacteria are released at a slower rate from calcium alginate beads when compared to peat inoculums.

Alginate entrapped VAM inoculum enhanced the plant biomass and P content significantly when compared to noninoculated control. It was statistically equal to the plants which were inoculated with sand:soil inoculum. The perlite based alginate entrapped VAM inoculum which contained high propagules of VAM could be used for inoculating crop plants.

Bashan (1981) working with azospirillam pointed out that dry alginate beads are easy to prepare, store and apply at field conditions. Hedge *et al.,* (1992) suggested that immobilized rhizobia in alginate beads provide a slow and constant release of the inoculant over long time and hence could most probably overcome the need for re-inoculation. The loose sand: Soil inoculum is known to clog the pipes of farm machinery (Powell *et al,*. 1982). Under such conditions the dry granular alginate beads could be used for large scale field inoculations. The alginate beads are non-toxic in nature, biodegradable by soil microorganisms and cause no ecological pollution (Bashan 1981). Further, the alginate beads could be used for the introduction of VAM fungi along with other beneficial soil microorganisms which promote plant growth.

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