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Mass production of Alginate Entrapped Arbuscular Mycorrhizal Inoculum and their influence on growth and nutrition of Onion

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Abstract

The present experiment was conducted to evaluate the possibility of sand: soil inoculum of Arbuscular Mycorrhizal fungus with different carrier materials and to determine their influence on plant growth and nutrition of Onion. Sand:soil mixture containing azygospores and infected root segments (chopped) of *Allium cepa* L. infected with ***Gigaspora margarita*** grown for four months served as the Mycorrhizal inoculum. The different carrier based alginate entrapped AM inocula were prepared. The various carrier materials used in the study were perlite, soilrite, talc and vermiculite. Wet and dry beads were examined for the number of propagules of AM fungi they harbour. Perlite based alginate entrapped AM inoculum contained higher number of propagules. A pot 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 AM inoculum was equal to plants inoculated with sand: soil inoculums and significantly higher than plants grown in un-inoculated soil. This study revealed that the alginate beads are non- toxic in nature, biodegradable by soil microorganisms and cause no ecological pollution. Further, the alginate beads could be used for the introduction of AM fungi along with other beneficial soil microorganisms which promote plant growth.

Keywords: *Allium cepa L., Gigaspora margarita*, Alginate beads.

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INTRODUCTION

The introduction of beneficial organism into soil is one of the present cruzes of applied Mycorrhizal research. Isolation and multiplication of native strains of AMF (Arbuscular Mycorrhizal fungi) are essential for mass production and tailoring of roots of medicinal cash crops to make them suitable for agricultural soils (Bagyaraj, 1992). Indigenous strains of AM fungi are found to be better colonizers of medicinal plants under pot culturing conditions (Selvaraj, 1989). At present considerable importance is being given to bio-inoculants because of the contemporary sensitivity to environmental pollution and health hazards resulting from the use of chemical fertilizers,Somani *et al*., (1990). Hence an investigation was carried out for mass multiplication of selected and efficient strains of AMF using Onion (*Allium cepa* L.) as the host plant and to evaluate the possibility of immobilishing sand:soil inoculum of Am fungus, *Gigaspora margarita* from infected root segments of *Allium cepa* along with different carrier materials and also to determine their infectivity and influence of plant growth of Onion under pot culturing conditions.

MATERIALS AND METHODS

Pot cultures were raised by inoculating the predominant AM fungi extracted from soil on Onion (*Allium cepa* L.) seedlings grown separately in steam sterilized soil in pots. After four months, the plants were harvested separately and the roots examined for AM colonization. Sand and soil mixture containing azygospores and infected root segments (chopped) of Onion (*Allium cepa* L.) infected with *Gigaspora margarita* served as mycorrhizal inoculum. The inoculum was air dried and passed through 400µm sieve. To an aqueous suspension of sodium alginate (2%) 10 per cent of the sieved sand: soil inoculum of the AM fungus plus 2 per cent of the carrier material (perlite, soilrite, talc, vermiculite, kaolinite and bentonite) were added separately and mixed using a magnetic stirrer. This mixture was passed through a sieve onto 0.1 M sterile calcium chloride solution to form beads. After 30 minutes the beaded inoculum was rinsed with tap water. A portion of the alginate- entrapped wet AM inoculum was dried to surface dryness and stored at 40° C. This formed the carrier based alginate entrapped wet AM inoculum (Kropacek *et al*., 1989, Strullu and Plenchette, 1991). A portion of the beads were air dried for 5 days to form dry AM alginate inoculums, packed in polythene bags and stored at room temperature (32±5° C). The pH of the carrier materials used in this

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study was estimated by using a digital pH meter (substrate: water ratio = 1:10 w / v).

The number of propagules in the different carrier based alginate entrapped AM inoculum was determined by the MPN method using four- fold dilutions (Sieverding, 1991). A pot culture experiment was also conducted to know the effect of alginate AM inoculum on the colonization of roots and growth of onion as the host plant. Observations were made on plant, fresh weight and dry weight of shoot and bulb 90 days after planting. The plant, were harvested 90 days after planting. Plant sample were oven dried at 60° C to a constant weight to get plant biomass.

Phosporous and potassium content of the shoot and bulb samples were determined by the Vanado molybdate phosphoric yellow colour method (Jackson, 1973) and flame photometric method respectively. Mycorrhizal colonization of the root determined by the grid line-intersects method (Giovannetti and Mosse, 1980) after staining the roots with trypan blue (Philips and Hayman, 1970). The data obtained from the pot experiment was subjected to analysis of variance by randomized complete block design and treatment means separated by Duncan's multiple (DMR) test (Lttle and Hills, 1978).

RESULTS AND DISCUSSION

Immobilization procdures can preserve the physiological properties of Mycorrhizal fungi (Strullu and Plenchetti, 1990) entrapped the intraradical forms and the root segments colonized by AM fungi in calcium alginate beads. The pH of the different carrier materials varied from an acidic to alkaline range (pH 4.62 to 8.02). The moisture content of wet beads was around 82% (Table 1).

Table 1. pH of the different carrier materials and moisture content of the wet alginate beads

Carrier material	pH	Moisture content of wet alginate beads %
Perlite	7.35	82.3
Soilrite	7.25	83.45
Talc	7.9	81.84
Vermiculite	8.02	86.62
Kaolinite	5.62	86.62
Bentonite	5.65	82.4
Sand and soil inoculum	7.46	65.05

Perlite based wet AM alginate beads had highest number of propagules of AM (3.78 per g) inoculum followed by soilrite based wet alginate entrapped AM inoculum (2.72 per g). Among the dry AM alginate beads, the beads with perlite as carrier ranked first with 21.28 propagules of AM per g of the inoculum, while Kaolinite based alginate entrapped AM beads had only 5.05 propagules of AM per g of beads (Table 2).

Table 2. VAM fungal infective (*Gigaspora margarita*) propagule numbers in the different carrier based alginate entrapped VAM inocula

Carrier Material	Wet beads	Dry beads
	* IPg^{-1}	
Perlite	3.78	21.28
Soilrite	2.72	18.05
Talc	2.19	12.65
Vermiculite	1.25	7.84
Kaolinite	0.95	5.05
Bentonite	0.96	5.12
Sand and soil inoculums	46.99	--

* at 95% confidence level

Inoculation with the AM fungus *Gigaspora margarita* either as sand:soil inoculum or as alginate entrapped inoculum produced plants with greater height and plant biomass. Inoculation of soil with vermiculite based dry beads resulted in plants with maximum height and plant biomass. There was a significant increase in plant P content because of inoculation with alginate entrapped AM inoculum of Gigaspora margarita compared to uninoculated control (Table 3).

Table 3. Effect of inoculation with different carrier based alginate entrapped VAM inocula on plant height, biomass, phosphorus content and mycorrhizal root colonization of Onion

Carrier Material	Plant height (cm)	Phosphorus content (mg $plant^{-1}$)	Plant biomass (g $plant^{-1}$)	Mycorrhizal colonization (%)
Wet beads				
Perlite	52.5^{cd}	4.20^{ab}	2.18^{abc}	70.9^{ab}
Soilrite	49.5^{cd}	3.76^{bc}	2.10^{abc}	68.2^{b}
Talc	48.5^{d}	3.42^{cd}	2.05^{cd}	67.4^{b}
Vermiculite	51.2^{cd}	3.27^{cd}	2.15^{cd}	67.5^{b}
Dry beads				
Perlite	75.5^{a}	4.17^{ab}	2.50^{a}	70.2^{ab}
Soilrite	62.5^{abc}	4.12^{ab}	2.42^{ab}	68.5^{b}
Talc	54.5^{cd}	4.10^{ab}	2.35^{ab}	69.5^{b}
Vermiculite	64.2^{ab}	4.15^{ab}	2.45^{ab}	70.2^{ab}
Sand and soil inoculum	65.2^{ab}	4.60^{a}	2.50^{a}	76.5^{a}
Control	46.5^{d}	2.91^{d}	2.05^{d}	52.9^{c}

* means with the same superscript do not differ significantly at P = 0.05 level by Duncan's multiple range test.

The results of the present study clearly brought out that perlite based dry alginate entrapped VAM inoculum performed better in improving growth and nutrition of onion. Further, the alginate beads could be used for the introduction of VAM fungi along with other beneficial soil microorganisms which promote plant growth.

In the present study root colonization was considerably high in all inoculated plants compared to uninoculated plants. The extent of colonization varied with different VAM fungi. Higher root colonization allows more host fungal contact and exchange of nutrients and helps in better plant growth (Abbott and Robson, 1982). In the present study inoculated onion plants had higher shoot and bulb dry weight, fresh weight and plant P content compared to uninoculated plants. This could be due to low populations of VAM fungi and / or inefficiency of the indigenous fungi. The present study reveals that onion plants differ in their response to inoculaton with different native VAM fungi and confirms that VAM fungi do have host preference (Mallesha *et al.*, 1994). Further it reveals that, ***Gigaspora margarita*** was the best native fungus for inoculating onion plants. The importance of proper selection of efficient VAM fungi for the right medicinal plants and environment may be key for successful use in agriculture.

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