Madras Agric. J., 86(4-6): 256 - 259 April - June 1999
https://doi.org/10.29321/MAJ.10.A00594

GRANULATION OF VA-MYCORRHIZAL INOCULUM

S. SUJATHA LILLY and P. SANTHANAKRISHNAN

Department of Agricultural Microbiology
TNAU, Coimbatore - 641 003.

SUMMARY

An attempt was made to develop a granular VA - mycorrhizal inoculum for easy application to polythene bags as well as nursery raised plants. The clay was used as a substrate for preparation of granules. The granules were prepared with VA-mycorrhizal fungi individually and in combination with *Azospirillum* and Phosphobacteria. The shelf life of granular formulation of VAM fungi with *Azospirillum* and Phosphobacteria. The shelf life of granular formulation of VAM fungi with *Azospirillum* and Phosphobacteria was tested and found that there is a possibility of storing the granules upto 60 days. The response to inoculation of granular formulation prepared with all the three inoculants was superior than two inoculants or uninoculated control.

KEY WORDS: Granular inoculum, VAM fungi, *Azospirillum*, Phosphobacteria

Mycorrhizal plants exhibit greater of phosphorus and other micronutrients particularly when those nutrient are sparingly soluble in soil (Abbott and Robson, 1984) which inturn increase the growth and yield of different crop plants. *Azospirillum*, an associative nitrogen fixing bacterium and phosphate solubilising bacterial are widely used in increasing the growth of crop plants. However these inoculants are used singly. Hall (1979) developed a VAM fungal inoculum which include a mixture of soil, roots and spores adhered to seeds with adhesives Sweet potato entrapped in responded to early and rapid colonization by effective strains of VAM fungi over a practical range of P fertility (Hung *et al.*, 1994). Strullu and Plenchette (1990, 1991) encapsulated the active intraradical form of *Glomus intraradices* in calcium alginate beads. But the disadvantage of this method is that hydrogels are expensive.

VA-mycorrhizal inoculum can come in a range of forms such as soil, spore preparation, dried mycelium, infected roots or more sophisticated products such as alginate beads with incorporated mycelial homogenates. The soil based inocula is too heavy for extensive use in agriculture. For efficient handling, vesicular-arbuscular mycorrhizal fungi may be processed into small and uniform inocula, however processing can reduce the inoculum density. One of the main problems in inoculant technology is the survival of microorganisms during storage. There is a need to develop a quality inoculum and formulation having VA-mycorrhizal fungi and other beneficial microorganisms for easy application to nursery raised plants. Hence a study was conducted to develop a granular formulation which consists of VAM along with *Azospirillum* and Phosphobacteria and tested the response in maize plant due to inoculation of granular formulation.

MATERIALS AND METHODS

(a) Procedure for preparation of granular VAM fungi inoculants

VA- mycorrhizal bulk inoculum (20 g) consisting of infected root, spores and hyphae was mixed with two gram of sterilized clay soil. These materials are mixed well and granules were prepared of pellet size I am size 96 hour old *Azospirillum* broth (5 x 10^8 cells/ml) / 72 hr old Phosphobacteria broth (2 x 10^7 cells/ml) were used instead of sterile distilled water for preparing inoculants which consist of *Azospirillum* or Phosphobacteria. For granules which consist of both inoculants equal quantity of mixer is used for preparing the granules.

(b) The effective response of granular VAM fungi inoculants incorporated with *Azospirillum* and Phosphobacteria were tested by using maize as a host plant.

The treatment details are as follows:

1. *Glomus fasciculatum*
2. *Glomus fasciculatum* + *Azospirillum brasilense*
3. *Glomus fasciculatum* + Phosphobacteria (Ps-1)

Table 1. Survival of *Azospirillum* population in VAM granular inoculum incorporated with *Azospirillum* and Phosphobacteria

Treatment	*Azospirillum* population x 10^{10} cells g^{-1}*				
	Days after storage				
	1	15	30	45	60
Glomus fasciculatum	-	-	-	-	-
Glomus fasciculatum + *Azospirillum*	5.4	4.3	2.1	1.5	0.84
Glomus fasciculatum + Phosphobacteria	-	-	-	-	-
Glomus fasciculatum + *Azospirillum* + Phosphobacteria	4.3	3.5	1.9	1.1	0.64
Control	-	-	-	-	-

* Average of five replications

4. *Glomus fasciculatum* + *Azospirillum brasilense* + Phosphobacteria
5. Uninoculated control

The treatments were replicated with 10 plants per replication. Plant samples were taken from each treatment on 45th day after sowing and the shoot length, root length, plant dry weight were recorded. The VA-mycorrhizal spore count was determined by wet sieving and decanting technique of (Gerdemann and Nicolson, 1963) and the percentage mycorrhizal colonization by staining roots with tryphan blue (Philips and Hayman, 1970) The dry weight of extramatrical hyphae was estimated using the membrane filter technique of Abbott *et al.* (1984). Population of *Azospirillum* in the granules and rhizosphere soil was estimated by adopting MPN technique (Okon

Table 2. Survival of Phosphobacteria population in VAM granular inoculum incorporated with *Azospirillum* and Phosphobacteria

Treatment	Phosphobacteria population x 10^{8} cells g^{-1}*				
	Days after storage				
	1	15	30	45	60
Glomus fasciculatum	-	-	-	-	-
Glomus fasciculatum + *Azospirillum*	-	-	-	-	-
Glomus fasciculatum + Phosphobacteria	10.5	8.5	7.5	4.0	2.5
Glomus fasciculatum + *Azospirillum* + Phosphobacteria	7.5	6.5	6.0	3.5	2.0
Control	-	-	-	-	-

* Average of five replications

et al. 1976) and Phosphobacteria by enrichment culture technique described by Katznelson and Bose (1959)

RESULTS AND DISCUSSION

Initial moisture content and water holding capacity on the day of preparation of granules was 12.1 percent and 54.69 percent which was reduced to 11.94 percent when the granules were stored for 60 days. Population of *Azospirillum* and Phosphobacteria survived well in the granular formulation even after a storage period of 60 days (Table 1 and 2). The efficiency of granular inoculum was tested in maize and the results revealed that by using formulation prepared with *Glomus fasciculatum, Azospirillum* and Phosphobacteria showed increased growth, plant dry weight (Table 3), VAM colonization (Table 4), and dry weight of extramatrical hyphae when compared to twin inoculants or uninoculated control at 45 days after sowing. In the present study, VAM fungi survived well in the dried granules and infected the maize roots even when 60 day old granules were used. The entrapment of microbial cells could enhance the shelf life of the organisms and there is a possibility for combining two or more organisms. VA-mycorrhizal inoculation significantly improved the growth and uptake of Phosphorus. Similar result was obtained by Hall (1979) who reported that white clover plants inoculated with infested soil pellet containing VAM fungi recorded maximum shoot length. VAM colonization and uptake of phosphorus when compared with uninoculated control. Better survival of *Azospirillum* in alginate formulations was reported by Roughley (1968) and Fages (1990). The present investigation clearly indicates that granules which consists of *Glomus fasciculatum, Azospirillum* and Phosphobacteria increased the growth, nutrient uptake and VA-mycorrhizal colonization than pellets having combination of two inoculants. The improved growth can be attributed to cumulative, greater nutrient uptake and production of growth promoting substances by *Azospirillum* and Phosphobacteria.

Table 3. Influence of VAM granular formulation incorporated with *Azospirillum* and Phosphobacteria on the dry weight of host plant (maize)

Treatment	Dry weight (g/plant)*					
	Days after storage					
	1	15	30	45	60	Mean
Glomus fasciculatum	0.696	0.689	0.635	0.617	0.625	0.653
Glomus fasciculatum + *Azospirillum*	0.731	0.732	0.725	0.723	0.719	0.726
Glomus fasciculatum + Phosphobacteria	0.831	0.836	0.828	0.819	0.821	0.828
Glomus fasciculatum + *Azospirillum* + Phosphobacteria	1.272	1.099	1.146	1.169	1.165	1.170
Uninoculated control	0.530	0.527	0.519	0.519	0.560	0.531
Mean	0.812	0.777	0.771	0.769	0.778	0.781

* - 45 days after Sowing

	SEd	CD
Treatment	0.017	0.033
Stages	0.017	0.033
Treatment x stages	0.037	0.073

Table 4. Influence of VAM granular formulation incorporated with *Azospirillum* and Phosphobacteria on per cent root colonization of *Glomus fasciculatum* in maize

Treatment	Per cent root colonization*					
	Days after storage					
	1	15	30	45	60	Mean
Glomus fasciculatum	34	32	32	29	27	31
Glomus fasciculatum + *Azospirillum*	35	33	34	27	26	31
Glomus fasciculatum + Phosphobacteria	38	35	34	28	27	32
Glomus fasciculatum + *Azospirillum* + Phosphobacteria	39	37	35	28	28	33
uninoculated control	14	12	12	13	15	13
Mean	32	30	30	25	25	28

* - 45 days after sowing

	SEd	CD
Treatment	0.60	1.18
Stages	0.60	1.18
Treatment x stages	1.32	2.64

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(Received : December 1997 Revised : August 1998)