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Survival of microbial consortium in granular formulations, degradation and release of microorganisms in soil

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Abstract: The green revolution bought amazing consequences in food grain production but with insufficient concern for agriculture sustainability. Biofertilizers are gaining importance in sustaining agriculture. Various complementing combinations of microbial inoculants for management of major nutrients are necessary for agriculture sustainability. The present investigation was conducted to study the survivability of granular formulations containing Nitrogen fixing bacteria (*Azotobacter chrococcum*), phosphate solubilizing bacteria (*Bacillus megaterium*) and plant growth promoting bacteria (*Pseudomonas fluorescens*) in consortium prepared using different flour. Maximum survival of microbial consortium was observed in Soybean, followed by Soybean + Semolina and Rice inoculant formulations. Minimum survival of population was observed in Ragi + Semolina inoculant formulation. Wheat, wheat + semolina, soybean, soybean + semolina granular formulations have shown better degradation compared to other granular formulations both in presence and absence of tomato and finger millet (*Eleusine coracana*). Among different granular inoculant formulations, Maximum release was observed in soybean granular inoculant formulations in presence of tomato and Finger millet plant and in absence of plant and minimum release of microbial consortium was observed in ragi + semolina granular inoculant formulations during incubation in soil.

Key words: Nitrogen fixing bacteria; Phosphate solubilizing bacteria; Plant growth promoting bacteria; Consortium; Granular formulations; Degradation and release of microorganisms

Introduction

Biofertilizers are living microorganisms; they themselves are not source of nutrients but can help the plant in accessing the nutrient available in its surrounding environments (Suman et al., 2015). *Azotobacter* is major free living in soils so that it can be cultured and produced in artificial medium. It stimulates the density and length of root hairs, increases the growth through hormonal production, increases biomass, increases survival rate and fixes nitrogen. Phosphate solubilizing Bacteria (PSB) plays a major role in the solubilization and uptake of native and applied soil P (Krishnaveni, 2010). The term "plant growth-promoting-rhizobacteria" (PGPR) has been coined to encompass bacteria with plant growth stimulating activity resulting from several mechanisms (Kundan 2015). The production of plant growth stimulating hormones and the suppression of minor plant pathogens by various mechanisms have been suggested to represent the main activities of PGPR. The best microorganisms with PGPR activity are *Pseudomonas* species have been successfully used worldwide as plant growth promoters that play an important role in enhancing growth of several crops.

Biofertilizers are usually prepared as carrier-based inoculants containing effective microorganisms. Assimilation of microorganisms in carrier materials enables easy-handling, long term storage and high effectiveness of biofertilizers. Unfortunately, liquid inoculants has to be applied immediately because it is not easy to handle and it have been limited due to inherent constraints like, least protection against desiccation, difficult transportation and application should not done under high temperature. In last decade, there has been increased interest in the development of granular inoculant formulation for agriculture (Hegde and Brahmaprakash 2012, Bashan *et al.,* 2013) by encapsulation with various polymers, followed by drying, have been proposed. A major advantage of the granular inoculants is the ability of control placements and application rate as needed, avoids damage to fragile seed coats and overcomes the adverse effects of pesticides and fungicides applied to seeds and it also reduces the risk of losing viable bacteria through seed drilling equipment or when the seed coat is lifted out of the ground during germination (Deaker *et al.,* 2004). In this context, preparation of granular inoculant formulation using different grain flours as substrate appears to be a better alternative than powder or liquid formulations.

Materials and Methods

The present study deals with methods of preparing granular formulations comprising of microorganisms and substrate. Granules were prepared by using eight types of grain flours as substrates, e.g. Rice flour, Rice flour + Semolina (Sooji) (2:1), Ragi flour, Ragi flour + Semolina (Sooji) (2:1), Wheat flour, Wheat flour + Semolina (Sooji) (2:1), Soybean flour, Soybean flour + Semolina (Sooji) (2:1). Each substrate was taken in a sterile container having 2 % CMC (carboxy methyl cellulose) and mixed well to get a dough.

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The granules were prepared and immersed in boiling water (100^0 C) for 2-3 minutes. Air dried granules were soaked overnight in *Waksman No. 77* broth (Waksmann, 1961) containing *A. chroococcum*, Pikovskaya's broth (Pikovskaya, 1948) containing *B. megaterium* and King's B broth (King *et al*., 1954) containing *P. fluorescens* and *mixed broth containing* microbial consortium of *A. chroococcum* + *B. megaterium* + *P. fluorescens*. These granules were air dried and packed in polythene cover. Initially the nitrogen fixing bacterium *(A. chroococcum),* Phosphate solubilizing bacterium (*B. megaterium)* and Plant Growth promoting rhizobacteria or PGPR (*P. fluorescens*) were tested for compatibility of growth by cross streak assay on Nutrient agar medium (Anandaraj and Delapierre, 2010*)*.

Granular prepared using different grain flour were stored for 240 days at room temperature (25 ± 3^0C). The number of viable cells of *A. chroococcum, B. megaterium and P. fluorescens* in consortium, were estimated at 0, 15, 30, 60, 90,120, 160 and 180 days interval. Viable counts were enumerated by plate count method **(**Aneja, 1996**)** and number of colony forming units (Cfu) were recorded at 10^{-7} and 10^{-8} dilutions, and converted as $\log_{10}$ cfu per granule.

Degradation of granular inoculant formulations:

The experiment was carried out in presence and absence of tomato (*Lycopersicum esculentum*) and finger millet (*Eleusine coracana*) plants, respectively. Microbial consortium containing three different microorganisms, *A. chroococcum* + *B. megaterium* + *P. fluorescens* in eight selected granular inoculants formulations like rice granules, ragi granules, wheat granules, soybean granules, rice + semolina granules, ragi + semolina granules, wheat + semolina granules and soybean + semolina granules was placed in a nylon cloth bag (3 granules in each bag) of 3 cm x 3 cm and 65 µm mesh size, then tied with a piece of thread. This was placed 2 cm below the soil near the germinating seeds in plastic pots containing 250 g of soil. Similarly, nylon bag containing mixed microbial consortium of *A. chroococcum* + *B. megaterium* + *P. fluorescens* in different granular inoculants formulations were placed 2 cm below the soil without germinating seeds in plastic pots containing 250 g of soil.

The observations were recorded at an interval of 3 days for 30 days and observations were recorded by observing under olympus stereo microscope for degradation with following scale.

0. No Degradation
1. Slight visible degradation on granule edges
2. One half to three-fourth of granules were degraded
3. The granules are fully degraded to the extent that they were not found in the nylon bag.

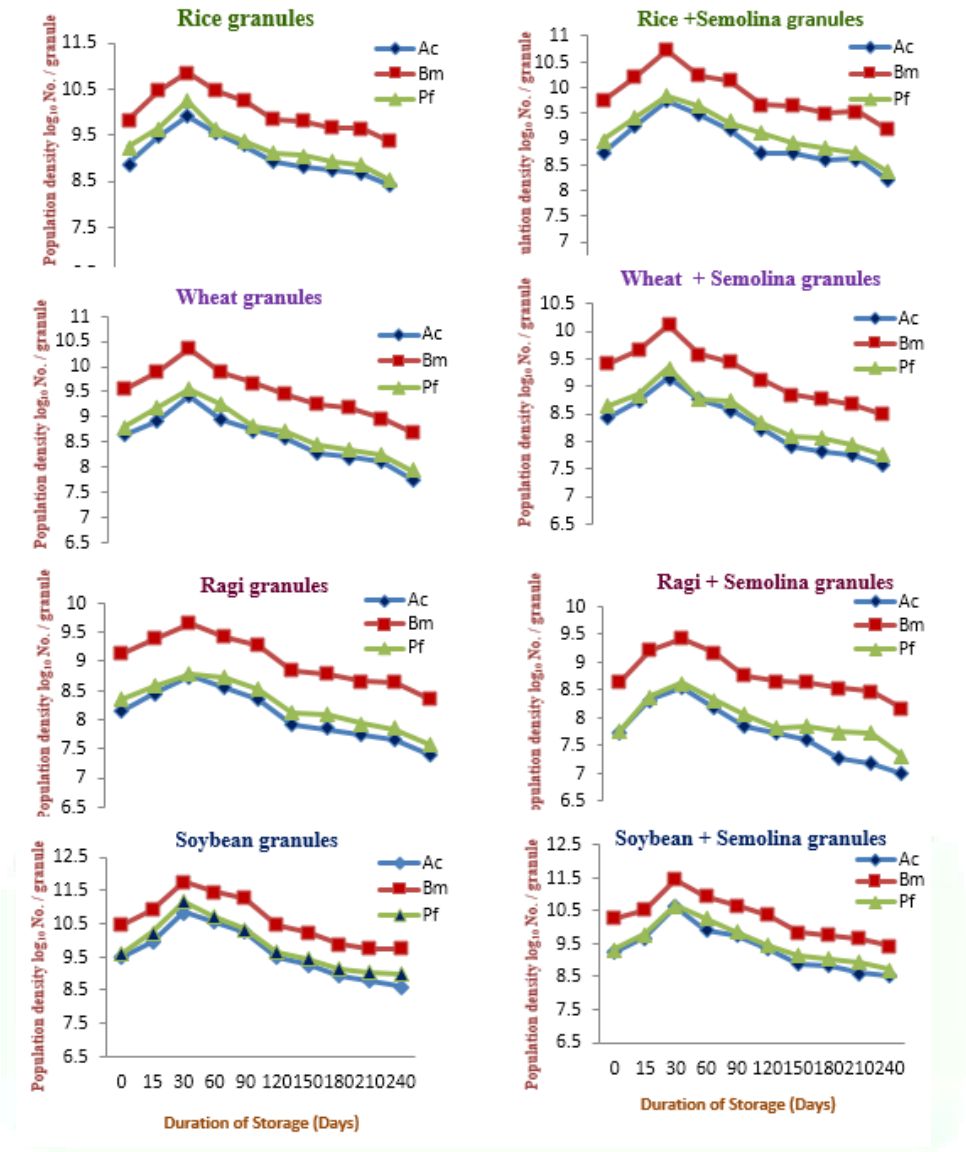
Release of microorganisms in soil:

The Release of *Azotobacter chroococcum* + *Bacillus megaterium* + *Pseudomonas fluorescens* in consortium from selected granular substrate inoculant formulations in presence of tomato and finger millet and in absence of plant were observed during degradation in soil. The release of microbial consortium (*Azotobacter chroococcum* + *Bacillus megaterium* + *Pseudomonas fluorescens*) in selected granular inoculant formulations during degradation in soil with tomato plant and finger millet plant and in absence of plant were monitored for 30 days at 3 day intervals. Viable counts were enumerated by plate count method and number of colony forming units (Cfu) were recorded at 10^{-7} and 10^{-8} dilutions, and converted as $\log_{10}$ cfu per granule.

Result and Discussion

The study revealed that Viable cells counts for total bacteria during storage was found to reduce from the original number during initial days of storage (Hasarin and Viyada., 2008). The quality of microbial inoculants depends primarily on the number of viable cells present in the inoculants (Lupwayi *et al*., 2000). Based on results, survival of microbial consortium in all the selected substrates during storage upto 240 days was increased during initial 30 days of storage and gradually declined at the end of 240 days of storage. Among the selected substrate, survival of *A. chroococcum, B. megaterium* and *P. fluorescens* in microbial consortium of three microorganisms in all the selected granular inoculant formulations was also highest in Soybean granular inoculant formulations compared to other inoculant formulations *i.e*., $\log_{10}$ 10.84 during initial 30 days of storage to 8.61 cfu per granule of *A. chroococcum* population at the end of 240 days of storage. In the same way, $\log_{10}$ 11.76 to 9.73 cfu per granule of *B. megaterium* population and $\log_{10}$ 11.18 to 8.97 cfu per granule of *P. fluorescens* population followed by Soybean + Semolina inoculant formulations and least was observed in Ragi + Semolina inoculant formulations. Similar, results were also reported by Shankar and Brahmaprakash (2005), (Gopalan *et al*., 2007), Rekha *et al*., 2007 and Gandhi and Saravanakumar (2009). These findings also support the reports of Kumar and Gupta (2010) who reported that viability of *A. chroococcum* in carrier formulations was increased upto nine months of storage. Similar results were also reported by Bashan *et al*., (2002) and Stella and Sivasakthivelan (2009). According to Bureau of Indian Standards (BIS) (Yadav, 2009), minimum viable cells counts for *A. chroococcum*, *B. megaterium* and *P. fluorescens* inoculants are cfu minimum $5x10^7$ cells/g of carrier material and no contamination at 10^{-5} dilution. In the present study, survival of granular inoculant formulations prepared using selected substrates is going to meet the BIS as prescribed by National Center of

Organic Farming, Department of Agriculture and Cooperation, Government of India. This could be due to soybean flour are rich in nutrients and they are organic in nature, thus they provide nutrition for growth and multiplication of bacteria inside the granules. The viability of *B. megaterium* population increased initially and declined rapidly during storage at room temperature for six months contradicting with reports of Kanjanamaneesathian *et al.*, (2000). These results are in agreement with the earlier reports of Trivedi *et al.*, (2005), Gomathy *et al.*, (2007) and Menaka and Alagawadi (2007).



Survival of Survival of microbial consortium (*Azotobacter chroococcum* +*Bacillus megaterium* + *Psuedomonas fluorescens*) in different granular substrate inoculants formulations

Degradation of eight selected granular inoculants formulations in soil occurred in both presence and absences of tomato and finger millet (*Eleusine coracana*). Among all these eight selected granular inoculant formulations, wheat, wheat + semolina, soybean, soybean + semolina granular formulations have shown better degradation compared to other granular inoculant formulations both in presence and absence of tomato and finger millet (*Eleusine coracana*). Result revealed that, wheat, wheat + semolina, soybean, soybean + semolina granular formulations started to degradation on 3rd day and complete degradation was observed on 12th day of incubation in presence of tomato and finger millet (*Eleusine coracana*) in soil. However, degradation in absence of plant started to disintegrate on 6th day and complete degradation was observed on 15th day of incubation. It might be due to the increased granular diameter during the first 20h in soil due to swelling after absorption of water, after that it

remains constant all the time and then they developed the potential to deliver their active components (Ivanova, 2005). Biodegradation of granules also depends on soil microflora and the rhizosphere effect. Higher the density of microflora surrounding the granules, faster is the biodegradtion. Biodegrdation is carried out from the outside layer inward, thus sequentially exposing different layers of the granules to the surrounding soil until degradation of the granules is complete. Rhizosphere is characterized by greater microbial activity than soil away from plant root. The intensity of such activity depends on the distance to which exudation of plant root system migrate. If the granules degrade early, entrapped microbial cells will be released into soil and colonize the plant rhizosphere. Similar results were reported by Bashan (1986), In all the eight selected granular inoculant formulation containing *A. chroococcum* + *B. megaterium* + *P. fluorescens in* consortium and the release of all these three microorganisms was observed maximum on 6th day of incubation and declined gradually in presence of tomato and finger millet plant in soil. The release of microbial consortium from soybean granular inoculant during 6 - 9 days of incubation was $\log_{10}$ 12.06 - 10.86 number per granule of *A. chroococcum,* $\log_{10}$ 12.28 -11.16 of *B. megaterium* and $\log_{10}$ 11.86 - 10.32 number per granule of *P. fluorescens,* followed by soybean + semolina granular formulation, rice granular formulation, rice + semolina granular formulation, wheat granular formulation, wheat + semolina granular formulations, ragi granular formulation. A minimum population density of microbial consortium was observed in ragi + semolina formulation which was $\log_{10}$ 9.96 to 8.21 number per granule of *A. chroococcum,* $\log_{10}$ 10.12 to 8.36 of *B. megaterium* and $\log_{10}$ 9.45 to 8.09 number per granule of *P. fluorescens*. Among *A. chroococcum* + *B. megaterium* + *P. fluorescens* in microbial consortium, population density of *B. megaterium* was higher followed by *P. fluorescens* and *A. chroococcum*.

Release of microbial consortium in selected granular inoculant formulations were monitored for 30 days at 3 days interval in absence of any plant. Observations recorded were based on viable cell counts of microbial consortium during degradation.

Maximum release of microbial consortium in presence of tomato plant during incubation in soil was observed in soybean inoculant formulations $\log_{10}$ 12.06 to 10.86 number per granule of *A. chroococcum,* $\log_{10}$ 12.28 to 11.16 number per granule of *B. megaterium* and $\log_{10}$ 11.86 to 10.32 number per granule of *P. fluorescens* followed by soybean + semolina inoculant formulations and minimum was observed in ragi + semolina inoculant formulations .Similarly, maximum release of microbial consortium in all the selected formulations during incubation in presence of finger millet (*Eleusine coracana*) was observed in soybean inoculant formulations $\log_{10}$ 11.86 to 10.32 number per granule of *A. chroococcum,* $\log_{10}$ 12.28 to 11.16 number per granule of *B. megaterium* and $\log_{10}$ 12.06 to 10.86 number per granule of *P. fluorescens* followed by soybean + semolina inoculant formulation and minimum was observed in ragi + semolina inoculant formulations. However, release of microbial consortium in absence of plant was also observed maximum in soybean inoculant formulation $\log_{10}$ 11.26 to 9.87 number per granule of *A. chroococcum,* $\log_{10}$ 11.66 to 10.25 number per granule of *B. megaterium* and $\log_{10}$ 11.43 to 10.10 number per granule of *P. fluorescens* followed by soybean + semolina granules $\log_{10}$ 11.08 to 9.53 number per granule of *A. chroococcum,* $\log_{10}$ 11.38 to 10.06 number per granule of *B. megaterium* and $\log_{10}$ 11.23 to 9.74 number per granule of *P. fluorescens* and minimum was observed in ragi + semolina inoculant formulations. Substrates are organic in nature and they are rich source of protein that provides nutrition for growth and multiplication of bacteria entrapped in granular substrate. These results are in agreement with the findings of Elsas *et al*., 1992, Ivanova *et al*., 2005 and Bashan (1986). Due to the rhizosphere effect, granule degrades early. Entrapped microbial cells after secondary multiplication have the ability to release the bacteria into soil and colonize the plant.

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