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A REVIEW

Biofertilizers - A noble technology for sustainable agriculture

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Green revolution played a major role in reducing food crisis by introducing and developing high yielding varieties (HYV). Since, these varieties are highly fertilizer responsive, the usage of fertilizers increased to maximum extent with an aim to get higher productivity. Indiscriminate use of chemical fertilizers, pesticides and other highly productive systems had a serious effect on our environment. To attain sustainable production from soil reduced use of chemical fertilizers by substituting it with some new technologies is highly required. The bio fertilizer is one such technology which is gaining importance in integrated plant nutrient system as it maintains soil health, substitutes the chemical fertilizers thereby reducing their usage, increases microbial population of soil and minimizes environmental pollution.

Key words : Biofertilizers, Chemical fertilizers, Environmental pollution, High yielding varieties, Mass production

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INTRODUCTION

Crop plant need mineral nutrients essential for their growth and development.Soil acts as initial source but nutrients given by soil are not sufficient to meet the requirement thereby creating a nutrient consumption gap, this gap has to be filled by manures only during pre-green revolution and substituted by fertilizers during post green revolution till date. This inclusion on one hand solved the food crisis of our country but on other had its synthetic origin had a serious negative impact on soil physical and chemical properties and sometimes even making the soil toxic. If this continues soil may turn unproductive in the near future. In order to tackle this problem we should be adopting an alternative strategy like INM for reducing the use of chemical fertilizers and substituting the remaining nutrient gap by using organic sources like bio-fertilizers, organic manures, green manures etc. Biofertilizers technology found to have greater scope in reducing nutrient consumption gap by increasing nutrient availability in soil and increasing the population of beneficial microbes in soil thereby improving soil properties as suggested by Rathore *et al.* (2011) during their experiment in black gram-wheat cropping system at Udaipur found that bulk density is getting lowered and nutrient uptake is improved when P_2O_5 is integrated with FYM @ 5 t/ha and uptake of phosphorus is increased by integrating PSB and VAM to FYM+P_2O_5. Generally, bio-fertilizers are either liquid suspensions or wettable powders with living or latent cells of microbial organisms obtained after mass multiplication of pure mother culture of a known strain. Knowledge on different types of bio-fertilizers known to us till date, their morphology, mode of action and their application methods are very important for a scholar inorder to solve the drawbacks in this technology and this knowledge generates faith in farmers and thereby technology penetration rate increases.Thus, in the development and implementation of sustainability in agriculture, biofertilization plays a major role in decreasing environmental pollution and the conservation

of nature. This biofertilizer production procedure basically includes collection of soil sample and isolation of required species of living organism from the soil by culturing it on a suitable medium and mass producing it by providing suitable temperatures for their incubation for a specified period of time. The resultant of mass production undergoes some quality control and then it is considered to be as biofertilizers.There are many biofertilizer companies in India who have commercialized their products in market and mostly they are available in two forms 1) liquid suspensions, 2) wet table powders. In order to convert a liquid biofertilizer to solid it has to be mixed with a suitable sterilized and neutral carrier. This article completely focused on these basic production procedures mentioned above.

Collection and isolation techniques of different biofertilizers from soil:

Azotobacter:

Collect the soil sample in a sterilized polythene bags as described by Reddy *et al*. (1986).Verma and Paul (2000) in a biofertilizer manual the isolation method which includes taking 10g of representative soil sample collected to be suspended in 90ml of distilled water and shaken for 10minutes. Aliquots of 0.1ml dilution was poured on Jensen's agar plates.These plates were incubated at 28-30^{0}C for 3 days in an incubator. Slimy, soft and mucoid colonies were observed after the incubation period. Purification was done by repeated sub-culturing using Jensen's agar.Pure cultures after isolation were subjected to Gram staining and Gram negative isolates were further subjected to biochemical tests by which we can identify the strain.

Rhizobium:

Collect some roots of legumes with nodules on it and then wash them with tap water to remove mud and soil particles on its surface and now washed samples are treated with 5% H_2O_2 for surface sterilization. There after they are washed for 3-4 minutes with sterile water to get rid of sterilant completely and then treat it with 70% ethyl alcohol for about one minute and then with 0.1% $HgCl_2$ for two minutes followed by washing the samples with distilled water under asceptic conditions and crushing inside sterile pestle and mortar. A suspension was made from these crushed nodules ,after sterilization plate that suspension on Yeast Mannitol Agar (YEMA) medium as described by Rajendran *et al*. (2008),which consists of 1% congored dye and incubate those plates at 28-30^{0}C for 1 day and growth is observed on the plate. Pure cultures after isolation were subjected to Gram staining and gram negative isolates were further subjected to biochemical tests by which we can identify the strain.

Azospirillum:

Collect the soil from rhizosphere region (top 2-3cm) and root sample by uprooting the plant and collecting them in a polythene bag and preserve in a refrigerator. Wash

Table 1 : Classification of types of biofertilizers

Sr. No.	Groups	Examples
Nitrogen (N_2) fixing biofertilizers		
1.	Free-living	*Azotobacter, Clostridium, Anabaena, Nostoc*
2.	Symbiotic	*Rhizobium, Frankia, Anabaena azollae*
3.	Associative Symbiotic	*Azospirillum*
P Solubilizing biofertilizers		
1.	Bacteria	*Bacillus megaterium* var. *phosphaticum, Bacillus circulans, Pseudomonas striata*
2.	Fungi	*Penicillium* sp, *Aspergillus awamori*
P Mobilizing biofertilizers		
1.	*Arbuscular mycorrhiza*	*Glomus* sp., *Gigaspora* sp., *Acaulospora* sp., *Scutellospora* sp. and *Sclerocystis* sp.
2.	*Ectomycorrhiza*	*Laccaria* sp., *Pisolithus* sp., *Boletus* sp., *Amanita* sp.
3.	*Orchid mycorrhiza*	*Rhizoctonia solani*
Biofertilizers for micro nutrients		
1.	Silicate and Zinc solubilizers	*Bacillus* sp.
Plant growth promoting rhizobacteria		
1.	Pseudomonas	*Pseudomonas fluorescens*

Source: Himachal Motghare and Rashmi Gauraha (2012);"Biofertilizers –types and their application".

the root samples with distilled water until it gets rid of soil particles adhered to it and cut them into small pieces and dip them in 70% alcohol for 2 minutes followed by washing with distilled water repeatedly and crush them in a sterile mortar and pestle and a suspension made is plated on a NFB medium by taking 0.1 ml of the the suspension and incubated for 3 days at 37^0C resulting in production of pellicle (Dobereiner, 1980). These pellicles were observed microscopically and sub-culture to obtain a pure Gram negative, vibroid and actively motile cells and then perform some biochemical tests and isolate the pure strains.Agitate it continuously for 4-9 days until its cell count reaches to 10^8-10^9 cells/ml.Then it is mixed with suitable carrier (Amrutha *et al.*, 2014).

Phosphorus solubilising bacteria(PSB) :

Collect soil sample from rhizosphere regions of different crops. Remove all plant residues,pebbles,gravels etc. from the soil, crush it with a mortar and pestle and then serial dilute 1g of soil sample and 10^{-3} and 10^{-4} dilution is plated using pour plate method on Pikovskaya's agar medium. The inoculated plates were incubated at 32^0C for 9 days (Ranjan *et al.*, 2013) . Purification was done by repeated sub-culturing. Pure cultures after isolation were subjected to Gram staining and biochemical tests by which we can identify the strain.

Mass production of biofertilizers:

Azotobacter:

Transfer identified pure strain to the liquid Jensen's broth,place it on a incubater shaker and set it to 28-30^0C and maintain 130 rpm for 3-4 days to prepare starter culture which is then transferred to fermenter in batch culture and maintain the strain at 30^0C by agitating it continuously for 4-9 days until its cell count reaches to 10^8-10^9 cells/ml.Then it is mixed with suitable carrier(activated charcoal mostly) to convert liquid fertilizer to solid fertilizer (Amutha *et al.*, 2014).

Rhizobium:

Transfer identified pure strains of rhizobium in a conical flask with yeast mannitol broth in a conical flask and incubate it at 30^0C in an incubator shaker and then transfer the starter solution to the fermenter in batch culture maintain the strain at 30^0C by agitating it continuously for 4-9 days until its cell count reaches to 10^6-10^8cells/ml.Then it is mixed with suitable carrier (activated charcoal mostly) to convert liquid fertilizer to solid fertilizer.

Phosphorus solubulising bacteria(PSB):

Make a starter solution Pikosvakaya medium broth and then transfer the solution to fermentor and agitate it for 7 days at about 28-30^0C temperature and after 1week test the cfu if it reaches 10^9cells/ml then stop the process and store the liquid fertilizers at cool temperatures.If the fertilizer is to be formulated as wettable powder then mix it with suitable neutral sterile carrier at 2:1 ratio.

Azolla:

Make 20*2m size pits in a field with suitable bunds and irrigation channels with a water depth of atleast 10cm and 20 l of water is added in each plot and inoculated with 8-10 kg azolla. Now add 100g single super phosphate in 2-3 split doses at an interval of 4 days to each plot and add furadoncarbendazim (3% a.i. granules)@100g/plot can be applied after a week of inoculation and this whole process results in production of 100-150 kg azolla after 15 days will get ready to harvest (Yadav *et al.*, 2014).

Carriers:

Incorporation of micro-organisms in a carrier enables easy handling, long term storage and high effectiveness of biofertilizers. Sterilize the carrier by any suitable method. Different materials that can be used as carriers are clay minerals, diatomaceous soil, rice and wheat bran, peat,lignite, activated charcoal, organic matter. Different adhesive substances used to hold the carrier + microbe mixture on the surface of seed are popularly gum Arabic,methyl ethyl cellulose and vegetable oils are in use (Bhattacharjee and Dey, 2014). According to a research by Sparrow and Han (1981) found that vermiculite supported 10^7 cells of rhizobia g of carrier even 30 weeks of period when compared with peat, powdered peanut shell, corn cobs. Gaind and Gaur (1990) suggested charcoal-soil mixture as the best carrier concerned with crop productivity, for phosphate solubilizing micro-organisms. Tilak and Subba Rao (1978) tested 11 carrier materials and used charcoal and coconut shell powder as amendments to some carrier materials for rhizobia and they concluded that lignite, pressmud and FYM amended with coconut shell powder or charcoal serve as good substitute for peat under Indian conditions.

Role of biofertilizers in agriculture:

The bio-fertilizers plays major role in improving soil properties, Nutrient use efficiency and thereby final yield. In addition, their addition also improves the soil biota and minimizes the use of chemical fertilizers.The uptake nitrogen by cereals through BNF ranges from 7–58 % (Baldani *et al.,* 2000); N uptake by rice through release of azolla ranges from 70–110 kg N ha^{-1} (Wagner, 1997). Many bacterial genus (e.g. *Pseudomonas*, *Burkholderia*, *Acidothiobacillus*, *Bacillus* and *Paenibacillus*) are able to release potassium from minerals such as mica, illite,muscovite, biotite and orthoclases (Bennett *et al.,* 1998).

Advantages of biofertilizers:

– Harnesses atmospheric nitrogen and makes it available directly to the plants.

– Increases phosphorus uptake by solubilising and releasing unavailable phosphorus.

– Enhances root proliferation due to release of growth promoting hormones.

– Increases the crop yields by 10 – 25 %.

– Improves soil properties and sustain soil fertility.

– Are cost effective and environment friendly.

– Benefit to cost ratio of bio-fertilizers is fairly high.

Constraints in biofertilizer production:

– Lack of region specific and appropriate strain

– Unavailability of suitable carrier

– Mutations during fermentation

– Lack of awareness of farmers

– Inadequate and inexperienced staff

– Lack of quality assurance

– Unassured demand

– Antagonistic effects of native microbes in soil whch effects biofertilizer microbes survival.

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