

Biofertilizers for Sustainable Production in Oil Seed Crops

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Abstract: Many oilseeds are cultivated as rainfed crop with poor input resources. This will have greater impact on plant health particularly plant nutrition and pests that include insect, non-insects pests, diseases and weeds. Further, these problems many a times are region specific and depends on the soil condition and climate. Many oilseeds are cultivated as rainfed crop with poor input resources. This will have greater impact on plant health particularly plant nutrition and pests that include insect, non-insects pests, diseases and weeds. Total oil seed production in India. Biofertilizer are the products containing cell of different types of beneficial microorganisms. Thus, biofertilizers can be important components of integrated nutrients management. Microorganisms that are commonly used as biofertilizers component are nitrogen fixers (N-fixer), solubilizer (K-solubilizer) and phosphorus solubilizer (P- solubilizer), or with the combination of molds or fungi. These potential biological fertilizers would play key role in productivity and sustainability of soil and also protect the environment as eco-friendly and cost effective inputs for the farmers. With using the biological and organic fertilizers, a low input system can be carried out and it can be help achieving sustainability of agricultural farms.

Keywords: Oil seed crops, biofertilizers , integrated nutrient management, sustainability

INTRODUCTION

Soil is a dynamic living body and contains enormous numbers of diverse living organisms. Organic agricultural practices aim to enhance biodiversity, biological cycles and soil biological activity so as to achieve optimal natural systems that are socially, ecologically and economically sustainable. Oil seeds contributed to yellow revolution and significant role has been played by introduction of soybean and sunflower. Oilseeds constitute a second largest agricultural commodity after cereals in India occupying 13% of gross cropped area and are cultivated in an area of 25.73 million hectares with a production of 26.67 million tonnes and productivity of 1037 kg per hectare. The oilseed form essential part of human diet. Besides it produces basic raw materials for agrobased industries. India ranks first in production of castor and sesame, second in groundnut, third in rapeseed & mustard and safflower, fifth in soybean and sixth in linseed in the world. In the last one decade, in spite of increase in production of vegetable oils, the imports also increased due to increase in consumption levels creating huge demand and supply gap. This resulted into import of edible oil worth Rs. 57,000/- crores to meet the domestic consumption. Many oilseeds are cultivated as rainfed crop with poor input resources. This will have greater impact on plant health particularly plant nutrition and pests that include insect, non-insects pests, diseases and weeds. Further, these problems many a times are region specific and depends on the soil

condition and climate. Many oilseeds are cultivated as rainfed crop with poor input resources. This will have greater impact on plant health particularly plant nutrition and pests that include insect, non-insects pests, diseases and weeds. Total oil seed production in India.

Microbes as biofertilizers

A group of rhizosphere bacteria (rhizobacteria) that exerts a beneficial effect on plant growth is referred to as plant growth promoting rhizobacteria or PGPR [1]. PGPR belong to several genera, e.g. *Agrobacterium*, *Alcaligenes*, *Arthrobacter*, *Actinoplanes*, *Azotobacter*, *Bacillus*, *Pseudomonas* sp., *Rhizobium*, *Bradyrhizobium*, *Erwinia*, *Enterobacter*, *Amorpho sporangium*, *Cellulomonas*, *Flavobacterium*, *Streptomyces* and *Xanthomonas* [2]. PGPR increased recently as a result of the numerous studies covering a wider range of plant species and because of the advances made in bacterial taxonomy and the progress in our understanding of the different mechanisms of action of PGPR. In all successful plant microbe interactions, the competence to colonize plant habitats is important. Single bacterial cells can attach to surfaces and, after cell division and proliferation, form dense aggregates commonly referred to as macro colonies or biofilms. Steps of colonization include attraction, recognition, adherence, invasion (only endophytes and pathogens), colonization and growth, and several strategies to establish interactions [3]. Plant roots initiate crosstalk with soil microbes by producing

signals that are recognized by the microbes, which in turn produce signals that initiate colonization [4]. PGPR reach root surfaces by active motility facilitated by flagella and are guided by chemotactic responses. This implies that PGPR competence highly depends either on their abilities to take advantage of a specific environment or on their abilities to adapt to changing conditions or plant species [3]. Use of bio-fertilizers in integrated nutrient management is important for optimization of plant nutrition.

Bio-fertilizers play vital role for increasing the number of microorganisms and accelerate certain processes in plants which can change the available form of many nutrients for crops. Although nitrogen is the key element in increasing productivity but large rates of fertilizer N loss to the environment could cause a serious environmental problem such as groundwater contamination. Nitrogen fertilizer application and seed biopriming with PGPR can increase quantitative and qualitative yield of Safflower. Bio-fertilizers have a positive effect on growth, yield and yield component of many oil seed crops. Fertilizer management is one of the most important factors in successful cultivation of crops affecting yield quality and quantity [5]. Overuse of different chemical fertilizers is one of the causes for the degradation of environment and soil. Bio fertilizers are the new and technically advanced way of supplying mineral nutrients to crops. Compared to chemical fertilizers, they supply nutrients for plant needs, minimizes leaching, and therefore improves fertilizer use efficiency. The study on the use of biofertilizers in different crops revealed that for getting the highest seed yield we should apply both nitrogen and phosphate biofertilizers. Although most agricultural soils have large amounts of inorganic and organic P, these are immobilized and mostly unavailable. Therefore, only a very low concentration of P is available to plants and many soils are actually P deficient. Hence, alternative strategies such as use of biofertilizers to increase P availability and crop yield are required.

Integrated effect of biofertilizers and plant nutrients on some major oilseed production

Groundnut

For sustained groundnut production the modern farming demands integrated use of organic and inorganic fertilizers along with bio-fertilizers. Biofertilizers enriched vermicompost (E2 - E6) shows increased growth attributes, yield, protein and oil content than Co and E7. The organic fractions of flower waste vermicompost and the microorganisms in the biofertilizers could be an alternative to chemical fertilizers to improving the growth and yield of groundnut [6]. Application of 50% RDF + 5 ton FYM/ha + *Rhizobium* + PSB to groundnut provided an alternate best option of nutrient management in groundnut- pigeonpea relay intercropping system [7]. Seed inoculation with biofertilizers (*Rhizobium* + PSM) significantly increased the plant height, nodules per

plant, yield attributing characters and yield of groundnut. Manuring the crop with FYM 6 t/ha + *Rhizobium* + PSM gave significantly 40.19 and 35.96 per cent higher pod and haulm yields of groundnut, respectively over no manuring. Fertilizing the crop with vermicompost 2.0 t/ha + *Rhizobium* + PSM and FYM 3.0t/ ha + *Rhizobium* + PSM found equally effective and significantly superior to control in respect to growth parameters, yield attributes and yield of groundnut [8]. The crop inoculated with biofertilizers (*Rhizobium* +phosphate-solubilizing bacteria) recorded significantly higher pod (1.74 t/ha) and haulm yields (3.64 t/ha) of groundnut and grain (5.34 t/ha) and straw yields (5.52 t/ha) of succeeding rice over the control. Inoculation of groundnut with biofertilizers significantly increased N, P, K uptake by groundnut and succeeding rice (except N uptake by rice straw) as well as total N, P and K uptake (393.1 kg/ha), rice–grain equivalent yield (9.92 t/ha), net returns (66.8×103 /ha) and benefit: cost ratio (2.01) in groundnut–rice cropping system over no inoculation [9].

Soybean

Biofertilizers can be used as Seed treatment: *Rhizobium* culture @ 500 g / 75 kg seed/acre. Cross inoculation group: *R.japonicum* slow growing type strain will form the root nodules in soybean crop and fixes nitrogen efficiently. Inoculation of soybean seeds with proper bacterial strains increased seed production by 70-75% Simanungkalit *et al.*, [10]. The application of phosphate-solubilizing bacteria in soybean crop revealed that most examined traits was better than chemical fertilizer [11]. Soybean plants, as inoculated *Bradyrhizobium japonicum* with plots appeared much greener [12]. Some of the popular varieties of soybean (J.S-335, PK-1029, MACS-124 and LSB-1) in the state were treated with commercially available *Bradyrhizobium* biofertilizer and compared with no *Bradyrhizobium* inoculation plus recommended dose of N fertilizer and a Control without fertilizer and without *Bradyrhizobium* inoculation. Different plant characteristics *viz.*, nodule number, nodule dry weight, plant dry weight and nitrogen content were determined on 45 day old plants to identify nitrogen fixation responsive soybean variety among the soybean varieties. Among the popular four soybean varieties J.S-335, PK-1029, MACS-124 and LSB-1 used in pot culture experiment conducted in the present study, variety J.S-335 showed significantly better response to *Bradyrhizobium japonicum* biofertiizer treatment compared to other varieties [13].

Sunflower

Inoculation of biofertilisers and sulphur have significant effect on yield and yield attributes of sunflower. However, PSB + VAM + *Azotobacter*, as well as application of sulphur @40 kg ha⁻¹ may be considered as the best treatment for sunflower, with respect to height, total chlorophyll content, thalamus diameter, weight of thalamus, filled seeds capitulum-1

and 100 seed weight, grain yield, stalk yield, biological yield, harvest index and oil content [14]. The crop was fertilized with respective dose of NPK of 80:100:100 kg ha⁻¹. Results revealed that inoculation of biofertilizers significantly affected plant height and total chlorophyll content. Biofertilizers also significantly increased yield attributes, viz. thalamus diameter, weight of thalamus, filled seeds capitulum-1, and 100 seed weight (g), as well as seed and biological yield and oil content. The biofertilizers used as inoculum for seeds treatment of sunflower cv. Geza 1 were *Azospirillum* (nitrogen fixing bacteria, N.F.B.) and *Bacillus polymyxa* (phosphate dissolving bacteria, P.D.B.) and their mixture. Both bacterial inoculants and their mixture showed an increase in growth parameters, nutrients content and yield when compared to the control (full dose of NPK chemical fertilization). The results revealed that biofertilization treatments of *Azospirillum* + *Bacillus* plus 100% chemical fertilizers produced the highest values in all growth and yield parameters compared with the control [15].

Safflower

It has been found that application of 100% RDF + *Azospirillum* + PSB improved the yield and significantly increased the uptake of NPK by safflower [27]. The effect of bio-fertilizer on yield and yield components of safflower under dry land conditions, a factorial experiment was conducted based on randomized completed block design with three replications in Ilam, Iran in 2008-2009. The factors consisted of three levels of nitrogen fertilizer (0, 30 and conventional consumption (60) kg ha⁻¹) and bio-fertilizer (non-inoculation, *Azotobacter* and *Azospirillum*). The results showed that nitrogen rates had significant effects on yield and yield components. According to results of present study, it can be concluded that farmers can obtain the same safflower yield if they apply half of conventional consumption of nitrogen with bio-fertilizers. In this way, decreasing nitrogen fertilizer can be reduced the environment pollution and developed sustainable agriculture [16]. Seed inoculation with *Azospirillum* alone resulted in enhanced growth in terms of plant height and other yield attributing characters and was on par with 50% recommended inorganic N application. Seed inoculation with *Azospirillum* both alone and in combination with 50% recommended inorganic N has resulted in on par seed yield with treatment supplied with 50% N and 100% inorganic nitrogen respectively. Interestingly, seed inoculation with *Azotobacter* did not cause any improvement in seed yield. This assumes that seed inoculation with *Azospirillum* could result in absolute saving of 50% of inorganic nitrogen [17].

Sesame

The oil yield increased by 33.3%, while protein yield increased by 47.5% with treatment of half dose of fertilizer along with LES 4 (*Pseudomonas aeruginosa*) bacterized seeds, as compared to full dose

of fertilizers [18]. Seed production of six sesame genotypes after seed inoculation with either *Azotobacter* or *Azospirillum* along with Phospho-bactrin and Potash Solubilising bacteria in both cases, un-inoculated control was included for comparison. *Azotobacter* was able to produce seeds with either significantly higher or similar 1000 seed weight. Variation in performance of individual genotypes after different bio-fertilizer application indicated existence of genotype specific response for the parameters studied [19]. Wayase *et al* [20] reported that highest number of seeds plant⁻¹, capsules plant⁻¹, seed yield plant⁻¹, weight of capsule plant⁻¹, test weight, seed yield (kg ha⁻¹), straw yield (kg ha⁻¹), biological yield (kg ha⁻¹) and harvest index was recorded from 30:60:00 kg NPK ha⁻¹ and the combined application of *Azotobacter* + PSB. The harvest index of alone application of *Azotobacter* and PSB are same *i.e.* 23.0. Thus, soil inoculation with micro-symbiont inocula (particularly *Azospirillum spp.*), may be suitable for improving sesame performance in the study areas, where soils are mostly very low in essential nutrients (particularly N) [21].

Rape seed and Mustard

The new techniques of applications particularly use of methylcellulose for seed coating and pellets for direct soil application should be encouraged. The responses usually depend on several environmental factors. The type of soil as measured by its water holding capacity, its levels of other nitrates, phosphate and even calcium and molybdenum that help in protein synthesis in Rhizobia and the alkalinity, salinity and acidity of soil, all affect the response. Higher dose of mineral N- as starter suppresses nodulation, reducing response of *Rhizobium* but phosphate deficiency can be an inhibitor also. The inadequacy of organic matter especially common in dry-land and agriculture is a deterrent more for the nonsymbiotic strains, which essentially depend on soil organic matter for energy. Phosphobactrin response was found to be positive only in soils with high organic content and low available phosphorous. Native microbial population opposes the inoculants. In general, predatory organisms, often already present in the soil are more adapted to the environment and out compete the inoculated population. The maximum yield was obtained by the sulphur application @ 40 kg/ha and by the source of biofertilizer (B1) @ *Azotobacter* /10 kg seed inoculate. The interaction between sulphur and biofertilizer was significant and the maximum increase in yield was obtained by applied sulphur @ 40 kg ha⁻¹ at biofertilizer (B0, B1) 0 and 200 g *Azotobacter*/10 kg seed inoculate. The soil samples collected after harvest of mustard crop showed the slight decrease in pH and EC and increase in organic carbon, available nitrogen, phosphorus, potassium and sulphur was recorded by the application of sulphur and biofertilizer applied alone or in conjunction with each other [22].

Large number of field trials and various experiments carried throughout India and whole world have convincingly established the importance of *Azotobacter* as microbial inoculant. Mustard and rapeseeds gave good response to *Azotobacter* growth and development, seed yield and oil yield. Incidence of some diseases of mustard and rapeseeds could be reduced by inoculating with *Azotobacter*. Better results were given when seeds were treated in combination with *Azotobacter* and N [25]. Farmers should be advised to mix Biofertilizers to get benefits of the synergistic effect on plant growth and ultimately on yield, but multi strains biofertilizers may be avoided [23]. Sulphur @40 kg ha⁻¹ and biofertilizer @200 g *Azotobacter* per 10 kg seed inoculated (S2B1) treatment combination was the best treatment as compared to others (Yadav and Thomas, 2010). Integrated use of bio fertilizers, FYM with 40 Kg of nitrogen gave seed yield equal to the 80 kg N ha⁻¹ alone. Maximum seed yield was obtained in the use of higher doses of N fertilizer in conjunctions with bio fertilizers and FYM in both years [24].

Types of biofertilizers

1. Nitrogen fixing microorganisms

Rhizobium fixes 30-50 kg N/ha. Used mainly in legume crops. **Frankia** :Non leguminous symbiosis. Mainly trees and shrubs are harbouring by *Frankia* and forms root nodules. Eg: *Alnus, casuarina, ceanothus, Myrica, Hippophae* **Azolla** : Free floating water fern.Symbiosis with *Anabaena azollae* (cyanobacterium). Fixes nitrogen average from 40-60 kg/ha/rice crop depending upon the soil, temperature, light intensity and phosphorus content. Eg: **Azolla pinnata** , **Azolla microphylla** (widely used) , **Azolla caroliniana** ,**Azolla filiculoides**

- **Azotobacter**

Free living nitrogen fixer.Helps in saving 10-20kg N/ha. Widely used in cereal crops (Sorghum, Maize, Mustard and cotton, vegetable crop (tomato, Potato etc).

- **Azospirillum**

These are associative nitrogen fixers. Fix nitrogen @15-30kg/ha and secrete growth regulating substances. Recommended for Rice, Maize, Wheat, Sugarcane and co-inoculant for legumes

- **Blue green algae(cyanobacteria):** Fixes 20-30kg nitrogen/ha

Eg: *Nostoc, Anabaena, Calothrix*

2. Phosphate solubilising microorganisms

P is important nutrient for plant growth. Most of our soils are low in available form of Phosphorus and require phosphorus application. P-solubilizing organism will render insoluble soil phosphates available to plants due to production and secretion of organic acids by them.

Eg : *Bacillus megatherium var phosphaticum*
B. subtilis, Pseudomonas rathoris, A. awamori, P. digitatum

3. Phosphate mobilizing organisms

- **Vesicular Arbuscular Mycorrhiza (VAM)**

This is mutualistic/symbiotic relationship between plant roots and fungi (Endomycorrhizae). Arbuscules are formed by repeated dichotomous branching and reductions in hyphal width from an initial trunk hypha that ends in a proliferation of very fine branch hyphae. They are considered to be the major site of exchange with the host plant.

4. K-solubilizing microorganism (KSMs)

There are considerable populations of K-solubilizing microorganisms in soil and in plant rhizospheres. It is generally accepted that the major mechanism of mineral potassium solubilisation is the action of organic acids synthesized by soil microorganism. Production of organic acids results in acidification of the microbial cell and its surroundings. Silicate bacteria were found to resolve potassium, silicon and aluminum from insoluble minerals [26].

Eg. : *Bacillus mucilaginosus, Bacillus edaphicus, Bacillus circulans, Acidithiobacillus ferrooxidans, Paenibacillus spp.*. **Fungal strains** (*Aspergillus spp.* and *Aspergillus terreus*).

Quality Control of biofertilizers

Till 2006 although BIS standards were followed for assessment of quality for four types of biofertilizers, but it was voluntary in nature. As per the details available, only eleven manufacturers out of 135 (during 1999-2000) opted for BIS certification of their inoculants. Concerned with the continued quality issues, the Government of India brought four biofertilizers namely *Rhizobium, Azotobacter, Azospirillum* and PSB under the ambit of Fertilizer (Control) Order 1985 (FCO) during 2006. With the picking up of mycorrhizal biofertilizer production through tissue culture technique, the same was also brought under the FCO with separate specifications. Recently, two more biofertilizers, namely potash mobilizing and zinc solubilizing biofertilizers have also been incorporated under FCO . Under the statutory provisions of FCO, biofertilizer production and its sales have been regulated and is a mandatory requirement of registration for every manufacturing unit with the State Fertilizer Controller (who is generally the Commissioner or Director of Agriculture Department). In every district, some officers of the Agriculture Department have been declared as Fertilizer Inspectors, who are authorized to inspect production and storage facilities and draw samples for quality analysis. National Centre of Organic Farming (NCOF), Ghaziabad and its six Regional Centres located at Bhubaneswar, Bangalore, Jabalpur, Nagpur, Hissar and Imphal have been declared as notified testing laboratories. Under the provisions of the act, State Governments can also develop their own quality control laboratories and notify them under the FCO 1985. So far, 11 State Governments have

developed their quality control laboratories and notified their own biofertilizer testing laboratories.

Table 1: Specifications of *Rhizobium* biofertilizer

Parameter	Requirements
Base	Carrier based* in form of moist/dry powder or granules, or liquid Based
Viable cell count	CFU minimum 5x10 ⁷ cells/g of powder, granules or carrier material or 1x10 ⁸ cells/ml of liquid
Contamination level	No contamination at 10 ⁵ dilution
pH	6.5-7.5
Particles size	in case of carrier based material All material shall pass through 0.15-0.212mm IS sieve
Moisture per cent by weight, maximum in case of carrier based	30-40%
Efficiency character	Should show effective nodulation on all the species listed on the packet

Table 2: Specifications of *Azotobacter* Biofertilizer

Efficiency character	The strain should be capable of fixing at least 10 mg of nitrogen per g of sucrose consumed
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Table 3: Specifications of *Azospirillum* Biofertilizer

Efficiency character	Formation of white pellicle in semisolid N-free bromothymol blue medium
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Table 4: Specifications of Phosphate Solubilizing Bacterial Biofertilizer

Efficiency character	The strain should have phosphate solubilizing capacity in the range of minimum 30%, when tested spectrophotometrically In terms of zone formation, minimum 5mm solubilization zone in prescribed media having at least 3mm thickness
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Table 5: Specifications of Mycorrhizal Biofertilizers

Parameter	Requirements
Base/form	Fine powder/tablets/granules/root biomass mixed with growing substrate
Total viable propagules/g of product, minimum	100/g of finished product
pH	6.0 to 7.5
Particles size	90% should pass through 250 micron IS sieve (60 BSS)
Moisture per cent by weight, maximum	8 -12%
Efficiency character	80 infection points in test roots/g of mycorrhizal inoculum used

Table 6: Biofertilizer production in India during the period from 2008-09 to 2014-15

State	Actual production of Biofertilizers in MT during years						
	2008-09	2009-10	2010-11	2011-12	2012-13	2013-14	2014-15
India	25065.04	20040.35	37997.61	40324.21	46836.82	51870.67	65,500

Promotion

- Bringing out awareness among scientists, officers of Agriculture department, dealers, sellers and farmers.
- Spreading awareness on biofertilizers through radio, meetings, pamphlets
- Conducting training programmes by University, production centres, sellers

Table 7: Biofertilizers recommended for oil seed crops

Type of biofertilizer	Recommended crop
Nitrogen fixers <i>Rhizobium</i>	Ground nut, Soybean,
<i>Azotobacter</i>	Sunflower, Safflower, Mustard, Sesamum
Phosphorous solubilizing bacteria P- Solubilizers: <i>Bacillus, Pseudomonas, etc.,</i>	Sunflower, Soybean
P-mobilizers VAM	Soybean Sunflower
K or Zinc Solubilizers <i>Bacillus, etc.,</i>	All crops
Sulphur solubilizers <i>Thiobacillus thiooxidans</i>	Sunflower, Ground nut etc.,

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