



K.L.E.Society's
BASAVPRABHU KORE ARTS, SCIENCE AND COMMERCE COLLEGE,
CHIKKODI – 591201 District – Belagavi (Karnataka state, India)
(ACCREDITED AT 'A' GRADE BY NAAC WITH CGPA OF 3.26 IN THE THIRD CYCLE)

Department of Zoology (2019 – 20)

PROJECT WORK COMPLETION CERTIFICATE

This is to certify that following six B.Sc Final year students have undertaken the project entitled **Production of selected biofertilizers** in-partial fulfillment of the syllabus of Rani Channamma University, Belagavi during the year 2019-20. Following six students have together successfully completed the said project under the guidance of Dr Sridevi I Puranik.

Sl. No	Gender	Name of the student	Fathers name	Roll Number	Exam Seat Number
1	Miss.	Chaitra Mathapati	Appayya	165	S1715635
2	Miss.	Rakshata Kamate	Vilas	177	S1715719
3	Miss.	Arati Chougale	Vishwanath	210	S1715622
4	Miss.	Rutuja Patil	Sanju	211	S1715735
5	Miss.	Rohini Molake	Laxman	179	S1715730
6	Miss.	Jyoti Boraganve	Maruti	240	S1715646


Dr Sridevi I Puranik
PROJECT GUIDE


Dr N R Birasal
HEAD
DEPARTMENT OF ZOOLOGY


Prof U R Rajput
PRINCIPAL
KLES'S Basavaprabhu Kore
Arts, Science and Commerce College
CHIKODI - 591 201

Project Team Members

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1.	Miss.	Chaitra Mathapati	Appayya	165	S1715635
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INTRODUCTION

In past 50 years history, the chemical pesticides and fertilizers have played a crucial role in busting the agricultural production, however they have a short history in modern agriculture. Their immediate action and low cost managed to bring them rapidly into the center of attention. Their toxic effects on environment, plant, animal and human life diverted the focus on ecofriendly plant protection. Moreover, the development of resistance in insects against common pesticides has not been solved yet.

Indiscriminate use of chemical pesticides contributed in loss of soil productivity along with addition of salts to the soil. To revive the soil health and living on alternate source has become essential concept of bio-fertilizer come forward, which can be a good supplement for a chemical fertilizer. Bio-fertilizers are nutrient availability systems in which biological process are involved.

The term biofertilizers includes selective microorganisms like bacteria, fungi and algae. Which are capable of fixing atmospheric nitrogen or convert soluble phosphate and potash in the soil into forms available to the plants. Biofertilizers is a cost effective, eco-friendly and renewable source of land nutrient they play a vital role in maintaining long term soil fertility and sustain ability.

The biofertilizer with nitrogen fixer and phosphate solubilizer fixes 20-40 Kg of nitrogen per acre. The biofertilizer maintain the soil fertility cost by using in the yield is assured with biofertilizer and continuous use of biofertilizer makes the soil very fertile for good yield. The biofertilizer can be manufacture in soil form or in liquid form for spraying on the plants.

Biofertilizer is a need of modern agriculture since demand for safe and residue free food is increasing. Therefore, to cater the need, it is necessary to promote the efforts for production of biofertilizers in the state in private sector to encourage the entrepreneurs.

The role of essential macronutrients such as nitrogen, phosphorus potassium and other secondary elements is well known for increasing the productivity of land.

Population explosion has escalated the fresher on higher productivity per unit of land. Modern agriculture emphasized using hybrid seeds. High yielding varieties that are highly responsive to large doses of chemical fertilizers and irrigation. This has resulted in soil being deprived of

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essential plant nutrients and nourishing organic matter that had always been available to plant when natural forming was being practiced historically.

Chemical fertilizers which are now being used extensively since the green revolution have depleted soil health by making the soil ecology non inhabitable for soil micro flora and micro fauna which are largely responsible for maintaining soil fertility and providing some essential and indispensable nutrients to plant. It has now become an imperative to restore the soil with a beneficial microbe population by using biofertilizers.

Biofertilizers contain live cells of specific isolated strains of bacteria and fungi which is formulated in suitable carriers. These microbes, upon application to soil under suitable conditions secrete metabolites and enzymes which make the deficient element available to the plant in an assimilable form. Nitrogen fixing bacteria fix atmospheric nitrogen in soil while phosphorus bacteria solubilize insoluble fixed phosphorus in soil, potassium mobilizing bacteria mobilize the immobile potassium in soil and similarly other microbes mobilize/solubilize the element in soil and make it available to the plant. VAM infected roots penetrate the soil effectively and make relatively unavailable elements such as phosphorus, copper and zinc available to the plant. These beneficial microorganisms work incognito to maintain the ecological balance by active participation in carbon nitrogen, Sulphur and phosphorus cycles in nature.

The current status of bio-fertilizer in India

The fertilizer consumption varies from 130, 125, 60 and 70 kg/ hectare (NPK) for north, south, west and east respectively making for a national average of approx. 90 kg per hectare. Some states like Punjab are using more than 167 kg nutrients per Hectare. Even the full potential of available technologies is not fully utilized due to the fact that nutrient input doesn't match the needs of crop and soil. In case of biofertilizers the production and the supply of microbial cultures, the quality of the culture and the lack of publicity are affecting the popularity as nutrient sources. The government has no control over manufacture of biofertilizers for any of the state of India. Only a few entrepreneurs possess ISI mark for their products and Most of the products are of substandard quality. Due to these laxities on a part of govt. the farmers are confused about their rates, availability and expiry dates. The necessary action by govt. and its policies will certainly go a long way in the further development of biofertilizers.

Biofertilizer production at Biocenter Belagavi

1. Nitrogen fixing bacteria
2. Phosphorus solubilizing bacteria

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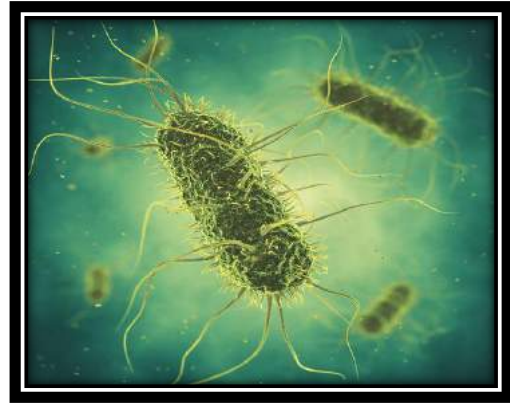
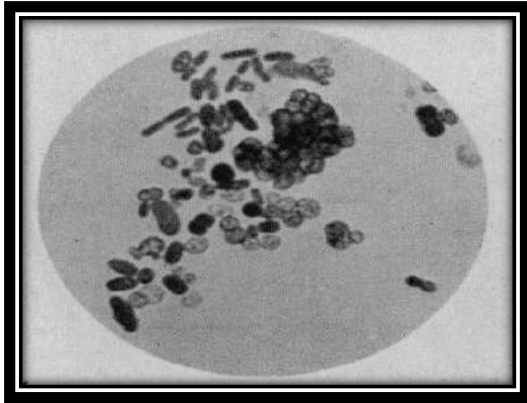
3. Potassium mobilizing bacteria
4. Ferrous mobilizing bacteria
5. Zinc mobilizing bacteria
6. Sulphur mobilizing bacteria
7. Manganese solubilizing microbe
8. Vesicular arbuscular mycorrhizae (VAM)

Biofertilizers are a suitable supplement to chemical fertilizers to meet the integrated nutrients demand of the crops. Application of biofertilizers result in increased mineral and water uptake, root development, vegetative growth and yield of good quality. They are ecofriendly, nontoxic, easy to use, economical biosolutions that improve soil health and crop productivity

Based on the type of microorganisms, the biofertilizers can be classified as

- 1) Bacterial biofertilizers: e.g. Rhizobium, Azospirillum, Azotobacter, Phosphobacteria
- 2) Fungal biofertilizers: e.g. Mycorrhiza.
- 3) Algal biofertilizers: e.g. Blue green algae and Azolla
- 4) Actinomycete biofertilizers: e.g. Frankia

Azotobacter



Azotobacter is one of the most important non-symbiotic N-fixing microorganisms present in neutral or alkaline soils and Azotobacter chorococcum is the most commonly occurring species in aerable soils. Apart from its ability to fix atmospheric nitrogen, Azotobacter is also known to synthesis biologically active growth promoting substances such as Indole Acetic Acid (IAA), Gibberellins and B-vitamins in culture media. The strains of Azotobacter also have fungistic properties against plant pathogens such as Fusarium, Alternaria and Helminthosporium. It produces growth promoting substances which improve seed germination and growth of extended root system. It produces polysaccharides which improve soil aggregation. Azotobacter suppresses the growth of saprophytic and pathogenic microorganisms near the root system of crop plants morphology.

Azospirillum



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It is a common inhabitant of tropics. There are four major species of Azospirillum: a. lipoferum, b. brasilense, c. amazonense and d. seropedicae. Among these physiological types occurring within the genus Azospirillum viz., a. lipoferum and b. brasilense, one group has an oxidative metabolism and the other has the ability to ferment certain sugars by producing acid. Azospirillum form associative symbiosis with many plants particularly with those having the C₄ – dicarboxylic pathway of photosynthesis.

Azospirillum are chemo heterotrophic in nature which secrete growth regulatory substances. The use of Azospirillum inoculants significantly increases the growth, chlorophyll contents and mycorrhizal infection in roots of plants which results in increased mineral and water uptake, root development, vegetable growth and crop yield. The optimum temperature for azospirillum growth is 32-35⁰C. It contains associative nitrogenfixing bacteria. These nitrogen fixing organisms are free living; they live association with the root system of crop plants and helps in fixing nitrogen, through reduction of Atmospheric nitrogen to ammonia.

Azolla

Azolla is an aquatic fern (pteridophyte), floating on water surface of flooded rice fields, small ponds, and canals. The nitrogen-fixing capability of Azolla has led to Azolla being widely used as a biofertiliser, its size is 1-5 cm except for a giant A. nilotica, generally it multiplies vegetatively, and often sexually. Seven extant Azolla species are recognized, and their distribution varies widely from temperate to tropical regions. A. nilotica, A. pinnata, A. filiculoides, A. rubra, A. cristata, A. Japonica and A. imbricate.



Azolla is useful as a “soya bean plant in rice field”, because it can assimilate atmospheric nitrogen gas owing to the nitrogen fixation by cyanobacteria (blue green algae) living in the cavities located at the lower side of upper (dorsal) lobes of leaf. Cyanobacteria are single or multi cell photosynthetic organisms. They are often called “algae”, but belong to “bacteria”. Most of them can assimilate atmospheric nitrogen under reduced oxygen pressure. This reaction is called Nitrogen fixation. Some can do nitrogen fixation under ordinary oxygen pressure and most of them have large cells, called Heterocyst specialized for nitrogen fixation.

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Azolla can, therefore, grow on the water deficient nitrogen compounds, and is high in nitrogen and protein. It fixes nitrogen as high as 3-5 kg N per ha per day under the optimum condition. Azolla multiplies at the daily mean temperature of 15-30⁰C. Optimum temperature is about 25⁰C.

Trichoderma viridae

Trichoderma viridae is an antagonistic filamentous fungus that is widely distributed in soil, plant material, decaying vegetation, wood which is highly effective for the control of seed and soil born diseases of majority of economically important crops especially vegetables, plantation crops, species, fruit crops, pulses and oil seeds, this bio-control agent when applied along with seed, colonizes the seed and multiplies on the surface of the seed and kills not only the pathogens present on the surface of the seed but also gives protection against soil-borne pathogens until life time of crop by action of mycoparasitism and antibiosis. Seed treatment with *Trichoderma viridae* has registered higher germination in a number of agriculture and horticulture crops

Its effective control of soil-borne diseases caused by *Rhizoctonia solani*, *Macrophomina phaseolina* and Fusarium species makes it very important weapon against disease such as root rot, seedling disease, charcoal rot, wilt, damping off, collar rot, etc., *Trichoderma viridae* is a potent biocontrol agent and used extensively for control of soil born diseases. Used in damping off caused by Pythium sp., Phytophthora sp., root rot caused by pellicularis filamentosa, Seedling blight caused by Pythium, Collar rot caused by Pellicularia rolfsii, Dry rot and Charcol rot caused by Macrophomina phaseoli, Loose smut caused by Ustilago segetum, Black scurf caused by *Rhizoctonia solani*, phytophthora foot rot in black pepper. Effective against silver leaf on plum, peach and nectarine, Botrytis caused by botrytis cineria, Effective against rots on a wide range of crops, caused by fusarium, Rhizoctonia, and pythium, and sclerotium forming pathogens such as Sclerotinia & Sclerotium. Apart from this also successfully in controlling damping off in cardamom and tomato, Fruit rot and leaf blight in Capsicum and Chili, Rhizome rot / soft rot in ginger, seedling disease of cotton, root rot of soybean, root rot of Cowpea, charcoal rot sorghum and root rot of mung bean caused by *Macrophomina phaseolina*.

Materials required

- Rice/Wheat/ Sorghum/ Maize
- Mother Culture (may be procured from CIPMCs or State biocontrol laboratory)
- 8" x 12 " plastic bag
- Cotton
- Rubber band

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- Plastic pipe of 1 ½ inch length and 1 1/2 cm - 2 cm diameter having both side open (or even a bamboo of same size and diameter, can be used removing the internodes)
- Pressure cooker of 5 Lts. or above
- Heating system (gas/electric heater)
- Stone/wood
- Fresh Water
- Candle
- Spoon

Protocol of production

a. Mass production of Biofertilizers

- Media preparation and starter culture

Bacteria require different nutrients for their growth. These include:

- a) Organic carbon source,
- b) nitrogen source and
- c) A variety of other elements dissolved in water.

Blue green algae that can fix atmospheric carbon dioxide, does not require any carbon source and the nitrogen fixing bacteria, which can fix atmospheric nitrogen, do not require any nitrogen source. A medium is an aquatic solution of a variety of organic and inorganic compounds that can supplement the above requirements for the growth of different microorganisms. Generally, media are of two types: a) general media and b) specific media. General media is constituted for the growth of most of the organisms. Such a media contains all the ingredients required for the growth of any microorganisms. Such a media contains all the ingredients required for the growth of any microorganism. A specific medium is constituted for the growth of specific group of microorganisms again, according to the physical appearance, media are of two types: a) liquid media and b) solid media. The liquid medium is solidified by the addition of solidifying agent – agar-agar. Liquid medium can harbor bacterial growth suspended in the media, whereas solid medium harbors microbial growth on the surface. Solid media may be prepared as slant or plate.

b. Sterilization pf medium in autoclave

The media is the autoclaved at 121⁰C temperature and 15LB pressure. The process is as follows.

1. Check the water level of the autoclave to note whether the heating coil is completely immersed in water, add water, if necessary.
2. Put the conical flasks, petridishes and the beaker with test tubes inside the basket of the autoclave.
3. Set the lid, tie the screws of the autoclave and switch on the power supply.
4. Keep the outlet of the steam open and wait until the inside air is completely released from the autoclave and only steam is coming out.

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5. When the inside air is completely released close the outlet and observe the meter indicating the inside pressure.
6. When the pressure rises to 15lbs, adjust the regulating valve to keep the pressure constant.
7. Keep this pressure for 30 min and then close the regulating valve and switch off the power supply
8. When the pressure comes down to zero. Open the regulatory valve to release the steam and then open the lid and take out the contents.

c. Prepare slants and Plates

The slants are necessary for culture storage. The slant preparation process is as follows

1. Put the test tubes containing medium in slanting position on the table with the help of a wooden blade and allow cooling down. The medium will be solid after cooling down thus slant are prepared.
2. Take the conical flask containing molten media with agar-agar and the petridish in the laminar flow cabinet and the allow the medium to cool down to 50c.
3. Open each petridish by slightly lifting the upper lid, pour 15-20 ml of medium and close the lid.
4. Keep to cool down and solidify and thus plates are prepared.

d. Preparation of starter culture:

The starter culture is a little amount of bacterial suspension, which is added to medium to start the growth of that bacterium, twin flask is a pair of flasks of identical size joined together by a latex tube, for the preparation of starter culture, this type of flask is used. Each flask contains a side arm below the neck position. The latex tube joining the two flasks is held together by this glass tube. The benefit of the use of the twin flask is the contamination can be avoided.

e. Fermentation:

A fermenter is a device in which the optimum conditions for the microbial growth and activity is established artificially. This device is used for the production of microbial metabolites such as antibiotics or enzymes: it may also be used for the growth of microorganisms i.e. production of microorganism itself.

A low-cost production unit has been developed for the production of microbial inoculants to be used as biofertilizers such as Rhizobium, Azobacter, and phosphate solublizing bacteria. This device can be prepared by investing small amount of money as compared to the scientific fermenter used in laboratories for research purposes.

Sterilization of the fermenter:

Fermenter is a metallic vessel for moist sterilization of any article. The principle of moist sterilization lies in the fact that when water is boiled in a closed system. The water vapor produced due to boiling accumulates within the vessel and increases the inside pressure. Thus, the boiling point of increases beyond 100°C, which is boiling point of water in normal

atmospheric pressure. In this condition, the steam, released from the boiling water is of higher temperature. If any article placed in this vessel in such condition, the high temperature destroys the microorganisms present in or on the article.

f. Inoculation, Growth, Quality Testing and Termination of Growth

Inoculation means addition of starter culture to the medium in the fermenter. For the production of microbial biofertilizers a small amount of suspension of the desired bacterium in pure form is inoculated to the medium. Care should be taken to maintain the quality of starter culture, as extent of purity (no contaminants should be allowed), size of the starter culture (in terms of culture volume and density of cell) and stage growth. Greater the size of starter culture, lesser the chance of contamination. If the starter culture is inoculated in its log phase, rapid initial growth will occur. Maintenance of proper physical and chemical environment inside the fermenter is essential for proper growth of microorganism. Quality testing, in this case, is enumeration of cell density and its purity broth. When the cell density reaches the desired level, growth is terminated and the culture ready for mixing with carrier. The time period required for optimum cell density is thus standardized.

g. Carrier Preparation:

Carrier is a medium, which can carry the microorganisms in sufficient quantities and keep them viable under specified conditions and easy to supply to the farmers a good carrier should have the following qualities:

- Highly absorptive (water holding capacity) and easy to process.
- Non-toxic to microorganisms
- Easy to sterilize
- Available in adequate amount and low cost
- Provide good adhesion to seed.
- Has good buffering capacity.

Different carriers are available in the market like, charcoal, peat, lignite, rice husk etc. but considering all the above qualities Azolla powder is the most suitable carrier in this region. This is due to

1. It has high water holding capacity (360%)
2. It has good pH buffering capacity.
3. It contains nutrient so bacteria can remain viable for long period.
4. It is easily available in this region.

h. Formulation:

Inoculation of the carrier with the culture broth means the mixing of broth and carrier. This operation must be done in aseptic conditions to avoid any contamination.

i. Quality control of formulation:

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The quality of the carrier- based inoculums depends upon the viable cell count and the presence or absence of contaminants. A good culture must contain about 10^7 - 10^8 viable cells or CFUs pre g of culture. No contaminants are permissible at 10^{-5} – 10^{-6} dilutions. These critical values differ according to the type of biofertilizer. As per I.S.I. in case of Rhizobium, the carrier-based culture must contain at least 10^7 cell or CFUs per g of culture and no contaminants are possible below 10^{-6} dilution. In case of Azobacter, so the enumeration of cells density and contaminants are important task in the production of carrier based microbial biofertilizer.

Bioactivity of Azobacter:

Nitrogenase is the enzyme catalyzing the reduction of nitrogen into ammonia. This enzyme can also reduce acetylene into ethylene as well. Acetylene and ethylene can easily be measured by a gas chromatography. In a closed system, if a portion of gas is substituted by acetylene and acetylene is allowed to be reduced for a certain period, the portion of acetylene and ethylene can be measured by passing the mixture of gas through the column of gas chromatograph and measuring the peak developed.

The nitrogenase activity (hence, the nitrogen fixation activity) of Azotobacter or other free-living bacteria can be extrapolated by Acetylene Reduction Assay (ARA) method, but this technique cannot be applied in case of Rhizobium as this bacterium cannot fix Nitrogen in free condition. In this case the plant containing the nodules is to be taken for assay.

Methodology

Take 200g of Rice/Wheat/Jower/Maize in the poly pack and add 200 ml of fresh water in the pack (if grains contain dust then wash it twice before adding fresh water). Place the plastic pipe/Bamboo in the middle of the plastic pack (opening end) ins such a way that level of the pipe and plastic remain equal. Tie it with the help of rubber band. Plug the opening end of the pipe tightly with the help of the cotton. Cover the cotton plug with a paper using rubber band. Place the thick paper inside the pressure cooker surrounding the cooker wall. Place the stone/wood in the cooker and add water into the cooker just below the stone/wood. Place the plastic pack inside the cooker and put it on heating system. Wait until 3 times gas release from the cooker (3 whistles). Remove the packet from the cooker until totally cool down.

Inoculation method

Place a candle at the corner of the room and wait for 3-4 min. Wash hand and the spoon with Dettol. Open the paper cover from the plastic pack. Take mother culture (Talc based) by using opposite end of the spoon and pour it in to the plastic pack, removing cotton plug in front of candle. Plug it again and keep the plastic pack in room temperature for 10-12 days. The entire grain-based medium will turn green due to sporulation of Trichoderma

Precautions

- Do not open the cotton plug until use.

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- Keep it in a cold place (Refrigerator preferably after sporulation)
- Avoid direct sunlight until use

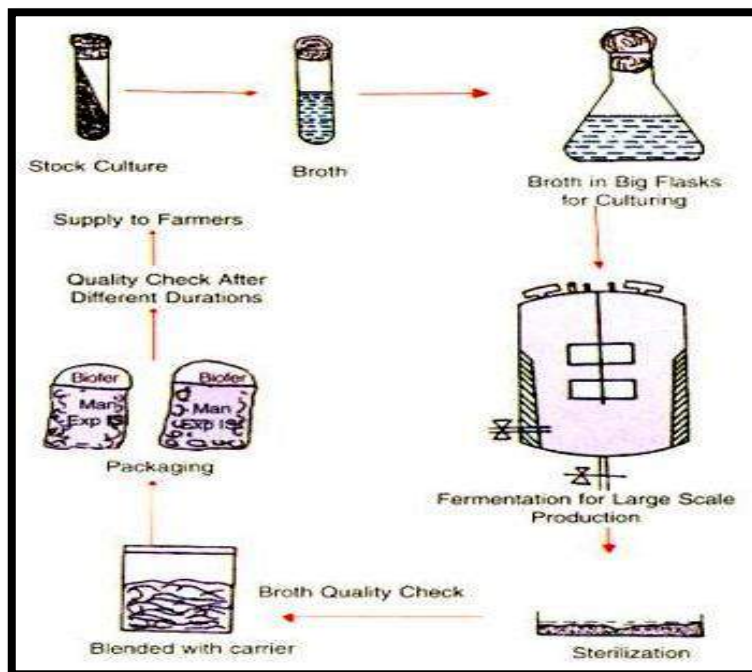


Figure1. Commercial production of Bio-fertilizer

Benefits of using bio-fertilizers

1. Increasing harvest yields
2. An average increase in crop yields by 20 to 37 percent.
3. Algae-based fertilizers give improved yields in rice at rates ranging between 10 and 45 %.
4. Improving soil structure
5. The use of microbial bio-fertilizers improves the soil structure by influencing the aggregation of the soil particles
6. Better water relation
Arbuscular mycorrhizal colonization induces drought tolerance in plants by:
 - ✓ Improving leaf water and turgor potential,
 - ✓ Maintaining stomata functioning and transpiration,
 - ✓ Increasing root length and development.
7. Lowering production costs
8. Made from easily obtained organic materials such as rice husks, soil, bamboo and vegetables etc.

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9. Reduce the input expenses by replacing the cost of chemical fertilizers.

10. Providing protection against drought and some soil-borne diseases v

Aquatic cyanobacteria provide natural growth hormones, proteins, vitamins and minerals to the soil.

Azotobacter infuse the soil with antibiotic pesticide and inhibit the spread of soil-borne pathogens like Pythium and Phytophthora.

11. Suppressing the incidence of insect pests and plant diseases Biofertilizers strengthen the soil profile, leave water sources untainted and improve plant growth without detrimental side effects.

Advantages

We can list the basic advantages of using biofertilizers:

- They help to achieve high yields of crops by enriching the soil with nutrients and useful microorganisms necessary for plant growth.
- They replace the chemical fertilizers, as the latter are not beneficial for plants. Chemical fertilizers decrease the plant growth and pollute the environment by releasing harmful chemicals.
- Plant growth can be increased because biofertilizers contain natural components which do not harm the plants but do the opposite.
- They are eco-friendly due to the fact that they protect the environment against pollutants.
- If the soil is free of chemicals, it will retain its fertility, which will be beneficial for the plants as well as the environment, because the plants will be protected against diseases and the environment will be free of pollutants.
- Biofertilizers destroy those harmful components from the soil which cause diseases in plants. By using biofertilizers, plants can also be protected against drought and other restrictive conditions.
- Biofertilizers are cost effective. They are not costly and even low-income farmers can make use of them.

Effects

- Gives much lower nutrient density - it requires large amounts to get enough for most crops
- Requires a different type of machinery to apply from that used for chemical fertilizers
- Sometimes is hard to locate in certain areas; odor; difficult to store
- Specific to the plants
- Requires skills in production and application.

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- There is inadequate awareness about the use and benefits of biofertilizers.

Bio-fertilizers and bio-control agents recommended for horticultural crops

Sl. No.	Crop	Plantations
01	Plantation/Spices a.Coconut, arecanut and others	25g Azospirillum, 25g PSM,100g VAM, 10g Trichoderma and 10g Pseudomonas per plant
02	Fruits a.banana	50g Azospirillum or Azatobacter,50g PSM 150-200g VAM, 10g Trichoderma and 10g Pseudomonas per plant
	b.Mango,Sapota and pomegranate	50g Azatobacter, 50g PSM,100g VAM,25g Trichoderma and 25g Pseudomonas per plant
	c.Grapes	30g Azatobacter, 30g PSM, 100g VAM,10g Trichoderma, and 10g Pseudomonas per plant
03	Vegetables a.Legumes	2-2.5 kg Rhizobium, 2.5 kg Azospirillum, 1 kg PSM, 20 kg VAM, 1 Kg Trichoderma and 1 kg Pseudomonas per ha
	b.Potato	3.5-4 kg Azatobacter, 20kg VAM, 2 kg Trichoderma, and 1 kg Pseudomonas applied per ha at the time of planting
04	Flowers a.Rose	25 g Azospirillum, 25 g PSM , 25 g VAM, 10g Trichoderma and 10g Pseudomonas per plant
	b.Jasmine	25g Azospirillum, 25g PSM, 25g VAM, 10g Trichoderma, 10g Pseudomonas per plant

CONCLUSION

Biofertilizers increase the availability of plant nutrients and can help in maintenance of the soil fertility over a long period. As discussed earlier, some microorganisms have the beneficial role of biological nitrogen fixation to supply nitrogen to crops, solubilizing insoluble phosphates to plant available (soluble) forms and synthesizing biomass for manuring of crops like rice, groundnut, maize etc. Biofertilizer are therefore economical, renewable and eco-friendly, but they cannot totally replace chemical fertilizers. Biofertilizers use is as important component of Integrated Nutrient Management and organic farming. These technologies are becoming vital in modern-day agricultural practices. The changing scenario of agricultural practices and environmental hazards associated with chemical fertilizers demand a more significant role of biofertilizers in coming years.

PHOTO GALLERY

Bio-fertilizer productions at Bio-center Belagavi



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