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BIOFERTILIZER AND MUSHROOM TECHNOLOGY

UNIT I: BIOFERTILIZERS

Definition, Advantages, Microbes used as Biofertilizers. Isolation, Characteristics, Identification, Mass inoculum production, Field application and marketing of *Rhizobium*, *Azospirillum*, *Azotobacter*.

UNIT II:

Cyanobacteria (BGA) - Isolation, Characteristics, Mass inoculum production and Field application. Azolla – Isolation, Characteristics, Mass inoculum production and Field application.

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Prepared by

**DR.R.SAGAYA GIRI,
ASSISTANT
PROFESSOR,
DEPARTMENT OF
BOTANY,
KN GOVT ARTS COLLEGE FOR
WOMEN, THANJAVUR-7**

UNIT I: BIOFERTILIZERS

Definition, Advantages, Microbes used as Biofertilizers

Introduction

Biofertilizer is a substance which contains living microorganisms which, when applied to seeds, plant surfaces, or soil and promotes growth by increasing the supply or availability of primary nutrients to the host plant. It adds nutrients through the natural processes of nitrogen fixation, solubilizing phosphorus, and stimulating plant growth through the synthesis of growth-promoting substances.



Definition

Biofertilizers are the substance that contains microbes, which helps in promoting the growth of plants and trees by increasing the supply of essential nutrients to the plants. It comprises living organisms which include mycorrhizal fungi, blue-green algae, and bacteria. Mycorrhizal fungi preferentially withdraw minerals from organic matter for the plant whereas cyanobacteria are characterized by the property of nitrogen fixation.

Types of Biofertilizers

Following are the important types of biofertilizers:

1. Nitrogen-fixing biofertilizers

Cultivated crop determine what type of nitrogen biofertilizer should be used:

- **Rhizobium for legume crops.**
- **Azotobacter/Azospirillum for non legume crops.**

- **Acetobacter** for sugarcane only.
- **Blue-Green Algae (BGA)** and **Azolla** for low paddy land used to grow rice.

2. Phosphorus fixing biofertilizers

Phosphorus biofertilizers are not dependent on the crops cultivated on the soil

- **Phosphatika** for all crops to be applied with
- **Rhizobium**
- **Azotobacter**
- **Azospirillum** and **Acetobacter**

Biofertilizers can use to enrich your compost and the bacterial processes breaking down the compost waste

- **Phosphotika** and **Azotobacter** culture.

MICROBES USED AS BIOFERTILIZERS

1.Symbiotic Nitrogen-Fixing Bacteria

Rhizobium is one of the vital symbiotic nitrogen-fixing bacteria. Here bacteria seek shelter and obtain food from plants. In return, they help by providing fixed nitrogen to the plants.

2.Loose Association of Nitrogen-Fixing Bacteria

Azospirillum is a nitrogen-fixing bacteria that live around the roots of higher plants but do not develop an intimate relationship with plants. It is often termed as rhizosphere association as this bacteria collect plant exudate and the same is used as a food by them. This process is termed as associative mutualism.

3.Symbiotic Nitrogen-Fixing Cyanobacteria

Blue-Green algae or Cyanobacteria from the symbiotic association with several plants. Liverworts, cycad roots, fern, and lichens are some of the Nitrogen-fixing cyanobacteria. **Anabaena** is found at the leaf cavities of the fern. It is responsible for nitrogen fixation. The fern plants decay and release the same for utilization of the rice plants. **Azolla pinnate** is a fern that resides in rice fields but they do not regulate the growth of the plant.

4. Free-Living Nitrogen-Fixing Bacteria

They are free-living soil **bacteria** which perform nitrogen fixation. They are saprotrophic anaerobes such as *Clostridium beijerinckii*, Azotobacter, etc.

Among all the types of biofertilizers, Rhizobium and Azospirillum are most widely used.

Importance of Biofertilizers

Biofertilizers are important for the following reasons:

1. Biofertilizers improve soil texture and yield of plants.
2. They do not allow pathogens to flourish.
3. They are eco-friendly and cost-effective.
4. Biofertilizers protect the environment from pollutants since they are natural fertilizers.
5. They destroy many harmful substances present in the soil that can cause plant diseases.
6. Biofertilizers are proved to be effective even under semi-arid conditions.

Advantages of Biofertilizers

1. It helps in maintaining environmental health by reducing the level of pollution.
2. Reduces human & animal hazards by reducing the level of residue in the product.
3. Increases the agricultural products and makes it sustainable
4. Ensures the optimum utilization of natural resources.
5. Reduces the risk of crop failure.
6. Improves the physical and chemical properties of soil
7. Biofertilizers are cost-effective when compared to synthetic fertilizers
8. Using biofertilizers can increase crop yield by 20 or 30 percent.

Drawback of biofertilizers

1. Slow-release
2. Crop specific
3. Strain-specific
4. Soil specific—lose effectiveness if soil too dry or hot
5. Lesser efficient than synthetic fertilizers

- 6. Crops show less response to biofertilizers than chemical fertilizers**
- 7. Much lower nutrient density — requires large amounts to get enough for most crops.**

Applications of Biofertilizers

Following are the important applications of biofertilizers:

1. Seedling root dip

This method is applicable to rice crops. The seedlings are planted in the bed of water for 8-10 hours.

2. Seed Treatment

The seeds are dipped in the mixture of nitrogen and phosphorus fertilizers. These seeds are then dried and sown as soon as possible.

3. Soil Treatment

The biofertilizers along with the compost fertilizers are mixed and kept for one night. This mixture is then spread on the soil where the seeds have to be sown.

Frequently Asked Questions on Biofertilizers

1. What do you understand by biofertilizers?

Biofertilizers are substances that contain microorganisms, which when added to the soil increases the crop yield and promotes plant growth.

2. What are the advantages of biofertilizers over chemical fertilizers?

- Biofertilizers are cost-effective.**
- They reduce the risk of plant diseases.**
- The health of the people consuming the vegetables grown by the addition of chemical fertilizers is more at risk.**
- Biofertilizers do not cause any type of pollution.**

3.What is the need of using biofertilizers?

Biofertilizers are required to restore the fertility of the soil. Prolonged use of chemical fertilizers degrades the soil and affects the crop yield. Biofertilizers, on the other hand, enhance the water holding capacity of the soil and add essential nutrients such as nitrogen, vitamins and proteins to the soil. They are the natural form of fertilizers and hence, widely used in agriculture.

4.Name a few microorganisms used as biofertilizers.

Microorganism used as biofertilizers are:

- **Rhizobium**
- **Azotobacter**
- **Azospirillum**

5.How do biofertilizers promote plant growth?

Biofertilizers utilise certain microorganisms. These microorganisms trap atmospheric nitrogen and convert it into nitrates and nitrites and make it available to the plants. They also convert insoluble phosphates into the forms required by the plants.

6.What are the main sources of biofertilizers?

The main sources of biofertilizers include bacteria, cyanobacteria and fungi.

7.Name an important nitrogen-fixing bacteria.

Rhizobium is an important nitrogen-fixing bacteria. Rhizobium lives in symbiotic association with the leguminous plants, specifically in their root nodules. It traps the atmospheric nitrogen and converts it into usable forms that enhance the growth of the plants.

Isolation, Characteristics, Identification, Mass inoculum production, Field application and marketing of *Rhizobium*

Rhizobium

***Rhizobium* is a gram negative, rod shaped, aerobic, nitrogen fixing , soil habitat bacterium which can able to colonize the legume roots and fixes the atmospheric nitrogen symbiotically.**

They have seven genera and highly specific to form nodule in legumes, referred as cross inoculation group.They are the most efficient biofertilizer as per the quantity of nitrogen fixed concerned. The morphology and physiology of Rhizobium will vary from free-living condition

The bacteria infect the legume root and form root nodules within which they reduce molecular nitrogen to ammonia which is reality utilized by the plant .This belongs to bacterial group and the classical example is symbiotic nitrogen fixation. Rhizobium inoculant was first made in USA and commercialized by private enterprise in 1930s.

Classification of Rhizobium

There are different types of Rhizobium that are categorized on the basis of the rate of growth and the type of plant they are associated with.

Some species of Rhizobium include:

- **R. leguminosarum**
- **R. alamii**
- **R. lentis**
- **R. japonicum**
- **R. metallidurans**
- **R. smilacinae**
- **R. phaseoli**
- **R. trifolii**

Along with Bradyrhizobium, Sinorhizobium, Mesorhizobium, Azorhizobium, and Allorhizobium, Rhizobium is a soil Rhizobia, which means that it consists of bacteria with the ability to fix nitrogen. As such, it presents a significant advantage to the plants it infects by contributing to their growth and development.

In addition to this classification, Rhizobium bacteria are also categorized based on the species of legume that they nodulate. This type of grouping is known as cross-inoculation.

Based on studies on a wide variety of legumes, it became evident that not all Rhizobia are capable of nodulating all types of legumes. This resulted in a need to group Rhizobium into appropriate groups (cross-inoculation groups) which are simply groups of legumes that given species of Rhizobium nodulate.

The cross-inoculation groups include:

- ❖ **Clover groups** - *R. trifolii* infects and nodulates plants of genus *Trifolium* (clovers/trefoil)
- ❖ **Alfalfa groups** - *R. meliloti* infects and nodulates the roots of medicago, melilotus and medicago
- ❖ **Bean group** - *R. phaseoli* infects and nodulates plants of genus *Phaseolus* (e.g. beans)
- ❖ **Lupine group** - *R. lupine* nodulates lupines and serradella (*Ornithopus*)
- ❖ **Pea group** - *R. leguminosarum* infects and nodulates pea, sweet pea, lentil, and vetch
- ❖ **Soybean group** - *R. japonicum* nodulates *Glycine* such as soybean
- ❖ **Cowpea group** - *Rhizobium* sp. nodulates cowpea, pigeonpea, lespedza, groundnut and kudzu among a few others.

* The word *Rhizobium* comes from the Greek words: "rhiza" which refers to root, and "bios" which refers to life.

Characteristics of *Rhizobium*

Rhizobium is discovered and described in 1889, *R. leguminosarum* is the type species of *Rhizobium* in the same way *Rhizobium* has been the type genus of family *Rhizobiaceae*. As such, it can be viewed as a representative of the genus (*Rhizobium*).

Some of the characteristics of the bacteria include:

- ❖ They appear as elongated rods when viewed under the microscope
- ❖ Like a number of other bacteria, *Rhizobium* do not form spores in their life cycle
- ❖ They possess several flagella on their polar end. This allows them to move from one location to another

- ❖ **They are aerobic. As such, they need oxygen for respiratory purposes**
- ❖ **There are various strains of the bacteria some of which have granules**
- ❖ **They are Gram-negative bacteria**
- ❖ **Although they can tolerate higher temperatures of about 38 degrees Celsius, Rhizobium ideally grow in temperatures of between 20 and 28 degrees Celsius**
- ❖ **Apart from various types of carbohydrates, the bacteria also uses nitrates and nitrite, ammonium salts and various amino acids among others for development**

Isolation of Rhizobium (Vincent, 1970)

The selected nodules were properly washed with running water to remove the soil particles. Then the undamaged root nodules were selected and again washed with distilled water. These selected nodules were kept immersed in 0.1% acidified potassium chloride solution for 5 minutes and washed repeatedly with sterile water. Then, they were immersed in ethyl alcohol solution. This treatment was followed by repeated washing with sterile distilled water. These sterilized root nodules were crushed simply with pestle and mortar. The homogenate was sieved through a fine sieve. The extract was serially diluted with distilled water and inoculated into a Yeast Extract Mannitol Agar (YEMA) medium (Vincent, 1970).

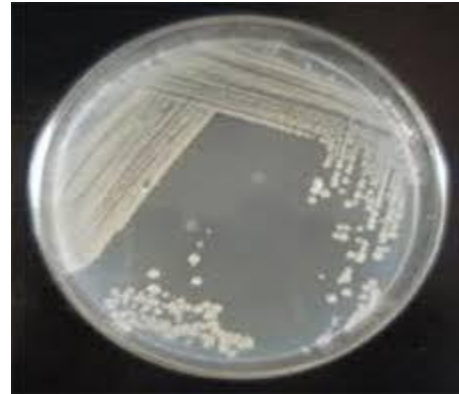
Composition of YEMA medium

Agar	- 20 g
Mannitol	- 10 g
Yeast Extract	- 1.0 g
K₂HPO₄	- 0.5 g
MgSO₄·7H₂O	- 0.2 g
NaCl	- 0.1 g
Distilled water	- 1000 ml
Congo red (1%)	- 2.5 ml (only for solid medium during isolation)

The pH of the medium was adjusted to 6.8 before adding agar.

The plates were incubated at 28 ± 2°C for 2 to 3 days. This medium allowed Agrobacterium and Rhizobium to grow and develop into colonies. The rhizobial colonies appeared as white translucent, elevated colonies on YEMA medium. They were removed and purified

by repeated streaking. Pure rhizobial cultures were maintained on YEMA slant



Identification of Rhizobium

The bacterium was identified by the following tests.

Growth on media: The pure culture from the slants was spread on peptone glucose agar and on YEMA media and the growth was observed.

Gram staining: The bacterial cultures were subjected to gram staining procedures and observed.

Congo red test (Hahn, 1966): An aliquot of 2.5 ml of 1 per cent solution of the dye in water was added to a litre of YEMA. The isolated bacterial culture was inoculated on to the plated YEMA and observed.

Hofer's alkaline broth test (Hofer, 1941): The isolated bacterial cultures were spread on YEMA with high pH 11 and observed for growth.

Lactose Agar Test (Bemaerts and Delay, 1963): Isolated nodule bacteria were grown on lactose plates for 4-10 days and the plates were flooded with Benedict's reagent. Finally the plates were observed for colour development.

Staining of poly P-hydroxy butyrate (PHB) (Mc Kinney, 1953): A loopful of selected rhizobia were spread out on a microscope slide in a drop of water and allowed to dry in air. The cooled smear was flooded with 5 times diluted carbol fuchsin for 30 seconds. The slide was washed in running water and allowed to air dry. The pinkish PHB granules were observed under the oil immersion microscope.

Methods of Mass Cultivation

Following are the steps of mass cultivation of *Rhizobium*.

- (a) sterilize the growth medium and inoculate with broth of mother culture prepared in advance,**
- (b) incubate for 3-4 days at 30 - 32°C,**
- (c) test the cultures for its purity and transfer to a large fermenter, wait for 4-9 days for bacterial growth (for good bacterial growth make the device for its aeration),**
- (d) allow to grow the bacteria either in a large fermenter containing broth or in small flasks as per demand,**
- (e) check the quality of broth,**
- (f) blend the broth with sterile carrier *e.g.* peat, lignite, farmyard manure and charcoal powder,**
- (g) pack the culture in polyethylene bags and keep at 25°C,**
- (h) check the quality of carrier culture,**
- (i) store at 4°C in a controlled-temperature room, and**
- (j) supply to farmers.**

During the blending of broth a variety of carriers are used, for example, peat, lignite, farmyard manure, charcoal powder, etc. In India powdered farmyard manure and charcoal powder are good carrier and an alternative to peat and lignite. Good quality of carrier culture is that which contains sufficient amount of rhizobial cells *i.e.* 1000 x 10⁶ to 4000 x 10⁶ rhizobia/g carrier. Seed inoculation with aqueous suspension of carrier culture during sowing has revealed the luxuriant nodulation and good yield of crops.

Methods of seed inoculation with rhizobial culture

The steps of seed inoculation with rhizobial culture are given below. Dissolve 10 per cent sugar or *gur* (jaggery) in water by boiling it for some time. Leave the content to cool down. Gum arabic solution (10%) may also be added to the solution. This serves as sticker for *Rhizobium* cells to seeds. Mix this carrier based culture of *Rhizobium* to form the inoculum slurry. For one hectare, 400 g charcoal based culture would be sufficient for mixing the seeds. Transfer the inoculum slurry on seeds and mix properly. The number of rhizobial cells/ seed should be between 10^5 to 10^6 . Spread the seeds in shade for drying on cement floor or plastic sheets.

While using rhizobial cultures, certain precautions are taken into account. For example, use of culture before expiry date, use of small amount of pesticides when required, immediate sowing of seeds after mixing, etc. Seeds must be stored at 4°C when not used immediately to protect the rhizobial cells.

Pelleting

When soil has the adverse conditions such as dryness, acidity, excess fertilizers and pesticides, etc., the rhizobial cells are protected by adopting special method of inoculation. One of these methods is pelleting *i.e.* preparation of pelleted seeds. This method involves the procedure as described earlier. High amount of gum arabic (40%) or carboxymethylcellulose (20%) is added to the inoculum slurry before mixing with seeds. Finally, pelleting agent is mixed when inoculated seeds are moist (before seed drying) to get the seeds evenly coated.

The commonly used pelleting agents are calcium carbonate, rock phosphate, charcoal powder, gypsum and betonite.

Effects of rhizobial inoculants on crop yield

Effect of rhizobial culture on yield of different pulses, and subsequent crops grown after harvesting the pulse crops are given in Table 12.1 based on the study made under All India Co-ordinated Pulse Improvement Research Programme of I.C.A.R., New Delhi. Yield of pulse crops can be substantially increased by rhizobial inoculation. Legume crops get benefit from rhizobial symbiosis. In addition, certain amount of N is left over in soil which is taken by the other plants also.

Subba Rao and Tilak (1977) studied the effect of residual nitrogen of many legumes on the yield of subsequent crops of wheat or rice. They always found more yield of subsequent crops in *Rhizobium* inoculated fields than in uninoculated control.

Application of biofertilizers to crops

1.Seed treatment

Each packet (200g) of inoculant is mixed with 200 ml of rice gruel or jaggery solution. The seeds required for one hectre are mixed in the slurry so as to have uniform coating of the inoculants over the seeds and then shade dried for 30 minutes. The treated seeds should be used within 24 hous. One packet of inoculant is sufficient to treat to 10 kg seeds. Rhizobium, Azospirillum, Azotobacter and Phosphobacteria are applied as seed treatment.

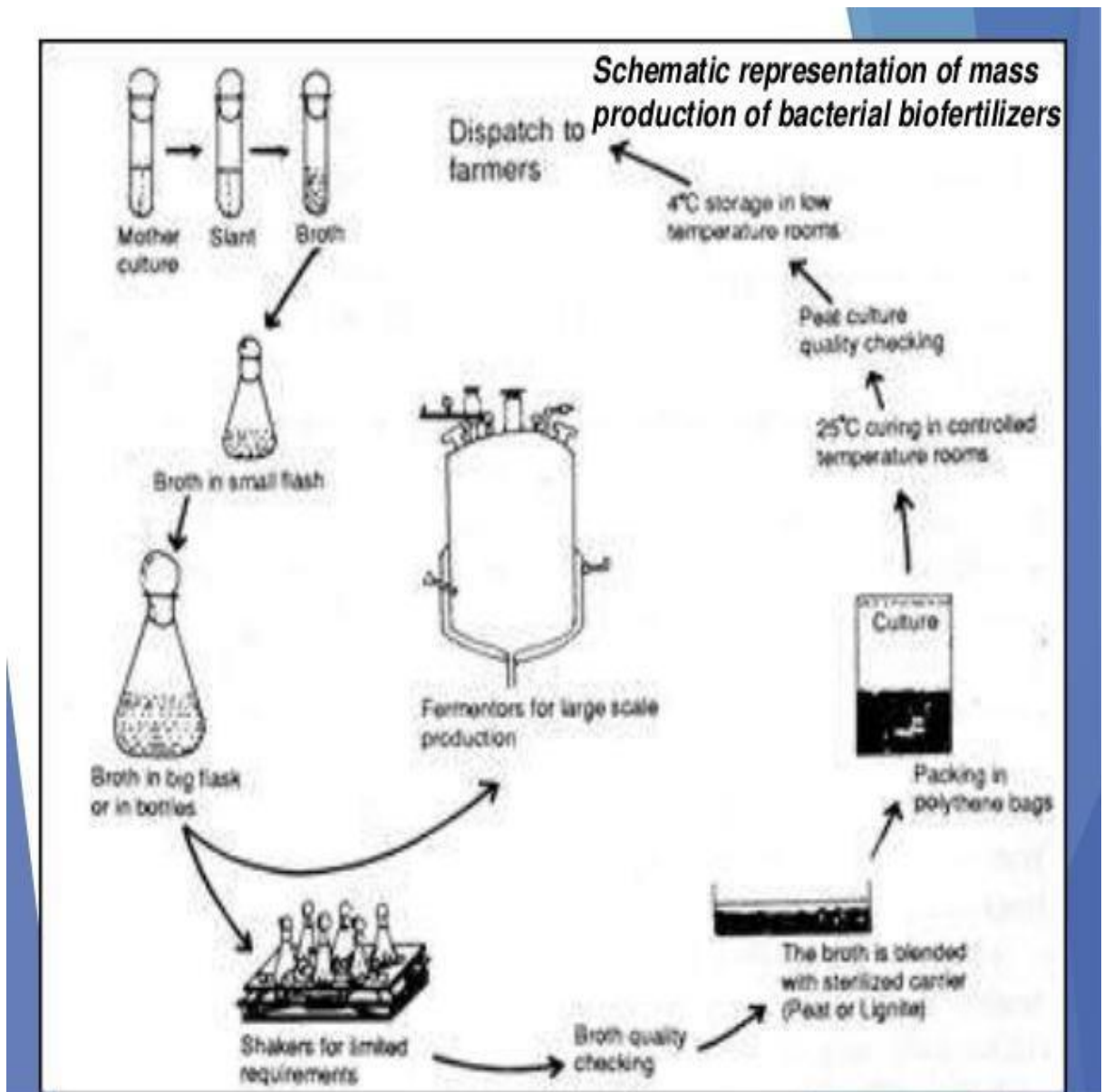
2.Seedling root dip

This method is used for transplanted crops. Five packets (1.0 kg) of the inoculants are required for one ha and mixed with 40 litres of water. The root portion of the seedlings is dipped in the solutions for 5 to 10 minutes and then transplanted. Azospirillum is used for seedling root dip particularly for rice.

3.Soil treatment

4 kg each of the recommended biofertilizers are mixed in 200 kg of compost and kept overnight. This mixture is incorporated in the soil at the time of sowing or planting.





Isolation, Characteristics, Identification, Mass inoculum production, Field application and marketing of *Azospirillum*,

***Azospirillum*, a free-living nitrogen-fixing bacteria closely associated with grasses. *Azospirillum* Bacteria is a Gram negative motile bacteria belonging to the order Rhodospirillales, associated with roots of monocots, including important crops, such as wheat, corn and rice.**

***Azospirillum* contains 109/gm spores of *Azospirillum* species. This *Azospirillum* bacterium fixes the atmospheric nitrogen and makes it available to plants in non-symbiotic manner that can replace 50-90% of the nitrogen fertilizer required by plants.**

Azospirillum biofertilizer also secretes some fungicides, enzymes but in minute amount. Use of Azospirillum biofertilizer increases the crop production in large scale. We are engaged in manufacturing and marketing of Bio control agents comprise of different types of beneficial Bacterial, Fungal and Viral cultures.

Azospirillum is mainly useful for monocot vegetables. It is an eco-friendly liquid biological fertilizer formulation containing bacteria, It contains large amount of lipid granules, which enters the cortical cells of the root and fix up atmospheric nitrogen and also produces biologically active substances like vitamins, nicotinic acid, indole acetic acid, gibberellins etc and helps in better retention of flowers and enhances the plant growth.

Azospirillum Biofertilizer is a nitrogen fixing biofertilizer. Nitrogen is a major nutrient for all plants. Azospirillum lipofereum is a very useful soil and root bacterium. It is an associative symbiotic nitrogen fixing bacteria. It is found in the soil around plant roots and root surface.

It also produces growth-promoting substances like indole acetic acid (IAA), gibberellins, pantothenic acid, thiamine and niacin and it promotes root proliferation and it improves the plant growth yield. It increases the rootlet density and root branching resulting in the increased uptake of mineral and water.

Suitable for:

Millets, oilseeds, fruits & vegetables, sugarcane, banana, coconut, oil palm, cotton, chilly, lime, coffee & tea, arecanut & rubber, flower, spices, & condiments, herbs, lawns & ornaments, trees etc.

It is suitable for all crops and applications including field crops, potting soil, vegetable and flower gardens, orchards and turf grass. Promotes balanced growth of crops, boosts the capacity of immunity and Greater nutritional value: Rapidly complement the nutrients, improve the quality of product.

Characteristics:

Azospirillum is a rod to spirillum-shaped nitrogen fixing bacterium and freely lives in soil forming nonspecific symbiotic associations with various plants in particular, corn. This genus

consists of species, namely, *A. lipoferum*, *A. brasilense*, *A. amazonense*, *A. halopraeferans*, *A. nitrocapta*, and *A. seropedica*. *Azospirillum lipoferum* was originally described and named *Spirillum lipoferum* by Beijerinck in 1922.

Azospirillum characteristically develops white, dense, and undulating pellicles on a semi-solid malate containing enrichment medium. The pellicle is formed 2 mm below the surface of the medium indicating the microaerophilic nature of the bacterium. *Azospirillum* is gram-negative, contains poly- β -hydroxy butyrate (PHB) granules, and shows polymorphism and spirillar movement.

It fixes atmospheric nitrogen (dinitrogen) in microaerophilic surroundings (low oxygen conditions) but possesses ability to grow profusely in ammonium-rich environment without fixing nitrogen. The bacterium also produces growth substances such as indole acetic acid (IAA), kinetins, and gibberellins.

Criteria for Strain Selection:

The efficient nitrogen fixing strain is evolved or selected in laboratory, maintained and multiplied on nutritionally rich artificial medium before inoculating the seed or soil. In soil, the strain has to survive and multiply to compete for infection site on roots against hostile environment in soil.

Steps for Preparing Bio-Fertilizer:

The isolated strain is inoculated in small flasks containing suitable medium for inoculum production. The volume of the starter culture should be a minimum of 1% to obtain at least 1×10^9 cells/ml. Now the culture obtained is added to the carrier for inoculant (bio-fertilizer) preparation.

Carriers carry the nitrogen fixing organisms to the fields. In some cases carrier is first sterilised and then inoculated, while in other cases it is first inoculated and then sterilised by UV irradiation. The inoculum is now packed with 10^9 - 10^{10} viable cells per gram. Final moisture content should be around 40-60%. For large scale production of inoculum, culture fermenters are used.

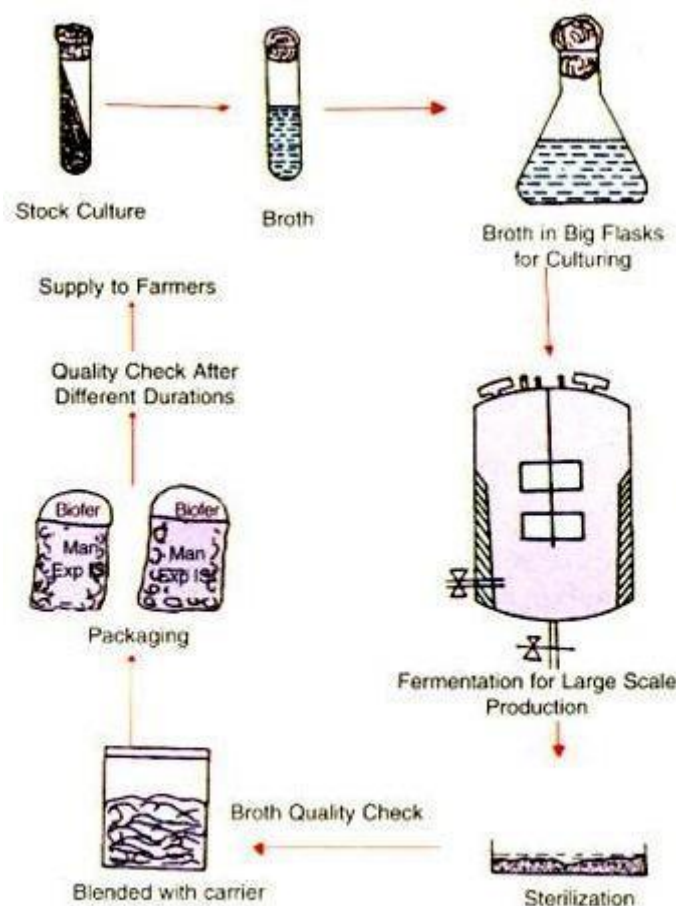


Fig. 34.1 : Commercial production of biofertilizer.

Application of biofertilizers to crops

1. Seed treatment

Each packet (200g) of inoculant is mixed with 200 ml of rice gruel or jaggery solution. The seeds required for one hectre are mixed in the slurry so as to have uniform coating of the inoculants over the seeds and then shade dried for 30 minutes. The treated seeds should be used within 24 hours. One packet of inoculant is sufficient to treat to 10 kg seeds. Rhizobium, Azospirillum, Azotobacter and Phosphobacteria are applied as seed treatment.

2. Seedling root dip

This method is used for transplanted crops. Five packets (1.0 kg) of the inoculants are required for one ha and mixed with 40 litres of water. The root portion of the seedlings is dipped in the solutions for 5 to 10 minutes and then transplanted. Azospirillum is used for seedling root dip particularly for rice.

3. Soil treatment

4 kg each of the recommended biofertilizers are mixed in 200 kg of compost and kept overnight. This mixture is incorporated in the soil at the time of sowing or planting.

Effects:

- **Better root systems: Promoting the development of roots**
- **Healthier foliage and fruit appearance: Thicken, enlarge and balance the leaf growth, supply well-balanced crop nutrients, stimulating cell division, improves the fruit set, improves blossom and fruit set**
- **Greater resistance to disease and pests: Containing antitoxins to fend off bacteria and viruses, and to repel insects. Helps plants to endure environmental stress**
- **Improved seed germination: Promoting the development of shoots**
- **As formulation: Seaweed Extract can be used not only on crops, but also to formulate types of fertilizers. A little addition of seaweed extract on common fertilizer will height the quality greatly resistance, improves crop quality and increase yield**

Isolation, Characteristics, Identification, Mass inoculum production, Field application and marketing of *Azotobacter*:

Azotobacter Structure and Characteristics:

Azotobacter is a soil-inhabiting bacterium and comprises large, gram-negative, obligately aerobic rods. This bacterium freely lives in soil and fixes atmospheric nitrogen nonsymbiotically. The first species of *Azotobacter* was discovered by the Dutch microbiologist M. Beijerinck in the beginning of 20th century, and was named by him *Azotobacter chroococcum*.

While growing, *Azotobacter* produces flat, slimy, paste-like colonies with a diameter of 5–10 mm, which may form films in liquid nutrient media. The colonies can be dark-brown, green, or other colors, or may be colorless, depending on the species. The growth is favored at a temperature of 20–30°C.

Subsequently, many other species of *Azotobacter* were isolated from different soils of the world and some important ones are: *A. agilis*, *A. vinelandii*, *A. beijerinckii*, *A. insignis*, *A. macrocytogenes*, *A. paspali*, etc. *Azotobacter* cells are large, many isolates being almost the size of yeasts, with diameters of 2-4 µm or more. Pleomorphism is common and a variety of cell shapes and sizes have been described. Some strains possess peritrichous flagella.

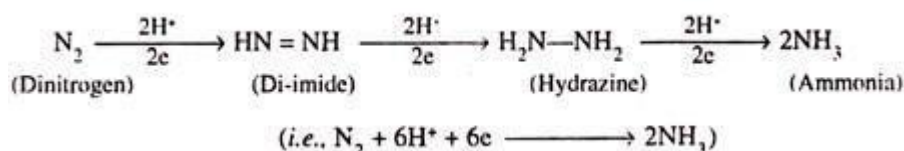
Although the *Azotobacter* is an obligate aerobe, its enzyme called nitrogenase that catalyzes atmospheric nitrogen fixation is oxygen-sensitive. It has been studied that the high respiratory rate characteristic of *Azotobacter* and the abundant capsular slime help protect nitrogenase from oxygen.

This bacterium grows on a wide variety of carbohydrates, alcohols, organic acids, ammonia, urea, and nitrate. *Azotobacter* forms cysts the resting structures, which are resistant to desiccation, mechanical disintegration, and ultraviolet and ionizing radiation. Each cyst measures about 3 µm in diameter.

Mechanism of N₂ Fixation:

Azotobacter (*A. chroococcum* and *A. vinelandii*) is one of the most extensively investigated member amongst free-living nitrogen fixing bacteria. The use of ¹⁵N tracer and acetylene reduction method have however enriched our knowledge regarding the biochemical pathway between atmospheric nitrogen (dinitrogen; N₂) and ammonia (NH₃) but the exact nature of intermediate products have eluded even critical investigators.

Nevertheless, the overall reaction in the enzymic reaction of N₂ to NH₃ can be postulated as under:



Nitrogenase of Azotobacter:

Nitrogenase, the enzyme that catalyzes atmospheric nitrogen fixation, consists of two protein fractions:

- (i) The Mo-Fe containing protein (molecular weight 220,000-2,70,000) and**
- (ii) Fe containing protein (molecular weight 55,000-66,800).**

In Azotobacter (A. vinelandii), two additional nitrogenases have been investigated. One of these possesses vanadium (V) instead of molybdenum (Mo) and the other has neither molybdenum nor vanadium.

The characterization of these nitrogenases has generated fresh problems in pinpointing evidences to demonstrate the essentiality of molybdenum for N₂-fixation and characterization of the site at which nitrogen binds to nitrogenase.

Production of azotobacter:

i. Mother culture:

A pure growth of any organism on a small scale is called as a mother culture. Mother culture is always prepared in a conical flask of 500 or 1000 ml. Capacity and then this mother culture is used for further production.

For this purpose, one liter conical flasks are taken to which 500 ml of broth of nitrogen free medium is added and these flasks are then plugged with non-absorbent cotton, sterilized in an auto slave for 15-20 minutes at 75 lbs pressure for 15 minutes. Flasks are then inoculated with mother culture with the help of inoculating needle aseptically. The flasks are transferred to shaker and shaking is done for 72-90 hours so as to get optimum growth of bacteria in broth. Bacteria are multiplied by binary method i.e. cell division. After about 90 days, the number of per milliliters comes to about 100 crores. Total growth of bacteria in this broth means starter culture or mother culture, which should carefully be done, since further purity of

biofertilizer or quality of biofertilizer depends upon how mother culture is prepared.

ii. Production on a large scale:

Azotobacter is multiplied on a large scale by two ways viz. Fermenter and Shaker. The fermenter is most automatic and accurate method of multiplication of any micro-organism. In this method, the medium is taken in a fermenter and then sterilized. After this pH of the medium is adjusted and 1% mother culture is added. In order to get an optimum growth of the Azotobacter required temperature and oxygen supply is adjusted so that concentrated broth is made. This concentrated broth of the culture is then mixed with a carrier previously sterilized and bio-fertilizers are prepared. Depending upon the demand and supply suitable fermenter is selected.

In the 2nd method i.e. shake method, a suitable medium is prepared transferred to conical flask of suitable capacity. These flasks are then sterilized in an autoclave at 15 lbs pressure for 15 minutes. Each flask is inoculated with 10 ml mother culture and they are transferred to shaker for multiplication where they are kept for 72-90 hours. This broth is mixed with a suitable carrier previously sterilized. Thus biofertilizer is prepared, filled in plastic bags and stored in cool place.

iii. Selection of carrier:

A carrier is nothing but a substance which has high organic matter, higher water holding capacity and supports the growth of organism. In order to transport the biofertilizer and becomes easy to use the suitable carrier is selected. Generally Lignite coal, compost and peat soil are suitable carriers for Azotobacter. Out of these carriers lignite is most suitable for this organism, since it is cheaper, keeps organism living for longer period and does not lower the quality of bio-fertilizers.

The lignite comes in clumps and hence it is ground in fine powder by grinding machine. Its fineness should be 250-300 mesh. The pH of the carrier is adjusted to neutral by adding CaCO_3 . The lignite naturally has a variety of micro-organism and hence it is sterilized in

autoclave at 30 lbs. Pressure for 30 minutes. After this the broth is mixed with lignite 1:2 proportion by following method.

Galvanized trays are sterilized and used. To these trays, previously sterilized lignite is transferred and broth is then added (lignite2: broth 1) and mixed properly. Trays are then kept one above the other for 10-12 hours for allowing the organism to multiply in the carrier. This mixture is then filled in plastic bags of 250 g or 500 g capacity. Plastic bags are properly sealed. Trays are then kept one above the other for 10-12 hours for allowing the organism to multiply in the carrier. This mixture is then filled in plastic bags of 250 g or 500 g capacity. Plastic bags are properly sealed. All the required information such as name of biofertilizer, method of use expiry date, etc. is printed on plastic bags. In this way biofertilizer is ready to sell or use. If biofertilizer is used immediately then bags are stored in cool place otherwise they should be stored in cold storage in order to keep biofertilizer in good quality.

As per ISI standards, one gram of biofertilizer immediately after it is prepared should have one crore cells of bacteria and 15 days before expiry date one gram of biofertilizer should have 10 lakh bacteria. If biofertilizer is stored at 15-20 °C then it will remain effective for 6 months. However, at 0 to 4 °C (cold storage) the bacteria will remain active for 2 years. The storage periods are decided after testing the biofertilizer for that particular storage conditions, such temperature and humidity.

How to apply Azotobacter bio-fertilizer?

1. Seed inoculation:

On the basis of efficiency of Azotobacter, other micro-organisms present in the soil, benefits obtained from biofertilizer and expenditure it has been fixed to use Azotobacter - bio-fertilizer at the rate of 250 g biofertilizer for 10-15 kg. If one knows this proportion then take a definite quantity of seed to be inoculated. The required

quantity of fresh biofertilizer is secured and slurry is made by adding adequate, quantity of water. This slurry is uniformly applied to seed, seed is then dried in shed and sown. Some stickers are used in order to adhere biofertilizer to seeds. Viz. Jaggery or gumarabica.

2.Seedling inoculation:

This method of inoculation is used where seedlings are used to grow the crop. In this method, seedlings required for one acre are inoculated using 4-5 packets (2-2.5 kg). For this, in a bucket adequate quantity of water is taken and biofertilizer from these packets is added to bucket and mixed properly. Roots or seedlings are then dipped in this mixture so as to enable roots to get inoculums. These seedlings are then transplanted e.g. Tomato, Rice, Onion, Cole, Crops, flowers.

c.Self inoculation or tube inoculation:

In this method 50 litres of water is taken in a drum and 4-5 kg of Azotobacter biofertilizer is added and mixed properly. Sets are required for one acre of land are dipped in this mixture. Potato tubers are dipped in the mixture of biofertilizer and planting is done.

d.Soil application:

This method is mostly used for fruit crops, sugarcane, and trees. At the time of planting fruit tree 20 g of biofertilizer mixed with compost is to be added per sappling, when trees became matured the same quantity of biofertilizer is applied.

In sugarcane after two to three months of planting i.e. before earthing up 5-6 kg of biofertilizer per acre is applied by mixing with compost or soil. Although, Azotobacter fixes nitrogen non-symbiotically, it also fixes atmospheric nitrogen in the rhizosphere region i.e. soil around the seedlings or trees. Biofertilizer applied to seed or seedlings bacteria remain around seeds or seedlings and use organic carbon for their metabolism. When seeds are germinated or seedlings set in soil they leave or exude root exudates which become food of these bacteria. They grow on these substances which include

sugars, organic acids, and amino acids and fix atmospheric nitrogen most efficiently. Nitrogen so fixed by these bacteria becomes available to plants after dead and degradation of bacterial cells.

Use of Azotobacter as Biofertilizer:

Plant needs nitrogen for its growth and Azotobacter fixes atmospheric nitrogen non-symbiotically. Therefore, all plants, trees, vegetables, get benefited. However, especially cereals, vegetables, fruits, trees, sugarcane, cotton, grapes, banana, etc. are known to get additional nitrogen requirements from Azotobacter. Azotobacter also increases germination of seeds. Seeds having less germinating percent if inoculated can increase germination by 20-30%.

Advantages of Azotobacter:

- 1. Azotobacter contributes moderate benefits**
- 2. Azotobacter is heaviest breathing organism and requires a large amount of organic carbon for its growth.**
- 3. It is poor competitor for nutrients in soil and hence its growth promoting substances, fungistatic substances.**
- 4. It can benefit crops by Nitrogen fixation, growth promoting substances, fungi static substances.**
- 5. Azotobacter is less effective in soils with poor organic matter content.**
- 6. It improves seed germination and plant growth**
- 7. It thrives even in alkaline soils.**
- 8. Azotobacter is tolerant to high salts.**

Conclusion:

Azotobacter is a broad spectrum biofertilizer and can be used as inoculant for most agricultural crops. Earlier, its utility as a biofertilizer was not a priority due to its relatively low population in the plant rhizosphere. However, seeding treatment with Azotobacter of several crops brought about an increase in yield. Besides, because of its well known N₂ nutritional function, it is now recognized to play a multiple role in helping crop plants to improve their growth potential, yield and maintenance of soil health for sustainable agriculture. Hence there is renewed interest in this rhizobacterium. However, quantitative understanding of the ecological factors that control the performance of biological N₂fixation systems of the bacterium in crop fields is essential for promotion and successful adoption of the bio-fertilizer production technology.

UNIT II:

Cyanobacteria (BGA) - Isolation, Characteristics, Mass inoculum production and Field application. Azolla – Isolation, Characteristics, Mass inoculum production and Field application

Cyanobacteria (BGA) - Isolation, Characteristics, Mass inoculum production and Field application.

The blue-green algae are photoautotrophic, prokaryotic algae. They are free living. They are called as cyanobacteria and are fixing atmospheric nitrogen and release it into the surroundings in the form of amino acids, proteins and other growth promoting substances . *e.g. Aulosira, Anabaena, Cylandrospermum, Ocillatoria, Nostoc, Plectonema, Tolypothrix* , and Gleocapsa.

Mass cultivation of cyanobacterial biofertilizers

For outdoor mass cultivation of cyanobacterial biofertilizers, the regional specific strains should be used. However, many germplasm collection laboratories have been established by the D.B.T. in different parts of the country for the development of starter inoculum. Mixture of 5 or 6 regional acclimatized strains of cyanobacteria, *e.g. species of Anabaena, Aulosira, Cylandrospermum, Gloeotrichia, Nostoc, Plectonema, Tolypothrix* are generally used for starter inoculum.

The following four methods are used for mass cultivation

- (i) cemented tank method.,**
- (ii) shallow metal troughs method,**
- (iii) polythene lined pit method, and**
- (iv) field method.**

The polythene lined pit method is most suitable for small and marginal farmers to prepared algal biofertilizer. In this method, small pits are prepared in field and lined with thick polythene sheets.

Mass cultivation of cyanobacteria is done by using any of the four methods under the following steps:

1. Prepare the cemented tanks, shallow trays of iron sheets or polythene lined pits in an open area. Width of tanks or pits should not be more than 1.5 m. This will facilitate the proper handling of culture.

2. Transfer 2 -3 Kg soil (collected from open place for 1m² area of the tank) and add 100 g of superphosphate. Water the pit to about 10 cm height. Mix lime to adjust the pH 7. Add 2 ml of insecticide *e.g.* malathion to protect the culture from mosquitoes. Mix well and allow to settle down soil particles.

3. When water becomes clear, sprinkle 100 g of starter inoculum on the surface of water.

4. When temperature remains between 35-40° during summer, optimum growth of cyanobacteria is achieved. Always maintain the water level to about 10 cm during this period,

5. After drying, the algal mat will get separated from the soil and forms flakes. During summer about 1 kg pure algal mat per m² area is produced. These are collected, powdered, kept in sealed polythene bags and supplied to the farmers.

6. The algal flakes can be used as starter inoculum if the same process is repeated.

PRODUCTION OF ALGAE FOR FIELD APPLICATION

The introduction of algal biofertilizer as a package of practices in rice cultivation will depend largely upon an efficient and economical system of large-scale production of blue-green algae, its preservation and transport. The method is credited with simplicity of operation and adaptability by small and marginal farmers. The algae used in this system is a mixture of species of *Tolypothrix*, *Aulosira*, *Anabaena*, *Nostoc* and *Plectonema*. The same system is reported to be working well in Burma. The procedure, starting in the laboratory and ending in the field, is described below. It may be noted that other efficient strains of algae may be selected and used, depending on the regions and locations.

1. Laboratory Culture

Maintain stock cultures of different nitrogen-fixing blue-green algae on 1 to 1.5% agar slants.

- ❖ **Maintain the same cultures also in a soil extract medium (1 g of soil + 10 cm³ of Fogg's medium, sterilized together in test-tubes). For Fogg's medium and soil extract medium**
- ❖ **Grow the algae in 250 cm³ flasks containing 100 cm³ of Fogg's medium in the light.**
- ❖ **Scale up the cultures in aspirator bottles or carboys.**
- ❖ **Transfer the algae to troughs to prepare soil-based starter material as described later under 'Trough Method'. Different algal forms are cultured and maintained separately before transferring them to the troughs. The pH of all the cultures should be between 7.0 and 7.5 unless acid or alkali-tolerant forms are used. All the cultures are grown in light.**

2 .Trough Method

- **Prepare shallow trays (2 m x 23 cm) of galvanized iron sheet or permanent tanks The size can be increased if more material is to be produced.**
- **Introduce 8 - 10 kg of soil and mix well with 200 g of super-phosphate**
- **Place from 5 to 15 cm of water in the trays depending on the local conditions and rate of evaporation. The reaction of the soil should be about neutral; if acidic correct by adding lime .**
- **To prevent insects, add Carbofuron (3% granules) at the rate of 25 g per tray or BHC or other suitable insecticide.**
- **After the soil has settled, sprinkle the algal culture on the surface of the standing water Keep the units in the open air and completely exposed to the sun.**
- **In hot summer months, the growth of the algae will be rapid and in about seven to ten days they form a thick mat . If the daily rate of evaporation is high, add water inter- mittently. When the algal growth becomes sufficiently thick, stop watering.**
- **Allow the water to evaporate completely in the sun the dry algae cracks into flakes.**
- **Collect the dry algal flakes from the trays and store in bags for use in the fields**

- **Fill the troughs again with water and add a small amount of the dry algal flakes as further inoculum. Continue the process as above. Once the soil in the troughs is exhausted (usually after three or four harvests) replace it with fresh soil mixed with superphosphate and continue as above. A single harvest of surface algae from one trough of the given dimensions will give about 1.5 to 2.0 kg of material.**

2. Pit Method

This method does not differ from the trough method except in magnitude. Instead of troughs or tanks, shallow pits are dug in the ground and layered with a thick polythene sheet to hold the water . Other procedures are the same as in the trough method. This method is easy and less expensive to operate by small farmers. Also the accidental collection of excess soil along with the algae during harvesting is avoided. Sometimes the addition of 200 g of superphosphate as a single dose results in problems of acidity. To avoid this, split the dose into two or three smaller doses; similarly the amount of soil can be split.

3. Field Production

The field production of algae is really a scaled up operation of the trough or pit methods to produce the material on a commercial scale: it is being adopted by a number of farmers in south India

- a. Demarcate the area in the field for algal production; the suggested area is 40 m². No special preparation is necessary although if algal production is envisaged immediately after crop harvest, the stubble is to be removed and if the soil is loamy it should be well puddle to facilitate water logging.**
- b. Bund the area with strong 15 cm earth bunds. Flood the area with water to a depth of about 2.5 cm . In the trough and pit methods, flooding is done only in the beginning; in the field, flooding is repeatedly needed to keep the water standing.**

- c. Apply superphosphate at 12 kg 40 m⁻²**
- d. If the field has previously received algal application for at least two consecutive cropping seasons, no fresh algal application is required. Otherwise, apply the composite algal culture at 5 kg 40 m⁻²**
- e. To control predators like daphnids, snails and mosquitoes, apply Carbofuron (3% granules) or Ekalux (5% granules) at 250 g 40 m⁻² or BHC or Furadon**
- f. In clayey soils, good growth of algae takes place in about two weeks in clear, sunny weather, while in loamy soils it takes about three to four weeks.**
- g. Once the algae have grown and form floating mats, they are allowed to dry in the sun in the field and the dried algal flakes are then collected and stored in sacks for further use. One can continually harvest algae from the same area by re-flooding the plot and applying superphosphate and pesticides.**
- h. Addition of algal inoculum for subsequent production is not necessary.**
- i. During summer months (April-June), the average yield of algae per harvest ranges from 16 - 30 kg per 40 m². Adopting this method, a record production of 15.6 tonnes/ha of wet blue-green algae has been obtained by farmers within three weeks**

4. Nursery-cum-algal Production

Farmers can produce algae along with seedlings in their nurseries. If 320 m² of land are allotted to prepare a nursery, an additional 40 m² alongside can be prepared for algal production as described in the preceding section. By the time the rice seedlings are ready for transplantation, an amount of 15 to 20 kg of algal material will be available and sufficient to inoculate about one and a half hectares. Transplantation is made in the nursery and algal plots and in this way land is neither wasted nor locked up exclusively for algal production during the growing season. The algae are first grown in

flasks containing sterile medium and are then transferred to large glass bowls under non-sterile conditions.

Nursery plots of 5-7 m x 1 m x 20 cm, containing 6-7 cm of water are inoculated at the rate of 150 g algae per m². After about seven days the algae attain a biomass of 500 - 1 000 g/m². The nursery plots are covered with transparent plastic sheets to protect them from low temperatures .The field inoculation is done by spreading the algae at the rate of 750 kg/ha, which grows to attain a biomass of 7.5 tonnes/ha within 10 - 15 days and if the temperature exceeds 303 kelvin (30 °C) 15 tonnes/ha can be reached.

RECOMMENDATIONS FOR FIELD APPLICATION OF BLUE-GREEN ALGAE

- 1.If mineral nitrogen fertilizers are not being used, apply blue-green algae in order to gain the benefit of from 20 - 30 kg N/ha.**
- 2. Broadcast the dry algal material over the standing water in the rice field at a rate of 10 - 15 kg/ha one week after transplanting the seedlings. Addition of excess algal material is not harmful and will accelerate the multiplication and establishment in the field. Providing that the field is not being used and that water facilities are available, algal application can be done well in advance of transplanting the rice.**
- 3. When mineral nitrogen fertilizer is used reduce its dose by 25 kg/ha and supplement with algal application.**
- 4.If so desired, algae can also be used along with high levels of nitrogen fertilizer.**
- 5. The sun-dried algal material can be stored for a long time in a dry state without any loss in viability.**
- 6. Do not store the algal material in direct contact with fertilizers or other chemicals.**

7. Apply algae for at least three consecutive seasons.
8. Recommended pest control measures and other management practices do not interfere with the establishment and activity of the algae in the field.

Azolla - Isolation, Characteristics, Mass inoculum production and Field application.

Azolla is an aquatic heterosporous fern which contains an endophytic cyanobacterium, ***Anabaena azollae***, in its leaf cavity. The significance of ***Azolla*** as biofertilizer in rice field was realized in Vietnam. Recently, it has become very popular in China, Indonesia, Philippines, India and Bangladesh. A total of six species of ***Azolla*** are known so far viz., ***A. caroliniana***, ***A. filiculoides***, ***A. mexicana***, ***A. microphylla***, ***A. nilotica***, ***A. pinnata*** and ***A. rubra***. Out of these ***A. pinnata*** is commonly found in India. The global collections of several species of ***Azolla*** are maintained at CRRRI (Cuttack). Within the leaf cavity filaments of ***Anabaena azollae*** are present. Dr. P.K. Singh, at CRRRI has done an outstanding work on mass cultivation of ***Azolla*** and its use as biofertilizer in rice and other crop fields.



Azolla cultivation procedure



- 1. Create an artificial pond for growing Azolla.**
- 2. For creating Azolla cultivation pond, select partially shaded area because Azolla needs 30% sunlight too much sunlight will destroy the plant. The area under the tree is preferable.**
- 3. If you decide to grow an Azolla for the large scale you can make small concrete tanks otherwise you can make the pond any size you want.**
- 4. Dig out the soil for pond and level the soil after that spread the plastic sheet around the ground to prevent water loss. Make sure the pond is at least 20 CM Deep.**
- 5. Add some soil uniformly on the plastic sheet in the pond. For 2M X 2M size pond add 10-15 kg soil.**
- 6. Azolla needs Phosphorus to grow well you can use Super Phosphate along with cow dung slurry. Cow dung increases the available nutrients. Use, cow dung 4-5 days old.**
- 7. Next, fill the pond with water to a level of about 10 cm this will allow the short route of the Azolla Plant to float freely then leave the pond for 2 to 3 days so the ingredients can settle.**
- 8. After 2-3 days add Azolla culture in the pond by gently rubbing Azolla in hands. It helps break Azolla into smaller pieces for faster multiplication.**
- 9. After two-week start harvesting. form pond of 2M X 2M size, you can harvest 1kg Azolla each day.**



Important tips to grow Azolla

1. Azolla rapidly grows so maintain Azolla biomass 300 gms – 350 gms /sq.meter hence harvest daily to avoid overcrowding.
2. Add once in 5 days mixture of Super Phosphate, and cow dung also add mixture containing magnesium, iron, copper, Sulfur etc. at weekly intervals to enhance the mineral content of Azolla.
3. Replace 25 to 30% old water with fresh water, once in 10 days; it helps to prevent nitrogen build up in the pond.
4. Replace complete water and soil, at least once in six months and then add Fresh Azolla seeds.
5. Maintain the water level of at least 10 cm, so Azolla root doesn't grow in the soil by keeping the roots floating it becomes easy to harvest.
6. Harvested Azolla wash thoroughly, so it removes dirt and smell of cow dung and then feeds them to animals.

Benefits of Using Azolla:

Azolla as a Nitrogen Fertilizer for expanding Crop Yields:

- Azolla plants are described through the Chinese and Vietnamese as being miniature nitrogen fertilizer factories.

- **The Vietnamese use them as nitrogen fertilizer since Azolla endured to supply nitrogen fertilizer for Vietnamese rice paddies.**
- **The nitrogen fertilizer mounted by way of Azolla turns into to be had to the rice after the Azolla mat is included into the soil and its nitrogen starts to be released through decomposition. For Azolla, it takes 25 to 35 days to supply enough nitrogen for a four to six ton/ha rice crop right through the wet season, or a five to eight ton/ha crop underneath irrigation all over the dry season.**

Maintaining Soil Fertility:

- **As a inexperienced manure, Azolla's affect on soil fertility is because of its natural subject and nitrogen.**
- **Incorporation and decomposition of Azolla will shape a humus compound. Humus will increase the water keeping capability of soil and promotes aeration, drainage, and the aggregation crucial for extremely productive soils, Organic matter can bind together soil debris and makes clayey soils extra friable.**
- **Apart from its influence on soil physical homes, Azolla is vital within the biking of vitamins, whilst Azolla is rising in the paddy, it fixes nitrogen and absorbs vitamins out of the water that might in a different way be washed away. When the Azolla is composed with the soil and humus is shaped, and these nutrients are slowly launched into the soil as decomposition progresses.**

Controlling the Growth of Aquatic Weeds:

- **Agricultural economists have calculated that the Asian farmers, particularly women, pay longer time on weeding than on another activity required for rice manufacturing. Although analysis is inadequate, it's frequently believed that Azolla suppresses the expansion of certain aquatic weeds.**
- **Weed enlargement is suppressed as soon as Azolla forms a thick, light-proof mat. There are mainly two mechanisms for this**

suppression, the most effective mechanism is the light-starvation of young weed seedlings by way of the blockage of daylight.

- **The opposite is the bodily resistance to weed seedling is exposed in a heavy, interlocking Azolla mat. In the case of weed-infested rice fields, the benefit from Azolla weed suppression may also surpass its get advantages as a nitrogen supply. Rice seedlings aren't affected by Azolla's weed suppression impact as a result of, when transplanted, they stand above the Azolla mat.**

Azolla Is Used as a Green Manure:

- **Azolla can be utilized as a inexperienced manure by way of growing it as a monocrop after which incorporating it as a basal manure earlier than the rice is transplanted; or transported to any other website for use on upland plants; growing it as an intercrop and incorporating it as a most sensible dressing manure after the rice is transplanted; or by way of rising it both as a monocrop and an intercrop. All those programs may also be a hit, however, and is not unusual in agriculture, use of the green manure crop requires some changes in the control of both the golf green manure and the main crop.**
- **Monocrop Azolla is used in China and Vietnam all the way through the iciness and spring to produce nitrogen for the spring rice crop. The identical methodology is used to provide nitrogen for the early summer season rice crop, but that is much less not unusual for the reason that growth of Azolla pinnata is affected by top temperature and heavy pest attack during mid to late summer.**
- **Azolla is most often grown with the rice in places the place there is no time available within the cropping gadget for the monocropping of Azolla.**
- **Azolla as an intercrop Azolla shall be to start with presented via hand or rotary rice weeder and then later killed by way of heavy shading and/or top temperatures, along side next decomposition**

and free up of nitrogen to the crop, which maximizes the grain production.

Unit:III

Mycorrhiza: A mycorrhiza (from Greek *μύκης* *mýkēs*, "fungus", and *ρίζα* *rhíza*, "root"; pl. mycorrhizae, mycorrhiza or mycorrhizas[1]) is a mutual symbiotic association between a fungus and a plant.[2] The term mycorrhiza refers to the role of the fungus in the plant's rhizosphere, its root system. Mycorrhizae play important roles in plant nutrition, soil biology and soil chemistry.



In a mycorrhizal association, the fungus colonizes the host plant's root tissues, either intracellularly as in arbuscular mycorrhizal fungi (AMF or AM), or extracellularly as in ectomycorrhizal fungi. The association is sometimes mutualistic. In particular species or in particular circumstances mycorrhizae may have a parasitic association with host plants.[3]

Definition

Typs:

Mycorrhizas are commonly divided into ectomycorrhizas and endomycorrhizas. The two types are differentiated by the fact that the hyphae of ectomycorrhizal fungi do not penetrate individual cells within the root, while the hyphae of endomycorrhizal fungi penetrate the cell wall and invaginate the cell membrane.[7][8]

Endomycorrhiza includes arbuscular, ericoid, and orchid mycorrhiza.

Ectomycorrhizae



***Leccinum scabrum*, an ectomycorrhizal fungus**



Ectomycorrhiza

Ectomycorrhizas, or EcM, are symbiotic associations between the roots of around 10% of plant families, mostly woody plants including the birch, dipterocarp, eucalyptus, oak, pine, and rose[9] families, orchids,[10] and fungi belonging to the Basidiomycota, Ascomycota, and Zygomycota. Some EcM fungi, such as many *Leccinum* and *Suillus*, are symbiotic with only one particular genus of plant, while other fungi, such as the *Armillaria*, are generalists that form mycorrhizas with many different plants.[11] An individual tree may have 15 or more different fungal EcM partners at one time.[12] Thousands of ectomycorrhizal fungal species exist, hosted in over 200 genera. A recent study has conservatively estimated global ectomycorrhizal fungal



species richness at approximately 7750 species, although, on the basis of estimates of knowns and unknowns in macromycete diversity, a final estimate of ECM species richness would probably be between 20,000 and 25,000.[13]

Ectomycorrhizas consist of a hyphal sheath, or mantle, covering the root tip and a Hartig net of hyphae surrounding the plant cells within the root cortex. In some cases the hyphae may also penetrate the plant cells, in which case the mycorrhiza is called an ectendomycorrhiza. Outside the root, ectomycorrhizal extramatrical mycelium forms an extensive network within the soil and leaf litter. Wheat is arbuscularmycorrhizal

Arbuscular mycorrhiza

Arbuscular mycorrhizas, or AM (formerly known as vesicular-arbuscular mycorrhizas, or VAM), are mycorrhizas whose hyphae penetrate plant cells, producing structures that are either balloon-like (vesicles) or dichotomously branching invaginations (arbuscules) as a means of nutrient exchange. The fungal hyphae do not in fact penetrate the protoplast (i.e. the interior of the cell), but invaginate the cell membrane. The structure of the arbuscules greatly increases the contact surface area between the hypha and the cell cytoplasm to facilitate the transfer of nutrients between them.



Ericoid mycorrhiza:Ericoid mycorrhizas are the third of the three more ecologically important types. They have a simple intraradical (grow in cells) phase, consisting of dense coils of hyphae in the outermost layer of root cells. There is no periradical phase and the extraradical phase consists of sparse hyphae that don't extend very far into the

Vesicular Arbuscular Mycorrhiza (VAM)

Isolation of VAM Spores from Soil



1. *Wet Sieving and Decanting:*

Principle:

When soil suspension is passed through sieves having smaller mesh size than VAM spores, they will be retained in the sieve and can be collected.

Requirements:

- a. Rhizosphere soil sample.***
- b. Beaker, glass rod, water, slides, etc.***



c. **Set of sieves (710-45 μm mesh size).**

d. **Binocular and microscope.**

e. **Lactophen**

ol.

Procedure:

1. **Place approximately 250 g of rhizosphere soil in a 500 ml Erlenmeyer beaker and add 400 ml of water.**
2. **Stir well in order to disperse the soil particles and allow heavier particles to settle down.**
3. **Decant the suspension through the set of sieves, 710-45 μm placed one below the other with 710 μm one on top and 45 μm and one at bottom.**
4. **Collect the residue of sieves 75 μm and 45 μm after removing them and washing them on a filter paper.**
5. **Examine the filter paper under a binocular and remove spores gently with the help of a flat needle to a drop of water on a slide and observe.**
6. **Blot water, place a drop of lactophenol on the slide, place a coverslip gently and observe under a microscope.**
7. **Count the number of spores and calculate for one gram of soil.**
8. **Permanent slides can be made by mounting spores in Hoyer's medium which preserves all characters of the spores in original form. Staining is not essential since most of the spores are brightly coloured.**

2. Sucrose Centrifugation:

Principle: Spore suspension when placed in 2(M) sucrose solution and centrifuged, they easily float in the supernatant and these are filtered and collected.

Requirements:

1. **Suspension of VAM spores from sieves.**
2. **Centrifuge.**
3. **2(M) sucrose solution.**
4. **Slides and coverslips.**
5. **Distilled water.**

Procedure:



1. ***Place the suspension from 75 and 45 μm sieves in a 50 ml centrifuge tube and make up the volume to 35 ml with distilled water.***
2. ***Centrifuge at 2000 rpm for 10 minutes and filter supernatant.***
3. ***Suspend the pellet in the centrifuge tube in 2(M) sucrose solution and make up the volume to 35 ml.***
4. ***Stir well and centrifuge at 2000 rpm for 10 minutes.***
5. ***Filter the supernatant and collect the spores.***



6. **Mount the spores and examine them.**

3. **Mass Collection VAM Spores:**

Principle: Larger spores like *Gigaspora* also can be collected by taking the fractions from 425 and 250 μm sieves besides 75 and 45 μm .

Requirements:

- a. **Rhizosphere soil sample.**
- b. **Beakers glass rod, etc.**
- c. **Set of sieves 710-45 μm .**
- d. **30% (W/V) aqueous sucrose gradients.**
- e. **Aspirator.**
- f. **Centrifuge.**
- g. **Distilled water.**
- h. **Sucrose (2M).**

Procedure:

1. **Subject the soil sample to wet sieving and decanting with cold water.**
2. **Collect spore fraction from 425 and 250 μm sieve with debris for *Gigaspora margarita* and for other genera from sieves 75 and 45 μm .**
3. **Decant and remove debris or vacuum aspirate the surface of the suspension in a 4 litre beaker which has the debris and spores from 250 μm sieve.**
4. **For the remaining portion from 75 and 45 μm sieve separate spores by using discontinuous 30% (W/V) aqueous sucrose gradients.**
5. **Take a one litre beaker add 600 ml of water over 200 ml sucrose.**
6. **Gently place the sieved material in layers on this large gradient.**
7. **Spores and debris get collected at the interface due to gravity.**
8. **Remove this layer by vacuum aspiration and rinse with cold water.**
9. **Centrifuge at 1600 rpm for 2 to 5 minutes on 2nd gradient (15 ml water and 20 ml sucrose).**
10. **Aspirate spore fraction from gradient interface and store in cold water.**

4. **Modification in Our Laboratory:**



A. Wax Coated Plates:

Principle:*Spores floating on the surface will move and stick to paraffin from where it can easily be collected.*

Requirements:

1. ***Debris from sieves.***
2. ***Petri dishes coated on the sides (inside) with paraffin.*** Procedure:



1. ***Suspend debris and spores in Petri plates coated with paraffin wax.***
2. ***Due to gravity debris gets settled and spores float on the surface and gets stuck to the paraffin coating.***
3. ***Decant water and collect spores in cold water.***

B. *Funnel Assembly:*

Principle: As water is let out, little by little, the air bubble that comes up push the spores through waves to the periphery.

Requirements:

1. ***Washing from sieves.***
2. ***Funnel fitted to a suction pump with closed stopcock. Procedure:***
 1. ***Place water in the funnel fitted to a suction pump and with the stopcock closed.***
 2. ***Suspend washings with water from sieve in funnel. Most of the spores move to the periphery and stick to the sides of the funnel.***
 3. ***Open the cock gently and allow a bit of water to go.***
 4. ***Spores present in the central region also now move to the periphery.***
 5. ***Repeat this process to reduce the quantity of water in the funnel and ultimately drain off the whole water by opening the cock.***
 6. ***Rings of spores get stuck to the sides of the funnel. Collect them in cold water. Assessment of Mycorrhizal infection in roots.***

Principle:

Using KOH the tissues of roots are macerated and fungal element which remains intact is stained and observed.

Requirements:

- a. ***Feeder roots of plants with VAM infection.***
- b. ***10% KOH.***
- c. ***1(N) HCl.***
- d. ***0.5% trypan blue/cotton blue in lactophenol.***
- e. ***H₂O₂***
- f. ***Slides, cover slips.***



g. ***Needles and forceps.***

h. ***Distilled water.***

i. ***Petri
dish.***

Procedure:

1. ***Wash infected roots thoroughly in water.***
2. ***Add 10% KOH and place them covered in a Petri dish in an oven at 90°C for 30 minutes to 1***



hour.

3. **Wash the root segments gently two to three times with distilled water.**
4. **Add H_2O_2 and leave aside for one to two hours.**
5. **Acidify by immersing in dilute in HCl for a few minutes.**
6. **Stain the roots with cotton blue in lactophenol and remove excess of stain with lactophenol. Mount on slides in lactophenol or acetic acid: glycerol (1:1v/v)**
7. **Place a cover slip seal and**

observe. For Pigmented Roots:

Principle:

Pigmented roots are decolourised by treating it with stronger solution of KOH and heating it for a longer time.

Requirements:

1. **10-20% KOH.**
2. **VAM infected feeder roots.**
3. **1(N) (HCl).**
4. **0.5% cotton blue in lactophenol.**
5. **H_2O_2 .**

6. **Slides and coverslips.**
7. **Needles, forceps.**
8. **Distilled water.**
9. **Petri plates.**

Procedure:

Same as above but depending on the colour of roots 10 or 20% KOH is used and this is kept for a longer period of at least one hour.

Measurement of Root Infection

Requirements:

1. **Stained segments of infected root.**
2. **Calibrated microscope.**
3. **Slides and cover slips.**
4. **Needle**



S.

Procedure:

1. ***Select at random, stained segments of roots and mount them on microscopic slides in groups of ten.***
2. ***Examine under the microscope and assess length of cortical colonization of VAM in μm for each segment.***



Identification of VAM Spores

i. ELISA

Method

(EISA)

Requirements:

ts:

- a. ***Freund's complete adjuvant.***
- b. ***PO₄ buffer.***
- c. ***Carbonate coating buffer.***
- d. ***0.05% Tween 20 in phosphate buffered saline (PBST).***
- e. ***Sterile saline.***
- f. ***Ig G-Horse radish peroxidase-labelled immunoconjugate.***
- g. ***Orthophenylenediamine.***
- h. ***H₂SO₄.4N***
- i. ***Cultures of VAM in sterile sand grown host plant maintained in glass house with 16 hour light per day.***
- j. ***Incubator.***
- k. ***Polyvinyl microlitre trays.***
- l. ***Rabbits.***
- m. ***Needle and syringe.***
- n. ***Centrifuge.***

Procedure:

1. ***Place roots of infected plant on a 250 µm sieve and wash with a strong jet of water (to remove external hyphae) into the 250 µm sieve.***
2. ***Place sieving in a Petri plate and remove soil particles by observing under a stereoscan binocular.***
3. ***Take hyphal mass, lyophilize, grind and store in desiccator at room temperature.***

This will be approximately 20 mg (dry weight) of hyphae from a single



heavily infected host.

- 4. Antigen when required for immunisation is prepared by resuspending 5 mg of powder in 1ml sterile saline and emulsifying it with equal volume of Freund's complete adjuvant.**
- 5. Inject antigen into white rabbits.**
- 6. A booster dose of 2 mg is given after 3 weeks in sterile saline intravenously.**
- 7. Take 5 ml of blood after seven days by cardiac puncture, allow the blood to clot and draw off the serum.**
- 8. 5 ml of pre-immune serum serves as control.**
- 9. For ELISA suspend 10 mg of powdered antigen in sterile distilled water, centrifuge at 15000 rpm for 20 minutes, discard surface lipids and lyophilize supernatant, suspend the powder in carbonate coating buffer (pH 9.6) at a concentration of 5 $\mu\text{g}\cdot\text{ml}^{-1}$.**
- 10. Disperse 50 μl aliquots of the antigen suspension into polyvinyl microlitre trays. Allow it to**



dry at 37°C for 12 hours.

- 11. Wash tray three times with phosphate buffered saline containing 0.05% Tween 20 (PBST).**
- 12. Make serial dilutions of serum in phosphate buffered saline containing 0.05% Tween 20 (PBST) (50µl) and add in quadruplicates to the wells.**
- 13. Seal the plates with tape, incubate for one hour at 37°C.**
- 14. Repeat washing with PBST.**
- 15. Add 50 µl of goat anti-rabbits Ig G-horse raddish peroxidase labelled immuno conjugate diluted to 1:1000 in PBST in each well and incubate at 37°C for 30 minutes.**
- 16. Give a final wash to the trays and add 50 µl of orthophenylene diamine substrate.**
- 17. Using NH_2SO_4 stop the reaction after 15 minutes.**
- 18. Read wells on a spectrophotometer at 490 nm using normal rabbit serum as control.**
- 19. From the absorbance of the test serum, the absorbance of control serum is to be deducted. This gives specific reading.**
- 20. Select a positive serum as reference to give an absorbance of 1.0. Compare other antisera to this reference.**

~~Production of VAM~~

Principle:

Since VAM cannot be grown in vitro, they are multiplied on the root system of host plants.

Requirements:

- 1. Seeds of host plant-Jowar.**
- 2. Plastic pots 7 X 10 X 10 cm.**
- 3. Sand (sterile).**
- 4. Inoculum obtained by wet sieving and decanting.**
- 5. Inorganic nutrient solution—Hoglands.**
- 6. Starter inoculum.**
- 7. Growth chamber.**



Procedure:

1. ***Wash Jowar seeds under running tap water to remove pesticides.***
2. ***Soak them over night and germinate.***
3. ***Add a 3 cm layer of sand (sterile) to the clean plastic pots.***
4. ***Then place 1 cm depth of substrate with VAM inoculum (starter).***
5. ***Place maximum 5-6 seeds on top of the inoculum per pot.***
6. ***Cover them with sand.***

7. ***Irrigate pots with Hogland's solution (composition given under N₂ fixation) at the rate of 20 ml***



per pot.

8. Keep the pots at 28°-30°C (room temperature) with a photo period of 14 hours light and 10 hours dark with a light intensity of 100 lux supplied with fluorescent tubes for 3 weeks. Now prepare second phase with expanded clay.

~~Mass Production of VAM:~~

Principle:

The pure inoculum produced must be transferred to expanded clay along with roots to be supplied as VAM inoculum.

Requirements:

- 1. Plants grown for pure inoculum.**
- 2. Ethanol 70%.**
- 3. Na hypochlorite-2%.**
- 4. Sterile expanded clay.**
- 5. 5 litre pots (clay ones).**
- 6. Growth room with photoperiod of 14 hours light and 10 hours dark.**
- 7. P free medium (liquid) / Hogland's**

solution. Procedure:

There are several steps involved in the production of VAM on a large scale.

a. Separation of host plants:

Plants grown with pure inoculum in pots in the above experiment should be uprooted carefully.

b. Sterilisation of host plants:

Wash thoroughly the uprooted host plants and surface sterilise the root system of these uprooted plants to prevent the transfer of pathogenic organisms to the expanded clay. For this, dip the root system in 70%



ethanol for 2 minutes or 2% Na hypochlorite solution. (This is optional for plants grown under controlled conditions in growth chamber or the ones that are upto three weeks old).

c. Preparation of expanded clay:

Either pasteurize or expose the clay to steam for 4 hours and cool to 20°C.

Place this in 5 litre or larger pots and plant two surface sterilised plants (Jowar) having maximum VAM colonisation in each pot carefully without damaging the root system and water the plants.



d. ***Parameters:***

Five parameters are to be followed:

- i. ***Optional illumination of host plant.***
- ii. ***Optional temperature range for fungi in the root.***
- iii. ***No water logging or drought.***
- iv. ***Plant nutrition should be optional for symbiosis.***
- v. ***Plants should be treated for***

pathogen. Illumination:

In temperate countries mass production should be done in green houses with an intensity of 5000 Lux for 16 hours per day with sodium vapour high pressure lamps; 10,000 Lux or more by mercury vapour lamps for 14 hours. In tropical countries 1000 Lux supplied by fluorescent mercury vapour tubes for 14 hours per day is sufficient.

Temperature:

Pots should be shaded by placing them in wide well-aerated trays.

Irrigation:

Since expanded clay has larger particles on top and smaller ones below, more moisture will be present in the lower layers. To avoid this, use clay pots instead of plastic ones and cover them with thin plastic sheets with holes to allow exchange of gases.

Plant nutrition:

Modified Hogland and Arnon (1938) Treatment against pathogens Water, plants once a month. Treat with fungicide (Previcur 0.15%) to prevent pathogens like Pythium.

e. ***Growth period:***



It takes about 3—4 months for mass production of inoculum in expanded clay. Stop irrigation, after checking if growth and colonisation is found good. A drought stress caused by stopping irrigation will induce formation of VAM spores.

f. Hence expose plants to drought for a week and then cut the plants.

g. After three weeks spread the substrate in a layer and air dry. Test for contamination.

h. Store the inoculum under cool dry conditions. They retain infectivity for several years. Vermicompost:

Vermicompost (vermi-compost) is the product of the decomposition process using various species of worms, usually red wigglers, white worms, and other earthworms, to create a mixture of decomposing vegetable or food waste, bedding materials, and vermicast. This process is called vermicomposting, while the rearing of worms for this purpose is called vermiculture.





Vermicomposting uses worms to decompose waste and make nutrient-rich "worm manure".

Vermicast (also called worm castings, worm humus, worm manure, or worm faeces) is the end-product of the breakdown of organic matter by earthworms.[1] These castings have been shown to contain reduced levels of contaminants and a higher saturation of nutrients than the organic materials before vermicomposting.[2]

Vermicompost contains water-soluble nutrients and is an excellent, nutrient-rich organic fertilizer and soil conditioner.[3] It is used in farming and small scale sustainable, organic farming.

Vermicomposting can also be applied for treatment of sewage. A variation of the process is vermifiltration (or vermidigestion) which is used to remove organic matter, pathogens and oxygen demand from wastewater or directly from blackwater of flush toilets.[4][5]

Harvesting[Edit](#)



Worms in a bin being harvested

Vermicompost is ready for harvest when it contains few-to-no scraps of uneaten food or bedding.[25] There are several methods of harvesting from small-scale systems: "dump and hand sort", "let the worms do the sorting", "alternate containers" and "divide and dump." [30] These differ on the amount of time and labor involved and whether the vermicomposter wants to save as many worms as possible from being trapped in the harvested compost.

The pyramid method of harvesting worm compost is commonly used in small-scale vermiculture, and is considered the simplest method for

single layer bins.[31] In this process, compost is separated into large clumps, which is placed back into composting for further breakdown, and lighter compost, with which the rest of the process continues. This lighter mix is placed into small piles on a tarp under the sunlight. The worms instinctively burrow to the bottom of the pile. After a few minutes, the top of the pyramid is removed repeatedly, until the worms are again visible. This repeats until the mound is composed mostly of worms.

When harvesting the compost, it is possible to separate eggs and cocoons and return them to the bin, thereby ensuring new worms are hatched. Cocoons are small, lemon-shaped yellowish objects that can usually be seen with the naked eye.[32] The cocoons can hold up to 20 worms (though 2-3 is most common). Cocoons can lay dormant for as long as two years if conditions



are not conducive for hatching.[33] Soil

- **Improves soil aeration**
- **Enriches soil with micro-organisms (adding enzymes such as phosphatase and cellulase)**
- **Microbial activity in worm castings is 10 to 20 times higher than in the soil and organic matter that the worm ingests[41]**
- **Attracts deep-burrowing earthworms already present in the soil**
- **Improves water holding capacity[42]**

Plant growth

- **Enhances germination, plant growth, and crop yield**
- **Improves root growth and structure**
- **Enriches soil with micro-organisms (adding plant hormones such as auxins and gibberellic acid)citationneeded**

Economic

- **Biowastes conversion reduces waste flow to landfills**
- **Elimination of biowastes from the waste stream reduces contamination of other recyclables collected in a single bin (a common problem in communities practicing single-stream recycling)**
- **Creates low-skill jobs at local level**
- **Low capital investment and relatively simple technologies make vermicomposting practical for less-developed agricultural regions**

Environmental

- **Helps to close the "metabolic gap" through recycling waste on-site**
- **Large systems often use temperature control and mechanized harvesting, however other equipment is relatively simple and does not wear out quicklycitationneeded**
- **Production reduces greenhouse gas emissions such as methane and nitric oxide (produced in landfills or incinerators when not composted).**



Semester	Course	Hours	Credit	Sub.Code	Marks		
					Internal	External	Total
I	MBE1	6	4	18KP1BELBI	25	75	100

BIOFERTILIZER AND MUSHROOM TECHNOLOGY

UNIT – IV & UNIT - V

Unit - IV

Introduction - History and Scope of edible and medicinal mushroom – Types of mushroom and Economic importance. Cultivation method – Isolation, Spawn preparation, Growth media, Spawn running and harvesting and Marketing of mushroom

Unit – V

Cultivation technology – Agaricus sp., Pleurotus sp., Volvariella sp., Storage – Short term storage and Long term storage. Nutritional value and Food preparation – Types of food prepared from mushroom (Cutlet, Omelet, Pickles, Curry, Soup & Biryani)

UNIT - IV & UNIT - V

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Prepared by

Dr. V. Latha,

Assistant professor,

KN. GOVT ARTS COLLEGE FOR WOMEN,

THANJAVUR -7



MUSHROOM TECHNOLOGY

INTRODUCTION

A mushroom or toadstool is the fleshy, spore-bearing fruiting body of a fungus, typically produced above ground, on soil, or on its food source. The standard for the name "mushroom" is the cultivated white button mushroom, *Agaricus bisporus*; hence the word "mushroom" is most often applied to those fungi (Basidiomycota, Agaricomycetes) that have a stem (stipe), a cap (pileus), and gills (lamellae, sing. lamella) on the underside of the cap. "Mushroom" also describes a variety of other gilled fungi, with or without stems, therefore the term is used to describe the fleshy fruiting bodies of some Ascomycota. These gills produce microscopic spores that help the fungus spread across the ground or its occupant surface. Forms deviating from the standard morphology usually have more specific names, such as bolete, puffball, stinkhorn, and morel, and gilled mushrooms themselves are often called agarics in reference to their similarity to *Agaricus* or their order Agaricales. By extension, the term mushroom can also refer to either the entire fungus when in culture, the thallus (called a mycelium) of species forming the fruiting bodies called mushrooms, or the species itself.

HISTORY

Mycophagy the act of consuming mushrooms, dates back to ancient times. Edible mushroom species have been found in association with 13,000-year-old archaeological sites in Chile. Ötzi, the mummy of a man who lived between 3400 and 3100 BCE in Europe, was found with two types of mushroom. The Chinese value mushrooms for supposed medicinal properties as well as for food. Ancient Romans and Greeks, particularly the upper classes, used mushrooms for culinary purposes. Food tasters were employed by Roman emperors to ensure that mushrooms were safe to eat

Edible mushrooms are the fleshy and edible fruit bodies of several species of macrofungi (fungi which bear fruiting structures that are large enough to be seen with the naked eye). They can appear either below ground (hypogeous) or above ground (epigeous) where they may be picked by hand. Edibility may be defined by criteria that include absence of poisonous effects on humans and desirable taste and aroma. Edible mushrooms are consumed for their nutritional and culinary value. Mushrooms, especially dried shiitake, are sources of umami flavor from guanylate. Mushrooms consumed by those practicing folk medicine are known as medicinal mushrooms. While psychedelic mushrooms are occasionally consumed for recreational or entheogenic purposes, they can produce psychological effects, and are therefore not commonly used as food. There is no evidence from high-quality clinical research that "medicinal" mushrooms have any effect on human diseases.

Edible mushrooms include many fungal species that are either harvested wild or cultivated. Easily cultivated and common wild mushrooms are often available in markets, and those that are more difficult to obtain (such as the



prized truffle, matsutake and morel) may be collected on a smaller scale by private gatherers. Some preparations may render certain [poisonous mushrooms](#) fit for consumption.

SCOPE

Mushroom production has tremendous scope in Tamil Nadu. Mushroom has excellent medicinal properties. It is rich in protein, fibre, and amino acids. Mushroom is a 100 per cent vegetarian food and is good for diabetes and joint pains

HISTORY OF MEDICINAL MUSHROOM

The ancestors have used mushrooms as medicine for thousands of years. The Greek physician Hippocrates, circa 450 bce, classified the amadou mushroom (Fomes fomentarius) as a potent anti-inflammatory and for cauterizing wounds.

TYPES OF MUSHROOMS

1. Button Mushrooms. Button mushrooms are also called baby mushrooms or white mushrooms.
2. Cremini Mushrooms.
3. Portobello Mushrooms.
4. Oyster Mushrooms.
5. King Oyster Mushrooms.
6. Chanterelle Mushrooms.
7. Porcini Mushrooms.
8. Hedgehog Mushrooms.

ECONOMIC IMPORTANCE

Nowadays, mushrooms are popular valuable foods because they are low in calories, carbohydrates, fat, and sodium: also, they are cholesterol-free. Besides, mushrooms provide important nutrients, including selenium, potassium, riboflavin, niacin, vitamin D, proteins, and fiber. General populace is less aware about the economic value of mushrooms. Mushroom is a saprophytic organism and hence it utilizes organic and agricultural waste. This reduces the burden of farmers to dispose his farm wastes. Additional income is obtained through quality mushrooms production by utilizing these residues. Mushroom cultivation both seasonal and commercial nature is giving handsome income to the growers. The employment generation through cultivation and associated allied activities is so immense. The value addition to mushrooms in terms of quality products is another economic avenue. The positive use of spent mushroom substrate viz., biofuel, biogas production, manures, potting medium, etc also generates additional revenue to the farmer.



CULTAVATION METHOD

Cultivation is the process of tilling or loosening soil to prepare it for planting. It is often an essential method for maintaining soil health, preventing weed development, and encouraging crop growth. Medicinal plants can be cultivated by two methods (applicable to non-medicinal plants). Sexual and Asexual method

Sexual Method (seed propagation).

In this method, the plants are raised from seeds. Such plants are known as seedlings. Seeds are sown in the fields by methods like broadcast, dibbling, or placing them in drills or holes. The seeds must be of good quality, capable of high germination rate, and free from diseases.

ADVANTAGES:

Seedlings are comparatively much cheaper and easy to raise. Seedlings are long-lived, bear more heavy fruits and plants obtained are more sturdy. In those plants where other methods of cultivation cannot be utilized, seed propagation becomes the only method of choice. There are chances of production of some chance-seedlings of very high superiority which may be of great importance for e.g., orange, papaya, etc.

DISADVANTAGES:

The seedlings obtained from this method require more time to bear and are not uniform in their growth and yielding capacity as compared to other methods like grafting. Also, the cost involved in harvesting and protection from pests is more.

Asexual Methods (vegetative propagation):

In this method, any of the vegetative part of the plant like root or stem is provided such an environment that it develops into a new plant. The environment is provided by setting various parts of the plant in well prepared soil.

Bulbs: A bulb is originally and structurally a bud, which possesses the capability of perennation. It consists of a very short stem ending in an apical meristem and enclosed by closely set leaves, which are thick and fleshy, being stored with reserves of food. Each of the leaves has of course its axillary bud.

Corms: In a corm, the storage organ is swollen base of the stem and this is wrapped in thin scale-leaves, each of which, of course, has an axillary bud e.g. colchicum, saffron.

Tubers: It is a swelling on an underground stem branch. The stem grows axillary buds formed low down on the aerial stem and push through the soil, swelling at their ends to form the tubers. The of the tuber are very small scale-leaves, each with three axillary buds e.g., jalap, aconite, potato.



Rhizomes: In underground stems, the older parts of rhizome die off. The buds borne on the detached younger portions thus become separate new plants e.g., ginger, turmeric.

Runners: The stem grows along the ground (horizontally over the surface of the soil), and produces roots and erect flowering shoots from lateral buds at many of its nodes. The growth of the creeping stem is continued by the terminal bud. Some of the older internodes die, and the detached rooted and shoot bearing parts become independent plants e.g., peppermint, strawberry.

Suckers: A shoot arising from a root of a woody plant e.g., mint, pineapple, banana.

Offsets: These originate from the axil of the leaf as short thick horizontal branches and also characterized by the presence of rosette type of leaves and a cluster of roots at their bottom e.g., aloe, valerian.

Stolons: A creeping stem that roots at nodes e.g., arrow-root, liquorice. Other methods,

Cutting: A clear cut is made preferably below the node and the lower leaves are removed. It is then placed in a suitable medium and provided with suitable conditions of moist atmosphere, temperature which favouring the development of roots e.g., mint, vanilla.

Layering: A layer is a branch or a shoot which is induced to develop roots before it is completely severed from the parent plant. It is done by a cut or ligature and embedding the part so treated in the soil e.g., cascara.

Grafting and Budding: Grafting is a process in which two cut surfaces of different but closely related plants are placed so as to unite and grow together. The rooted portion is called the stock and the cut off is the scion or graft e.g., female scion of *Myristica fragrans* on male stock to increase fruit bearing proportion. Budding consists of the introduction of a piece of bark bearing a bud into a suitable cavity or shaped slit made in the bark of the stock e.g., citrus species, sweet and sour oranges. **Aseptic methods of propagation:** In this method, the plants are developed in an artificial medium under aseptic conditions from very fine pieces of plants like single cells, callus, seeds, embryos, root tips, shoot tips, pollen grains, etc. They are provided with nutritional and hormonal requirements.

Advantages: (Asexual method): There is no variation between the plant grown and plant from which it is grown. As such, the plants are uniform in growth and yielding capacity. Seedless varieties of fruits can only be propagated vegetatively e.g., pomegranates, grapes, lemon. Plants start bearing earlier as compared to seedlings. Budding or grafting encourages disease-resistant varieties of plants.

Disadvantages: In comparison to seedling trees, these are not vigorous in growth and are not long lived. No new varieties can be evolved by this method.

ISOLATION

In microbiology, the term isolation refers to the separation of a strain from a



natural, mixed population of living microbes, as present in the environment, for example in water or soil flora, or from living beings with skin flora, oral flora or gut flora, in order to identify the microbes of interest.

SPAWN PREPARATION

In the spawn-production process, mycelium from a mushroom culture is placed onto steam-sterilized grain, and in time the mycelium completely grows through the grain. This grain/mycelium mixture is called spawn, and spawn is used to seed mushroom compost.

250 ml Flasks, 50 ml beaker level full of rye grain, 1/2 tsp. Calcium Carbonate, powder (lime), 1/4 tsp. Calcium Sulfate (gypsum), 60 ml warm water Autoclave 35 minutes at 121°C - Fast Exhaust

GROWTH MEDIA

A growth medium or culture medium is a solid, liquid or semi-solid designed to support the growth of a population of microorganisms or cells via the process of cell proliferation, or small plants like the moss *Physcomitrella patens*. Different types of media are used for growing different types of cells. The two major types of growth media are those used for cell culture, which use specific cell types derived from plants or animals, and microbiological culture, which are used for growing microorganisms, such as bacteria or fungi. The most common growth media for microorganisms are nutrient broths and agar plates; specialized media are sometimes required for microorganism and cell culture growth. Some organisms, termed fastidious organisms, require specialized environments due to complex nutritional requirements. Viruses, for example, are obligate intracellular parasites and require a growth medium containing living cells.

SPAWN RUNNING

After spawning, comes spawn-run or the stage where the spawn or the mycelium is allowed to grow through the substrate. At this stage, the mushroom mycelium starts to produce enzymes that will further break down the macromolecules of the substrates and absorb simple molecules into the mycelium for further growth and development. Thus, as the mycelium develops, the substrate starts to break down and becomes available to the growing mycelium. This period is also called the vegetative growth or incubation period. The length of the time for spawn running depends upon the mushroom being cultivated: 10-15 days for *Volvariella volvacea*, 2-3 months for *Lentinula edodes* and *Auricularia* and 2-4 weeks for *Pleurotus* and *Agaricus*.

As soon as spawning has been completed, the farmer has been advised to maintain the growing conditions at the optimum level and as uniform as possible inside the spawning room. For white button mushroom which is most common in India. The room should be maintained at about 25°C temperature. The humidity should be built up by frequently watering the floor and walls. The room may be kept closed as only a small amount of fresh air is needed for spawn growing and mushroom mycelium is quite tolerant to carbon-dioxide. The temperature of the bed is more important than the temperature of the



atmosphere. The bed temperature must not be allowed to exceed 24°C. The temperature rises particularly when compost beds are thick, and this leads to the development of fruit bodies later only at the edges of the bed. This problem can be dealt with in two ways: by providing thinner compost beds, and by lowering the room temperature.

HARVESTING

Harvesting is the process of gathering a ripe crop from the fields. Reaping is the cutting of grain or pulse for harvest, typically using a scythe, sickle, or reaper. On smaller farms with minimal mechanization, harvesting is the most labor-intensive activity of the growing season. On large mechanized farms, harvesting utilizes the most expensive and sophisticated farm machinery, such as the combine harvester. Process automation has increased the efficiency of both the seeding and harvesting process. Specialized harvesting equipment utilizing conveyor belts to mimic gentle gripping and mass transport replaces the manual task of removing each seedling by hand. The term harvesting in general usage may include immediate postharvest handling, including cleaning, sorting, packing, and cooling. The completion of harvesting marks the end of the growing season, or the growing cycle for a particular crop, and the social importance of this event makes it the focus of seasonal celebrations such as harvest festivals, found in many religions.

MARKETING OF MUSHROOM

The mushroom market, in terms of value, is projected to reach \$50,034.12 million by 2019, at a CAGR of 9.5% from 2014. The growth of this market is primarily triggered by factors such as a rise in the consumption of processed food and growing awareness about health and wellness.



UNIT - V

CULTIVATION TECHNOLOGY

Mushroom cultivation is a process utilizing waste materials such as horse manure, chicken manure, pig manure, wheat straw, rice straw, corn cobs, wood bark, sawdust, and cottonseed hulls to produce a delicious and nutritious food. Therefore, it can be considered as a twofold beneficial operation

Agaricus sp.,

Agaricus is a genus of mushrooms containing both edible and poisonous species, with possibly over 300 members worldwide. The genus includes the common (button) mushroom (*Agaricus bisporus*) and the field mushroom (*A. campestris*), the dominant cultivated mushrooms of the West. Members of *Agaricus* are characterized by having a fleshy cap or pileus, from the underside of which grow a number of radiating plates or gills on which are produced the naked spores. They are distinguished from other members of their family, Agaricaceae, by their chocolate-brown spores. Members of *Agaricus* also have a stem or stipe, which elevates it above the object on which the mushroom grows, or substrate, and a partial veil, which protects the developing gills and later forms a ring or annulus on the stalk.

Pleurotus sp.,

Pleurotus is a genus of gilled mushrooms which includes one of the most widely eaten mushrooms, *P. ostreatus*. Species of *Pleurotus* may be called oyster, abalone, or tree mushrooms, and are some of the most commonly cultivated edible mushrooms in the world. *Pleurotus* fungi have been used in mycoremediation of pollutants such as petroleum and polycyclic aromatic hydrocarbons. Oyster mushrooms contain lovastatin, a form of cholesterol lowering statin.

Vovariella sp.,

Vovariella volvacea (also known as paddy straw mushroom or straw mushroom) is a species of edible mushroom cultivated throughout East and Southeast Asia and used extensively in Asian cuisines. They are often available fresh in Asia, but are more frequently found in canned or dried forms outside their nations of cultivation. Worldwide, straw mushrooms rank third in consumption, although their use in the West is somewhat uncommon and usually confined to use in Oriental cooking.

Storage

Storing: Store mushrooms in their original packaging or in a porous paper bag for prolonged shelf-life. Some mushrooms may keep for up to one week in the refrigerator. Fresh mushrooms should never be frozen, but frozen sautéed mushrooms will keep for up to one month.

Short term storage

Short term storage is generally defined as storage for three months or less, but it's really just storage where you know you want to be able to access your belongings sooner rather than later



Long term storage

With long term, you are looking at prolonged periods of time of storage, which goes well with seasonal storing of clothes, furniture, or storing during the winter. When comparing short-term vs. long-term storage, it is good to know just how much of a difference

Natural value

In mathematics, the natural numbers are those used for counting (as in there are six coins on the table) and ordering (as in this is the *third* largest city in the country). In common mathematical terminology, words colloquially used for counting are cardinal numbers, and words used for ordering are ordinal numbers. The natural numbers can, at times, appear as a convenient set of codes (labels or names) that is, as what linguists call nominal numbers, forgoing many or all of the properties of being a number in a mathematical sense. The set of natural numbers is often denoted by the symbol $\mathbb{N}$. Some definitions, including the standard begin the natural numbers with, corresponding to the non-negative integers 0, 1, 2, 3, (often collectively denoted by the symbol $\mathbb{N}_0$ for emphasizing that zero is included), whereas others start with 1, corresponding to the positive integers (sometimes collectively denoted by the symbol $\mathbb{N}^+$ for emphasizing that zero is excluded).

Food preparation

Mushrooms can easily be regarded as nature's hidden treasure. It was once considered an exotic ingredient but is now rated as a new superfood. What's so super about them? Mushrooms are one of the few natural sources of Vitamin D. They contain no fat and are a valuable source of fiber. They are also packed with selenium which you don't find in most fruits and vegetables. According to a latest study, lentinan found in shiitake mushrooms may increase the survival rate in cancer patients. Slowly but surely mushrooms are gaining prominence on an Indian platter. All the goodness along with its unique earthy texture is reason enough for you to let them adorn your dinner table more often. The versatility of mushrooms can work its magic in almost any dish. Not only are there a number of varieties to cook with, but the culinary possibilities are almost inexhaustible. You can choose to grill, bake, stir-fry, sauté, stuff, roast or experiment with something new. With more than 90 percent water content, the best way to cook mushrooms is to allow them to release all their moisture. Pick a pan with a wide surface area because you should be able to spread them out which will make the moisture evaporate quickly.

Types of food prepared from mushroom

- Wild Mushroom Ragout. A ragout is a stew like preparation. ...
- Mushroom Risotto. ...
- Mushroom Khichda. ...
- Mushroom Samosa. ...



- Mushroom Chettinad. ...
- Whole Wheat Pasta in Mushroom Sauce. ...
- Herb Coated Mushrooms with Cheese Sauce. ...
- Stuffed Masala Mushrooms.

Cutlet

1. thin slice of meat from the leg or ribs of veal, pork, chicken, or mutton
2. dish made of such slice, often breaded (also known in various languages as a *cotoletta*, *Kotelett*, *kotlet* or *kotleta*)
3. croquette or cutlet-shaped patty made of ground meat
4. a kind of fish cut where the fish is sliced perpendicular to the spine, rather than parallel (as with fillets); often synonymous with steak
5. prawn or shrimp with its head and outer shell removed, leaving only the flesh and tail
6. mash of vegetables (usually potatoes) fried with bread

Omelet

In cuisine, an omelette or omelet is a dish made from beaten eggs, fried with butter or oil in a frying pan (without stirring as in scrambled egg). It is quite common for the omelette to be folded around fillings such as cheese, chives, vegetables, mushrooms, meat (often ham or bacon), or some combination of the above. Whole eggs or egg whites are often beaten with a small amount of milk, cream, or water.

Pickles

Pickling is the process of preserving or extending the shelf life of food by either anaerobic fermentation in brine or immersion in vinegar. The pickling procedure typically affects the food's texture, taste and flavor. The resulting food is called a *pickle*, or, to prevent ambiguity, prefaced with *pickled*. Foods that are pickled include vegetables, fruits, meats, fish, dairy and eggs. A distinguishing characteristic is a pH of 4.6 or lower, which is sufficient to kill most bacteria. Pickling can preserve perishable foods for months. Antimicrobial herbs and spices, such as mustard seed, garlic, cinnamon or cloves, are often added. If the food contains sufficient moisture, a pickling brine may be produced simply by adding dry salt. For example, sauerkraut and Korean kimchi are produced by salting the vegetables to draw out excess water. Natural fermentation at room temperature, by lactic acid bacteria, produces the required acidity. Other pickles are made by placing vegetables in vinegar. Like the canning process, pickling (which includes fermentation) does not require that the food be completely sterile before it is sealed. The acidity or salinity of the solution, the temperature of fermentation, and the exclusion of oxygen determine which microorganisms dominate, and determine the flavor of the end product



Curry

Mushroom gravy is a simple, usually red sauce that can be composed from stock (beef is typical, but chicken may be used), roux (a mixture of equal parts butter and flour to thicken), and mushroom base.

Soup

Soup is a primarily liquid food, generally served warm or hot (but may be cool or cold), that is made by combining ingredients of meat or vegetables with stock, or water. Hot soups are additionally characterized by boiling solid ingredients in liquids in a pot until the flavors are extracted, forming a broth. Soups are similar to stews, and in some cases there may not be a clear distinction between the two; however, soups generally have more liquid (broth) than stews. In traditional French cuisine, soups are classified into two main groups: *clear soups* and *thick soups*. The established French classifications of clear soups are *bouillon* and *consommé*. Thick soups are classified depending upon the type of thickening agent used: *purées* are vegetable soups thickened with starch; *bisques* are made from puréed shellfish or vegetables thickened with cream; cream soups may be thickened with béchamel sauce; and *veloutés* are thickened with eggs, butter, and cream. Other ingredients commonly used to thicken soups and broths include rice, lentils, flour, and grains; many popular soups also include pumpkin, carrots, potatoes, pig's trotters and bird's nests.

Biryani

I have removed the paragraph in the etymology section talking about vegetarian biryani and pulao as it does not belong there. There is already an extensive section talking about the difference between biryani and pulao, which led to inconsistency in this article with that extra paragraph in the etymology section. I also had it's only source as an opinion piece done only just a few months ago with no actual factual evidence for the claim. I'm perfectly fine with people saying veg biryani is not biryani, but if you're going to put that in the article you need a factual source rather than a source that is just an opinion. It also needs to be in the proper section. The etymology section is there to explain the origin of the word, not to advance personal opinions.





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