Semester – IV

16MBU404A **BIOFERTILIZERS AND BIOPESTICIDES** **(3H – 3C)**

Instruction Hours / week: L: 3 T: 0 P: 0 **Marks:** Internal: **40** External: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

This course has been designed to provide the student knowledge about eco friendly product which play a crucial role in determining its future use and applications in environmental management. Provides detailed idea about biofertilizer production and plant disease.

OBJECTIVES

To study about the biofertilizers in increasing soil fertility and usage of Biopesticides for plant disease.

Unit I

General account of the microbes used as biofertilizers for various crop plants and their advantages over chemical fertilizers. Symbiotic N2 fixers: *Rhizobium* – Isolation, characteristics, types, inoculum production and field application, legume/pulses plants. *Frankia* – Isolation and characteristics, Alder, Casurina plants, non-leguminous crop symbiosis. Cyanobacteria, *Azolla* – Isolation, characterization, mass multiplication, their role in rice cultivation, crop response and field application.

Unit II

Free living *Azospirillum*, *Azotobacter* – isolation, characteristics, mass production and field application.

Unit III

Phosphate solubilizing microbes – Isolation, characterization, mass production, field application.

Unit IV

Importance of mycorrizal inoculum, types of mycorrhizae and associated plants, Mass production of VAM, field applications of Ectomycorrhizae and VAM.

Unit V

General account of microbes used as bio-insecticides and their advantages over synthetic pesticides, *Bacillus thuringiensis*, production, Field applications, Viruses – cultivation and field applications.

SUGGESTED READINGS

1. Kannaiyan, S. (2003). Bioetchnology of Biofertilizers, CHIPS, Texas.
2. Mahendra K. Rai (2005). Hand book of Microbial biofertilizers, The Haworth Press, Inc. New York.
3. Reddy, S.M. *et. al.* (2002). Bioinoculants for sustainable agriculture and forestry, Scientific Publishers.
4. Subba Rao N.S (1995) Soil microorganisms and plant growth Oxford and IBH publishing co. Pvt. Ltd. New Delhi.
5. Saleem F and Shakoori AR (2012) Development of Bioinsecticide, Lap Lambert Academic Publishing GmbH KG.
6. Aggarwal SK (2005) Advanced Environmental Biotechnology, APH publication.

KARPAGAM ACADEMY OF HIGHER EDUCATION
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COIMBATORE - 641 021

LECTURE PLAN
DEPARTMENT OF MICROBIOLOGY

STAFF NAME: Dr. P. AKILANDESWARI
SUBJECT NAME: BIOPESTICIDES AND BIOPESTICIDES SUB. CODE: 16MBU404A
SEMESTER: IV CLASS: II B. Sc (MB)

S. No	Lecture Duration Period	Topics to be covered	Support material/Page Nos
UNIT - I			
1	1	General account of the microbes used as biofertilizers in various plant crops	R2: 166-172
2	1	Advantages of biofertilizers	R2: 172
3	1	Symbiotic nitrogen fixers - Rhizobium isolation, characteristics, mass production and field application	R2: 166-173
4	1	Frankia isolation and characteristics	W1
5	1	Alder and Casurina – characteristics and uses	W2
6	1	Cyanobacteria – isolation, characteristics, mass production and field application	R2: 151-165
7	1	Azolla - isolation, characteristics, mass production and field application	R2:160-163
8	1	Crop response and field application	R2: 381
9	1	Unit Revision	
Total No. of Hours Planned For Unit I=9			
UNIT - II			
S. No	**Duration**	**Topic**	**Support material/Page Nos**
1	1	Non – symbiotic nitrogen fixers – Introduction	R2: 166-173
2	1	Azotobacter as nitrogen fixation	R2: 116-128
3	1	Azotobacter – isolation, characteristics, mass production	
4	1	Azotobacter – field applications	
5	1	Azospirillum as nitrogen fixation	R2: 133-135
6	1	Azospirillum – isolation, characteristics	R2: 133-134
7	1	Azospirillum – mass production	R2: 133-134
8	1	Azospirillum – field applications	R2: 133-135
9	1	Unit Revision	

Total No. of Hours Planned For Unit II=9			
UNIT - III			
S. No	**Duration**	**Topic**	**Support material/Page Nos**
1	1	Phosphate solubilizing microbes – introduction	R1: 57-58
2	1	Phosphate solubilizing microbes – isolation	
3	1	Phosphate solubilizing microbes – characteristics	R1: 58-60
4	1	Phosphate solubilizing microbes – mass production	
5	1	Phosphate solubilizing microbes – field application	
6	1	PSM as biofertilizer	R1: 60-64
7	1	Effect of phosphate solubilizing microbes on different crops	W3
8	1	Interaction of PSM with other beneficial soil microorganisms	W3
9	1	Unit Revision	
Total No. of Hours Planned For Unit III=9			
UNIT – IV			
S. No	**Duration**	**Topic**	**Reference Support material/Page Nos**
1	1	Mycorrhizae – introduction	R1: 85
2	1	Taxonomy and ecology of VAM	
3	1	Importance of mycorrhizal inoculum	R1: 85-87
4	1	Role of VAM	R1: 89
5	1	Types of mycorrhizae and associated plants	R1: 84-85
6	1	Mass production of VAM	
7	1	Field application of ectomycorrhizae	R1: 84-86
8	1	Field application of VAM	R1: 84-86
9	1	Unit Revision	
Total No. of Hours Planned For Unit IV=9			
UNIT - V			
S. No	**Duration**	**Topic**	**Support material/Page Nos**
1	1	General account of microbes used as bioinsecticides	W4
2	1	Advantages of bioinsecticides over synthetic pesticides	
3	1	*Bacillus thuringiensis* – introduction	W5

4	1	*Bacillus thuringiensis* – production	W5
5	1	*Bacillus thuringiensis* – field applications	W5
6	1	Viral pesticides	W5
7	1	Cultivation of viral pesticides	W5
8	1	Field application of viral pesticides	W5
9	1	Unit Revision	
Total No. of Hours Planned For Unit V=9			

REFERENCES

1. K. V. B. R. Tilak, K.K. Pal & R. Day, Microbes for sustainable agriculture. I. K. International Publishing Pvt. Ltd.
2. Subba Rao, NS. Soil Microbiology, Fourth Edition, Oxford & IBH Publishing Co. Pvt Ltd.

WEBSITES

W1: https//en.m.wikipedia.org//wiki/Frankia
W2: https://en.m.wikipedia.org/wiki/Alder
W3: https//en.m.wikipedia.org/wiki/Phosphate_solubilizing_bacteria
W4: https://en.m.wikipedia.org/wiki/biopesticides
W5: https://en.m.wikipedia.org/wiki/Bacillus_thuringiensis

UNIT – 1

BIOFERTILIZERS

Biofertilizers

Biofertilizers are defined as preparations containing living cells or latent cells of efficient strains of microorganisms that help crop plants uptake of nutrients by their interactions in the rhizosphere when applied through seed or soil. They accelerate certain microbial processes in the soil which augment the extent of availability of nutrients in a form easily assimilated by plants.

Biofertilizer is defined as the microbial inoculation contains living cells of efficient strain of microorganisms such as cellulolytic N_2 fixing or phosphate solubilizing microbes. Biofertilizers increases the fertility and thus enhances the growth of plants. Biofertilizers are used to reduce the use of chemical fertilizers in agriculture. Chemical fertilizers are much harmful to man, whereas the biofertilizers are harmless. The microbial conversion is of two types namely simple organic conversion and complex conversion. In simple conversion, the insoluble organic substances are directly converted into organic acids or nitrogenase compounds in the soil. In complex reactions, the conversion is carried out by a series of reactions catalyzed by a number of enzymes produced by microorganisms.

Use of biofertilizers is one of the important components of integrated nutrient management, as they are cost effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture. Several microorganisms and their association with crop plants are being exploited in the production of biofertilizers. They can be grouped in different ways based on their nature and function.

Rhizobium

Rhizobium is a soil habitat bacterium, which can able to colonize the legume roots and fixes the atmospheric nitrogen symbiotically. The morphology and physiology of *Rhizobium* will vary from free-living condition to the bacteroid of nodules. They are the most efficient biofertilizer as per the quantity of nitrogen fixed concerned. They have seven genera and highly specific to form nodule in legumes, referred as cross inoculation group. *Rhizobium* inoculum contains the viable cells of *Rhizobium* which fixes the atmospheric nitrogen when the roots of higher leguminous plants are injected by *Rhizobium*.

***Rhizobium*-characters**: This belongs to bacterial group and the classical example is symbiotic nitrogen fixation. 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 to produce valuable proteins, vitamins and other nitrogen containing compounds. The site of symbiosis is within the root nodules. It has been estimated that 40-250 kg N / ha / year is fixed by different legume crops by the microbial activities of *Rhizobium*. The percentage of nodules occupied, nodules dry weight, plant dry weight and the grain yield per plant the multistrain inoculant was highly promising.

Isolation of *Rhizobium*:

The leguminous plants are uprooted and tested of any nodule is present in the root. The root nodule which are white brown to pink green in color and washed in water in order to eradicate the soil particles. Then a pinkish green nodule is selected and washed in distilled water. The washed root nodule is kept immersed in 0.1 acidified Kcl solution for 5 min. This Kcl is used in a disinfectant to sterilize the contaminate found on the surface of the nodule. Then again wash the nodule to remove the disinfectant. Finally the nodule is immersed in ethyl alcohol and later washed with sterile H_2O. The *Rhizobium* is isolated either by washing the nodule in pestle and morter or by cutting the nodule and streaking. The washed juice is collected by a sieve and serially diluted and plated. The nodule is streaked in a solid media to obtain proper growth of the bacteria. The media used for the growth of *Rhizobium* is yeast extract mannitol agar medium. The rhizobial cells from the culture are identified and mass cultured for the preparation of inoculum. The correct strain of Rhizobia is identified by nodule formation, cultural tests, Microscopic observation and staining techniques.

Mass culture of *Rhizobium*:

The selected rhizobial strain is cultured in YEMA medium for about 7 days in order to establish better growth. The *Rhizobium* culture is tested. The tested Rhizobial culture is transferred to a large container containing the sterile YEMA medium are incubated at 30 ° C for 9 days. Sufficient nutrients should be supplied at regular intervals of 24hrs. The rhizobial culture is checked to detect the presence of contaminants in the culture. pH of the medium and the growth rate are used to determine the presence of contaminants in the culture.

Carried-based inoculum for storage:

The term 'carrier' is generally used for a medium that carries the live microorganisms. The use of ideal carrier material is necessary in the production of good quality biofertilizer. Peat soil, lignite, vermiculite, charcoal, press mud, farmyard manure and soil mixture can be used as carrier materials. The neutralized peat soil/lignite are found to be better carrier materials for biofertilizer production The following points are to be considered in the selection of ideal carrier material.

- Cheaper in cost
- Should be locally available
- High organic matter content
- No toxic chemicals
- Water holding capacity of more than 50%
- Easy to process, friability and vulnerability.
- The carrier material (peat or lignite) is powdered to a fine powder so as to pass through 212 micron IS sieve.
- The pH of the carrier material is neutralized with the help of calcium carbonate (1:10 ratio) , since the peat soil / lignite are acidic in nature (pH of 4 - 5)
- The neutralized carrier material is sterilized in an autoclave to eliminate the contaminants.

The cultured Rhizobial cells can be added to the carrier like lignite to store the inoculum. This storage increases the efficiency of the strain. This carrier is used to preserve the inoculum in a viable condition.

Field Application:

1. The cultured *Rhizobium* is diluted with H_2O and applied on seeds. The suspension is sprinkled over on seeds. Sucrose solution (10%) is used to enhance the surviving potential of *Rhizobium* on the seed coats.
2. Inoculum is diluted with H_2O and slurry is uniformly mixed with seeds. Then the inoculum is pellatized on the seed coats. The inoculum is protected from the agricultural chemicals and acids and alkaline reaction of the soil. Thus the inoculum is spread over the field along with the seeds during sowing.
3. Pelleting agents like dolomite, gypsum, charcoal rock phosphates are used along with the inoculum. They increase the sedimentation potential of the inoculum on the surface of seeds. It protects the seeds from winter season.
4. The inoculum is stored at 4 °C in a refrigerator. The stored inoculum is sprayed over the soil directly to increase the fertility of the soil.

Frankia

Frankia belongs to Actinomycetes group of N-fixing organisms forming root nodules with non-leguminous plants. *Frankia* strains are heterotrophic aerobes having generation times of 15 or more hours. As a consequence of their filamentous morphology, the growth kinetics of *Frankia* strains generally consist of a stationary phase after transfer, followed by a short 'exponential' phase, and then by a slower increase in biomass over time. Problems typical of growing other filamentous organisms apply to *Frankia* strains. Care must be taken to avoid nutrient and waste gradients across mycelia and a flocs or pellet formation should be avoided by frequent homogenization.

Isolation of *Frankia*

Frankia is difficult to isolate directly from soil, so most strains originate from root nodules. Two factors limit success, one is that *Frankia* strains grow slowly, and the other is that fast-growing contaminants are common. To minimize the second problem, nodules are disinfected with dilute sodium hypochlorite and then peeled. Vesicle clusters can be separated from plant tissue by differential screening or density centrifugation. Clusters are best pour-plated on a variety of media and followed microscopically until they begin to grow over a period of ten days to three weeks. Monitoring the outgrowth of hyphae microscopically improves the chances of obtaining a monoculture. Contaminants are spatially removed from the slower-growing *Frankia* colony.

The medium used in isolating new *Frankia* strains is important but universal, or selective media can be used. Effective media range from defined propionate media to the complex QMod medium of Lalonde and Calvert. Antifungal agents, like cycloheximide or nystatin, can minimize fungal contamination. Virtually all *Frankia* strains isolated require no growth factors, and thus grow well in defined minimal medium (FDM). Some are inhibited by undefined media additives such as yeast extract. Another general medium for *Frankia* is defined propionate minimal medium (DPM). Most strains are grown and maintained in liquid culture, and generally grow slowly on solid media.

Alder

Alder is the common name of a genus of flowering plants (***Alnus***) belonging to the birch family Betulaceae. The genus comprises about 35 species of shrubs, a few reaching a large size, distributed throughout the North temperate zone with a few species extending into Central America, as well as the Northern and Southern Andes. *Alders* are commonly found near streams, rivers, and wetlands.

Nitrogen fixation

Alder is particularly noted for its important symbiotic relationship with *Frankia alni*, an actinomycete, filamentous, nitrogen-fixing bacterium. This bacterium is found in root nodules, which is large with many small lobes, and light brown in colour. The bacterium absorbs nitrogen from the air and makes it available to the tree. *Alder*, in turn, provides the bacterium with sugars, which it produces through photosynthesis. As a result of this mutually beneficial relationship, alder improves the fertility of the soil where it grows, and as a pioneer species, it provide additional nitrogen.

Uses

The catkins of some alder species have a degree of edibility and may be rich in protein. Reported to have a bitter and unpleasant taste, they are more useful for survival purposes. The wood of certain alder species is often used to smoke various food items such as coffee, salmon and other seafood. Most of the pilings that form the foundation of Venice were made from alder trees.

Casuarina

Casuarina is a genus of 17 tree species in the family Casuarinaceae, native to Australia, the Indian subcontinent, Southeast Asia, and Islands of the Western Pacific Ocean. They are evergreen shrubs and trees growing to 35 m (115 ft) tall. The foliage consists of slender, much-branched green to grey-green twigs bearing minute scale-leaves in whorls of 5–20. The apetalous flowers are produced in small catkin-like inflorescences. Most species are dioecious, but a few are monoecious. The fruit is a woody, oval structure superficially resembling a conifer cone, made up of numerous carpels, each containing a single seed with a small wing.

Uses

The wood of this tree is used commercially for shingles or fencing, and is said to make excellent, hot burning firewood. The wood of this tree is used for building-timber, furniture and tools, and makes excellent firewood. The tree's root nodules are known to fix nitrogen, and it is traditionally prized for its ability to increase the soil's fertility. Its abundant leaf-fall is high in nitrogen.

Cyanobacteria

Cyanobacteria, otherwise called as blue-green algae are ubiquitous in distribution. BGA fixes nitrogen in the soil. BGA such as *Anabena*, *Polypothium*, *Oscillotrian* actively fixes the nitrogen in soil. The BGA induces the growth of higher plants with the help vitamin B12; auxins etc and thus they form an effective biofertilizer in agriculture. The blue green algal inoculum may be produced by several methods viz., in tubs, galvanized trays, and small pits and also in field conditions. However the large-scale production is advisable under field condition which is easily adopted by farmers.

Mass multiplication

Preparation of the inoculum in trays:

Cyanobacteria are cultured in open trays exposed to air. The culturing tray is made of Zn or Fe and is filled with sieved nice soil, supper phosphate, sodium molybdate and water to keep the mixture or medium wet. The pH is adjusted neutral. A culture of *Cyanobacteria* is sprinkled over the soil mixture and the tray is kept in the open sunlight for about 10-20 days for proper growth.

Regular water is necessary which favor the better growth of *Cyanobacteria* in culture tray. Sometimes mosquitoes bread and the breeding can be stopped by the application of carbofuran. Owing to rapid growth, the *Cyanobacteria* cover the entire surface of soil mixture. The algal biomass is then separated from soil and air dried. The dried biomass is powdered and stored in polythene bags for future use.

Cyanobacteria culture in open:

The field is ploughed well and leveled properly for the culture of *Cyanobacteria*. The field is watered in order to facilitate the growth of *Cyanobacteria*. To induce the rapid growth of *Cyanobacteria,* super phosphate is sprayed over the surface of the soil. Clayey soil is prepared to sandy soil for proper and quick multiplication of *Cyanobacteria*. Application of carbofuran prevents the invasion of snails and mosquitoes. When the sufficient growth of *Cyanobacteria* is achieved, the field is ploughed well for the proper mixing of *Cyanobacteria* in the soil. Then the field is used as usual for agriculture.

Application:

1. The powdered *Cyanobacteria* mixture is simply spread over the agriculture field.
2. The application of *Cyanobacteria* after one week of transplantation of seedlings of paddy gives more beneficial result, because *Cyanobacteria* can be able to receive more sunlight.
3. Such paddy plants grow well in the field by consuming the nitrogen fixed by the *Cyanobacteria*.
4. The application of *Cyanobacteria* in the field increases the yield of crops.

Azolla

Azolla is a free-floating water fern that floats in water and fixes atmospheric nitrogen in association with nitrogen fixing blue green alga *Anabaena azollae*. Rice growing areas in South East Asia and other countries have recently been evincing increased interest in the use of the symbiotic N2 fixing water fern *Azolla* either as an alternate nitrogen sources or as a supplement to commercial nitrogen fertilizers. *Azolla* is used as biofertilizer for wetland rice and it is known to contribute 40-60 kg N ha-1 per rice crop. The agronomic potential of *Azolla* is quite significant particularly for rice crop and it is widely used as biofertilizer for increasing rice yields. The common species of *Azolla* are *A. microphylla, A. filiculoides, A. pinnata, A. caroliniana, A. nilotica, A. rubra* and *A. mexicana*.

Mass multiplication of *Azolla* under field conditions

A simple *Azolla* nursery method for large scale multiplication of *Azolla* in the field has been evolved for easy adoption by the farmers.

The potential *Azolla* species are maintained in concrete tanks keeping soil under flooded conditions. Partial shade helps during summer months. From these, *Azolla* is harvested and used as inoculum in bigger size plots or in small ponds generally found in rice growing areas. Its large scale production is carried out in a nicely prepared field divided into small sun plots with good irrigation facility (4-50 sqm plot with 5-10 cm water depth). *Azolla* is inoculated at the rate of 0.5 to 1.0 t/ha. Inoculation with higher doses ensures rapid multiplication. Superphosphate at the rate of 4-8 kg/ha stimulates fern growth. Animal dung (1.0-15 t/ha) or cattle slurry (2000- 3000 t/ha) can also be used in place of P_2O_5. Insecticides like furadon are applied. Under optimum conditions, *Azolla* forms a thick mat on the water surface in 15-20 days. Two-third of it is harvested and the remaining is left for further multiplication. It again multiples and forms a thick mat in 2-3 weeks. About 100 kg fresh *Azolla* inoculum can be obtained every week from 100 m^2 nursery. Superphosphate at the rate of 60 kg/ha can be split into 2-3 doses or added at weeks interval to have better results. If *Azolla* multiplication is good even without addition of P, then there is no need to add it.

Applications in field

Inoculation of *Azolla* to rice crop

The *Azolla* biofertilizer may be applied in two ways for the wetland paddy. In the first method, fresh *Azolla* biomass is inoculated in the paddy field before transplanting and incorporated as green manure. This method requires huge quantity of fresh *Azolla*. In the other method, *Azolla* may be inoculated after transplanting rice and grown as dual culture with rice and incorporated subsequently.

***Azolla* biomass incorporation as green manure for rice crop**

Collect the fresh *Azolla* biomass from the *Azolla* nursery plot. Then prepare the wetland well and maintain water just enough for easy incorporation. Apply fresh *Azolla* biomass (15 t/ha) to the main field and incorporate the *Azolla* by using implements or tractor.

***Azolla* inoculation as dual crop for rice**

Select a transplanted rice field and collect fresh *Azolla* inoculum from *Azolla* nursery. Broadcast the fresh *Azolla* in the transplanted rice field on 7^{th} day after planting (500 kg/ha). Maintain water level at 5-7.5cm. Note the growth of *Azolla* mat four weeks after transplanting and incorporate the *Azolla* biomass by using implements or tranctor or during inter-cultivation practices. A second bloom of *Azolla* will develop 8 weeks after transplanting which may be incorporated again. By the two incorporations, 20-25 tonnes of *Azolla* can be incorporated in one hectare rice field.

Unit – I
Possible Questions

Two Marks

1. Define biofertilizer.
2. What are the uses of biofertilizer?
3. Write the two microbial conversions in biofertilizer.
4. What is simple and complex reaction?
5. What are the characters of *Rhizobium*?
6. What is symbiosis?
7. Why potassium chloride is used in the isolation of *Rhizobium*?
8. Name a few carrier materials used for inoculum storage of *Rhizobium*.
9. Which basis the carrier material is selected?
10. Write two field applications of *Rhizobium*?
11. Write the two factors that limit the use of *Frankia* as biofertilizer.
12. Name the antifungal agents that minimize the fungal growth in the isolation of *Frankia.*
13. Write the uses of *Casuarina.*
14. Write the uses of carbofuran.
15. What is *Azolla*?
16. Mention three species name of blue green algae.

Eight Marks

1. Write an essay on *Rhizobium* as symbiotic nitrogen fixers.
2. Give an elaborate note on the isolation, mass multiplication and field application of *Rhizobium*.
3. Describe the mass multiplication and field application of *Cyanobacteria.*
4. What is biofertilizer? Write the field applications and crop response of biofertilizer?
5. Explain the methods of *Azolla* to rice crop.
6. Describe the isolation, mass cultivation and field application of *Azolla.*
7. Write the characteristics and uses of *Alder* and *Casuarina.*

UNIT - I

______ are aerobic and free-living nitrogen nitrogen fixers
______ are genes encoding enzymes involved in the fixation of atmospheric nitrogen
________ catalyze conversion of atmospheric nitrogen to ammonia
________ is a typical example of symbiotic nitrogen fixation seen in paddy fields
_________ recycles the H_2 produced during N_2 fixation, thereby minimizing the loss of energy
A free-living anaerobic photosynthetic bacterium
A free-living soil bacteria that is involved in nitrogen fixation
Amount of ATP needed to form 2 moles of ammonia from 1 mole of nitrogen gas during biological n
Apart from biological nitrogen fixation by microbes, _________ can fix atmospheric nitrogen
Bacteria that forms root nodules in legume plants
Biological nitrogen fixation was discovered by
Chemicals produced by the Rhizobia called _________ that cause the colonized root hairs to curl
Example of associative nitrogen fixation
Frankia is a
Group of irregularly shaped bacteria in root nodules are called as
In biological nitrogen fixation, ____ moles of ammonia are produced from one mole of nitrogen gas
In Cyanobacteria, nitrogen fixation occurs in terminally differentiated cells known as
In root nodules, ___________ bind and regulate the levels of oxygen in the nodule
Legume plants belongs to
Most abundant gas in atmosphere
Nitrogenase enzyme consists of
Rhizobia are attracted to ___________ released by the host legume's roots
The enzyme nitrogenase is inhibited by ________
Which is not true about *Anabaena* and *Nostoc*
The majority of hydrogenases in prokaryotes are ________ containing enzymes
With associative nitrogen fixation, which one of the following genera is associated?
The conversion of nitrogen to ammonia or nitrogenous compounds is called as______
Symbiotic nitrogen cyanobacteria are present in all except______
All the following are free living nitrogen fixers except________
Anabena is a nitrogen fixer present in the root pockets of_______
Splitting of dinitrogen molecule into free nitrogen atom in biological nitrogen fixation is carried out by _________
Which of the following aid plants in the acquisition of nitrogen from nitrogen gas of the atmosphere?
A major plant macronutrient found in nucleic acids and proteins is _________
Organisms capable of converting nitrogen to nitrate are_______

The conversion of amino acids to ammonium by soil decomposers is called______
To fix one molecule of nitrogen _______
Conversion of nitrite to nitrate is carried out by _______
All of the following are examples of negative symbiosis________
The reservior for nitrogen is __________
Degree of compost maturity can be assesed by _______
Which one of the following nacterium peodices nodule in alfalfa
In non leguminous plant, nodules are formed by which one of the following
Which one of the following component is the limiting and critical for soil
Which of the following is a classical example of a rhizobial species having biovars
Which of the following compound is known as the most resistant to microbial degradation during org
Which of the following forms symbiotism in soyabean crops
In1888, a Dutch microbiologist Beijerinck succeeded in isolating which one of the following bacterial strain from root nodules?
Ammonia produced in the bacteriod needs to be transported to the plant through which one of the following membrane
Which one of the following is the first species of rhizobia, identified in 1889
The fixation of the inert atmospheric elemental nitrogen by microorganisms through a reductive process accounts for about______
Which one of the following is nonleguminous
Nif gene is associated with _________
What are the cofactors needed for nitrogen fixation?

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OPTION 1	OPTION 2	OPTION 3	OPTION 4
Frankia & *Azospirillu*	*Clostridium* & *Desulfo*	*Beijerinckia* & *Klebs*	*Rhizobium* & *Anabaena*
mif	*nif*	*sif*	*nod*
Kinase	Hydrogenase	Nitrogenase	Phosphatase
Azolla-*Anabaena*	Alder-*Frankia*	Legume-*Rhizobium*	Higher plants-*Mycorrhizae*
Reductase	Catalase	Nitrogenase	Hydrogenase
Anabaena azollae	*Clostridium thermocel*	*Rhodospirillum rubr*	*Klebsiella pneumoniae*
Alcaligenes	*Acetobacter*	*Pseudomonas*	*Azotobacter*
8	16	32	64
Cyclone	Thunder	Raining	Lightning
Rhizobium	*Azotobacter*	*Azospirillum*	Cyanobacteria
Winogradsky	Beijerinck	Pasteur	Koch
Pod factors	Nod factors	Sod factors	Mod factors
Legume-*Rhizobium*	Rice-*Azospirillum*	Higher plants-Mycor	Azolla-*Anabaena*
Bacteria	Actinomycete	Fungi	Algae
Bacteroids	Asteroids	Mesteroids	Histeroids
2	4	6	8
Cyanocysts	Nitrocycts	Heterocysts	Homocysts
Teghemoglobin	Peghemoglobin	Leghemoglobin	Hemoglobin
Solanaceae	*Rosaceae*	*Astraceae*	*Fabaceae*
Nitrogen	Oxygen	Carbon dioxide	Hydrogen
Iron protein	Molybdenum-iron prot	Iron protein and a mo	Hemoglobin
Flavonoids	Enzymes	Toxins	Chemicals
CO_2	Sulfur	Hydrogen	Oxygen
Filamentous	Nitrogen fixing	Cyanobacteria	Symbiotic
Nickel	Copper	Molybdenum	Sulfur
Azotobacter	Escherichia	Rhizobium	Anabena
Nitrogen assimilation	Nitrogen fixation	Denitrification	Nitrification
Anthoceros	Azolla	Cycas	Gnetum
Rhizobium	Azotobacter	Rhodospirillum	Clostridium
Marselia	Salvinia	Pistia	Azolla
hydrogenase	nitrogenase	dinitrogenase	nitrate reductase
Bacteria	Algae	Nematodes	Moulds
calcium	nitrogen	sulphur	iron
yeast	bacteria	roundworms	moulds

ammonification	mineralization	deamination	both a and b
6 ATP molecules are	12 Atp molecules are	16 ATP molecules	20 ATP molecules
Nitrosomonas	Nitrosococcus	Nitrobacter	Clostridium
amensalism	competition	commensalism	parasitism
the atmosphere	rocks	ammonia	nitrates
infrared tehnique	germination test	both a and b	MPN test
Bradyrhizobium sp.	Rhizobium	Rhizobium	Rhizobium
Anabaena	Frankia	Ralstonia sp.	Sinorhizobium
Carbon	Nitrogen	Oxygen	Phosphorous
Rhizobium borbori	Rhizobium	Rhizobium lupini	Rhizobium vignae
Cellulose	chitin	Hemicellulose	Lignin
Azotobacter paspali	Bradyrhizobium	Nostoc	Rhizobium
Bradyrhizobium japon	Rhizobium leguminosarum	Sinorhizobium meliloti	Nostoc
Lipid membrane	Periplasmic membrane	Symbiosome membrane	Cytoplasmic membrane
Rhizobium borbori	Rhizobium	Rhizobium	Rhizobium lupine
60%	70%	90%	50%
Casuarina	Bacillus	Sesbania	Penicillium
Rhizobium bacteriod	Arthrobacter	Myrica	Bacillus
Cobalt	Molybdenum	Zinc	Copper

ANSWER KEY

Beijerinckia & *Klebsiella*
nif
Nitrogenase
Azolla-*Anabaena*
Hydrogenase
Rhodospirillum rubrum
Azotobacter
16
Lightning
Rhizobium
Beijerinck
Nod factors
Rice-*Azospirillum*
Actinomycete
Bacteroids
2
Heterocysts
Leghemoglobin
Fabaceae
Nitrogen
Iron protein and a molybdenum-iron protein
Flavonoids
Oxygen
Symbiotic
Nickel
Azotobacter
Nitrogen fixation
Gentum
Rhizobium
Azolla
nitrogenase

Bacteria

nitrogen
bacteria

both a and b
16 ATP molecules are required
Nitrobacter
competition
the atmosphere
both a and b
Rhizobium melliloti
Frankia
Phosphorous
Rhizobium leguminosarum
Lignin
Bradyrhizobium

Rhizobium leguminosarum

Symbiosome membrane
Rhizobium leguminosarum

70%
Casuarina
Rhizobium bacteriod
cobalt

UNIT – 2
NON-SYMBIOTIC NITROGEN FIXERS

Free living Nitrogen fixers

Free living and associative nitrogen fixers are important inoculants for non-leguminous crop particularly graminaceous and vegetable crops. Nitrogen fixing bacteria colonizing graminaceous plants can be grouped into three categories.

1. Rhizosphere organism - The species that colonize the root surfaces such as *Azotobacter* sp.
2. Facultative endophytes - Colonize the surface and interior of the roots such as *Azospirillum* sp.
3. Obligate endophytes - Includes *Gluconacetobacter, Herbaspirillum* sp. and *Azoarcus.*

Azotobacter

Beijerinck discovered an aerobic bacterium capable of fixing molecular nitrogen. Azotobacter species are known to influence plant growth through their ability to fix nitrogen, production of growth promoting substances like IAA, gibberellins or gibberellin like compounds and excretion of ammonia in the rhizosphere through exudates, production of antifungal metabolites and phosphate solubilization. Ecological or agro climatic factors like fertility level, moisture, temperature, acidic and alkaline condition and the carbon content of the soil seem to influence the proliferation of Azotobacter in the soil or in the rhizosphere.

Characteristics of Azotobacter

Azotobacter a soil habitant bacterium is a free living, nonsymbiotic nitrogen fixing bacteria. Azotobacter is rod shaped, relatively large organisms measuring 2.0-7.0µ x 1.0-2.5 µ. The cell size and shape vary considerably with species, strains, age of culture and growth conditions. For several species, the vegetative cells may give rise to specialized spherical resting cells known as cysts. Each cyst is produced from a single vegetative cell. Motility in most of the Azotobacter cells is carried out by means of peritrichous flagella. A unique differentiating character of Azotobacter is its ability to form pigments. Azotobacter species are known to influence plant growth through their ability to fix nitrogen, production of growth promoting substances like compounds and excretion of ammonia in the rhizosphere through exudates, production of antifungal metabolites and phosphate solubilization.

Factors influencing Azotobacter growth

Temperature: Azotobacter is typical mesophilic bacteria. The optimal temperature they can withstand is between 25 °Cand 30 °C.

Humidity: Requires high humidity. They have a lower intracellular osmotic pressure than fungi and Actinomycetes. Hence the moisture requirements resemble that of higher plants.

Aeration: Being aerobic, Azotobacter needs continous supply of oxygen, but unique in its needs.

pH: Optimal pH for its growth is near or slightly above neutrality. (7.2-7.6).

Salts: The main ecological factor affecting the viability (metabolism) of microorganisms in saline soils is the high salt concentration

Isolation of Azotobacter

Azotobacter species are isolated by soil dilution plating method. One gram of soil sample are transferred to 100ml sterile distilled water and mixed thoroughly by shaking the flask for 5 minutes. Serial dilution of the suspension is made using sterilized distilled water. Any one of the nitrogen free agar media specific for Azotobacter is prepared and poured into sterile petriplates. 0.1 ml samples from the appropriate dilutions are spread evenly over cooled agar medium in petriplates. The plates are incubated at 30 °C for 3-4 days. Azotobacter colonies appear as flat, soft, milky and mucoid on agar plates.

Mass production of Azotobacter

Jensen's N-free medium is routinely used for the mass multiplication of Azotobacter. For mass production of Azotobacter, the bacterial strain isolated preserved in slants were transferred to liquid broth of selective as well as optimized medium in the rotary shaker for 4 days to prepare starter culture. Later on the starter culture is transferred to the fermenter in batch culture is transferred to the fermenter in batch mode with proper maintenance of 30 °C and continuous agitation for 4-9 days. When the cell count has reached to 108-109 cells/ml, the broth is used as inoculants. For easy handling, packing, storing and transporting broth is mixed with an inert carrier material which contains sufficient amount of cells.

Carrier based medium

Powdered peat soil, lignite are used as carriers. The Azotobacter prefers 4 °C for its long term storage. Sometimes the powdered carriers are neutralized with $CaCO_3$ and autoclaved for proper sterilization. This is mixed with culture and dried in air before storage.

Applications

1. **Seed treatment:**

   The cultured inoculum is diluted with H_2O and the seeds are kept dipped in the inoculum for one night. This seeds are sown in the main field. The slurry is directly poured over the nursery bed or in agricultural field.

   The seeds are spread on a polythene bag and the inoculum is sprinkled over the seeds for the mixing of the inoculum with the seeds. The inoculum-coated seeds are then dried in the air before sowing.

2. **Seedling treatment:**

   In this method, the inoculum in diluted with the H_2O and the roots of the seedlings are kept dipped in the inoculum for about 10-15 min. Paddy field gets benefited by this process.

3. **In paddy field:**

   A required amount of inoculum is mixed with farmyard manure. Then this mixture is properly mixed with soil. The resulting carrier based inoculum is directly used in the cultivation of rice. Azotobacter synthesizes biologically active substances such as nicotinic acid, panthothenic acid, pyridoxine, biotin, giberellic acid. These are plant growth promoting substances (PGPS). Azotobacter provides a favorable micro environment to the root system of higher plants and induces the better growth of the roots which participates in the growth of root systems in higher plants.

Azospirillium

Characteristics of Azospirillium

Azospirillum is a free living nitrogen fixing bacteria closely associated with grasses. Azospirillum is a Gram negative, rod-shaped and motile bacteria associated with roots of monocots including important crops such as wheat, corn and rice. Azospirillum bacterium fixes the atmospheric nitrogen and makes it available to plants in nonsymbiotic manner that can replace 50-90% of the nitrogen fertilizer required by plants. The nitrogen source used by *Azospirillum* for their growth is ammonium, nitrate, amino acids and elemental nitrogen. *Azospirillum* sp. is highly adaptable, being able to grow under anaerobic conditions (nitrate used as eletron acceptor), microaerobic (elemental or ammonia used as N source) and fully aerobic conditions (ammonia, nitrate, amino acid or combined N only). *Azospirillum* as a "biofertilizer" is particularly important in agricultural systems where fertilizer inputs are either impractical (rangelands), undesirable (organic farming), or not possible (subsistence agriculture). Experiments on inoculation of crops with *Azospirillum* or other diazotrophs often resulted in enhanced plant growth or nitrogen content under environmental conditions, improve nutrient assimilation, alter root size and function.

Benefits

1. Promotion of root hair development and branching
2. Increased uptake of N, P, K and microelements
3. Improved water status of plants and
4. Increased dry matter accumulation and grain yield.

Isolation of *Azospirillum*

The roots are separated from the plants and thoroughly washed in running tap water. Then transferred into 1 L flask containing 500 mL of sterile tap water and shaken for 30 min. The procedure is repeated three times, after which the same procedure is repeated with distilled water three times. The washed roots are transferred to sterile petridish and are cut into pieces with sterile scissors. Half centimeter long root pieces are surface sterilized in 70% alcohol for 3-5 seconds. The root pieces are repeatedly washed in phosphate buffer (pH 7.0) and then they are plated in semi solid, nitrogen free medium. The plates are incubated at 35 °C for 3 days. Characteristic growth of *Azospirillum* is indicated by the formation of white pellicles 2-4 mm below the surface of the medium.

Mass multiplication of *Azospirillum*

For mass multiplication of *Azospirillum*, the organism is allowed to grow in flasks containing NH_4Cl and malic acid medium and incubated at 35-37 °C for 3 days. When there is good growth, the broth culture is mixed with the carrier and carrier based inoculum is packed in polythene pouches. The preparation of carrier based inoculant and for inoculating the seed or seedlings with *Azospirillum* culture are allowed to multiply be an important factor in *Azospirillum* culture preparation.

Carrier for *Azospirillum*

Soil and farmyard manure in the ratio of 1:1 sterilized for 3 hours consecutively for 3 days were found to be best suited as a carrier for *Azospirillum*. The bacterium was able to survive up to 6 months in the soil and farmyard and gave counts of10^6 cells/g of carrier materials.

Application

1. The cultured *Azospirillum* is diluted with H_2O and applied on seeds. The suspension is sprinkled over on seeds. Sucrose solution (10%) is used to enhance the surviving potential of *Azospirillum* on the seed coats.
2. Inoculum is diluted with H_2O and slurry is uniformly mixed with seeds. Then the inoculum is pellatized on the seed coats. The inoculum is protected from the agricultural chemicals and acids and alkaline reaction of the soil. Thus the inoculum is spread over the field along with the seeds during sowing.
3. Pelleting agents like dolomite, gypsum, charcoal rock phosphates are used along with the inoculum. They increase the sedimentation potential of the inoculum on the surface of seeds. It protects the seeds from winter season.
4. The inoculum is stored at 4 °C in a refrigerator. The stored inoculum is sprayed over the soil directly to increase the fertility of the soil.

Unit – II
Possible Questions

Two Marks

1. Define free living nitrogen fixers.
2. Write the three groups of nitrogen fixing bacteria colonizing graminaceous plants.
3. Write the characteristics of *Azotobacter*.
4. What is rhizosphere?
5. Name the carrier material used for *Azotobacter*.
6. Write the benefits of *Azospirillum* as biofertilizer.
7. What is nitrogen fixation?
8. Differentiate between symbiotic and nonsymbiotic nitrogen fixation.
9. Define siderophore.

Eight Marks

1. Give brief note on *Azotobacter* and *Azospirillum* as non symbiotic microorganism.
2. Describe the mass multiplication, field application of *Azospirillum*.
3. Describe the mass multiplication and field application of *Azotobacter*
4. Write the characteristics and application of *Azotobacter* and *Azospirillum*.

UNIT - II

Nitrogen fixing bacteria colonizing graminaceous plants can be classified into categories
Beijerinck discovered an aerobic bacteria capable of fixing molecular nitrogen in the year _____
Subramoney and Abraham isolated Azotobacter chroococcum strains from ________ soil
Azotobacter chroococcum grows in ________ soil
Azotobacter are _________ shaped bacterium
The incubation period for the isolation of Azotobacter is______
Aged cultures of Azotobacter chrococcum form an insoluble _______ colored pigment
The melanin formed by Azotobacter is due to the presence of ________ enzyme
The optimal temperature for Azotobacter are between _________
Inoculation with Azotobacter was found to increase vitamin ____ in tomatoes
The coinoculation of Azotobacter with other bioinoculant like Rhizobium enhance the growth of
Seed inoculation of Azotobacter chrococcum increases the yield of field crops by about _________
Low grade of ________ was applied at the intial step of filling up of compost pit
Homologous selection of a pigmented strain of Azotobacter established better on ______ part of th
Azotobacter has ability to produce ________ compounds
The optimum pH for the growth of Azotobacter is _________
Azotobacter are _________ bacterium
Azotobacter species require __________humidity
____________ species is being used as inoculum for seed bacterization of agricultural crops
The multiple action of __________ contributes to better germination percentage of seeds
____________ species is specific to the rhizosphere of Paspalum notatum
Example of associative nitrogen fixation
Azospirillium are _________ shaped bacterium
The root piece for the isolation of Azospirillium are surface sterilized in ______% of alcohol
Who reported nitrogen fixing bacterium under the name spirillum?
Azospirillum grows free with nitrogen as microaerophilic and when supplied with nitrogen as ____
The strains of some Azospirillum fail to grow in the absence of particular nitrogen source _______
In which year Azospirillum was described
Which one of the following a-proteobacteria is known as nitrogen fixers
Azospirillum amazonense peoduce __________ colored colonies in potato dextrose agar
Azosprillium irakense was found in association with _________ roots
Azospirillum rugosum a new species isolated from ________ soil
____________ is used as carrier for Azospirillum
The ratio of carrier used in Azospirillum is _________
__________ is used in the mass multiplication of Azospirillum
Which of the following are nitrogen fixing bacteria associated with roots of C4 plants like maize su
Which of the following is nitrogen fixing bacterium living in association with sugarcane?

All are free living nitrogen fixers except_______
Anbena a nitrogen fixer is present in the root pockets of ___________
Organism associated with sorghum and cotton which provide nutrition to them are
To fix one molecule of nitrogen
The major enzymes involved in biological nitrogen fixation are__________
Majority of nitrogen fixation occurs by
________ species of Azospirillum has pectinolytic activites
Which Azospirillum species are acid tolerant?
Pure culture of Azospirillum brasilense synthesized auxin from ________
Azospirillum brasilense produced ________ in defined media amended with malate, gluconate or f
The ability of Azospirillum to synthesize siderophores may contribute to improved _________ nut
Azospirilla are capable of living in soils or as __________
Azospirillum could fall prey to certain macrofauna like _______
The digestive fluid of Pachyinlus flavipes had a strong ______________
Salt tolerance among species of Azospirillum was the lowest in
Strains of ___________ exhibit higher tolerance to salt content
Azospirillum are highly sensitive to _____________
Soil salinity plays vital role in diversity of native strains of __________

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OPTION 1	OPTION 2	OPTION 3	OPTION 4
5	3	2	6
1906	1901	1899	1910
Red soil	Red loamy soil	Black soil	Clay
Acidic	Neutral	Basic	Neutral and alkaline
Rod	Cocci	Sprillium	Comma
3 Days	5 Days	1 Day	7 Days
Yellow	Red	Black brown	Black
Tyrosinase	Maltase	Trypticase	Pectinase
25 - 30 °C	27 - 30 °C	25 - 35 °C	25 - 40 °C
A	B12	C	E
Legumes	Fruits	Pulses	Seeds
10	30	50	20
Sulphur	Carbon	Rock phosphate	Nitrogen
Stem	Leaf	Flower	Roots
Antifungal	Antibacterial	Antimalarial	Antiviral
7.2-7.6	7	6	4
Anaerobic	Facultative anaerob	Aerobic	Obligate
High	Low	Extreme	Moderate
Azotobacter chrococ	Azotobacter paspali	Azotobacter vin	Azotobacter beijerinckii
Azospirillium	Azotobacter	Azolla	Anabena
Azotobacter chrococ	Azotobacter beijeri	Azotobacter vin	Azotobacter paspali
Legume-*Rhizobium*	Rice-*Azospirillum*	Higher plants-M	Azolla-*Anabaena*
Vibriod	Rod	Cocci	Bacilli
80	50	70	100
Beijerinck	Schroeder	Dobereiner	Tilak
Anaerobic	Aerobic	Facultative anae	Microaerophilic
Peptone	Sodium nitrate	Yeast extract	Ammonium chloride
1954	1966	1984	1983
Acetobacter	Azospirillum	Gluconobacter	Rhodospirillum
White	Pink	Red	Black
Wheat	Rice	Grass	Barley
Sewage contaminate	Water	Oil contaminate	Waste contaminated
Farmyard manure	Lignite	Charcoal	Press mud
02:01	01:10	01:01	02:02
NH4Cl	NH4So4	H2NH4Cl	Na2Cl
Azospirillum	Clostridium	Azotobacter	Bacillus polymyxa
Acetobacter	Azotobacter	Frankia	Azospirillum

Azospirillum	Clostridium	Azotobacter	Bacillus polmyxa
Salvinia	Marselia	Azolla	Pistia
Azospirillum, Azoto	Azotobacter, Aceto	Anabena, Rhizo	Rhizobium, Azotobacter
6 ATP molecules are	12 ATP molecules a	16 ATP molecu	20 ATP molecules are required
Nitrogenase and hex	Nitrogenase and hy	Nitrogenase and	Nitrogenase and peptidase
biological nitrogen fi	Haber Bosch proces	Lightning	Volcanisc eruption
Azospirillum irakens	Azospirillum amaz	Azospirillum do	Azospirillum zeae
Azospirillum oryzae	Azospirillum iraker	Azospirillum an	Azospirillum zeae
Tryptophan	Tyrosin	Alanine	Methionine
Minerals	Proteins	Vitamins	Carbohydrates
Magnesium	Sulphur	Carbon	Iron
Ectophytes	Endophytes	Mesophytes	Pteridophytes
Earthworms	Snail	Snake	Frog
Antifungal activity	Antibacterial actiiv	Antiviral activit	Antimalarial activity
A. zeae	A. irakense	A. amazonense	A. doebereinerae
A. doebereinerae	A. halopraeferens	A. irakense	A. zeae
Minerals	Salts	Heavy metals	Sugars
A. braslense and A. l	A. zeae and A. halo	A. amazonense	A. amazonense and A. doebereinerae

ANSWER KEY

3
1901
Red loamy soil
Neutral and alkaline
Rod
3 Days
Black brown
Tyrosinase
25 - 30 °C
C
Legumes
10
Rock Phosphate
Roots
Antifungal
7.2-7.6
Aerobic
High
Azotobacter chrococcum
Azotobacter
Azotobacter paspali
Rice-*Azospirillum*
Vibriod
70
Beijerinck
Aerobic
Yeast extract
1983
Azospirillum
White
Rice
Oil contaminated
Farmyard manure
01:01
NH_4Cl

Azotobacter

Azospirillum
Azolla
Azospirillum, Azotobacter
16 ATP molecules are required
Nitrogenase and hydrogenase
biological nitrogen fixing organisms
Azospirillum irakense
Azospirillum amazonense
Tryptophan
Vitamins
Iron
Endophytes
Earthworms
Antibacterial actiivty
A. amazonense
A. halopraeferens
Heavy metals
A. braslense and A. lipoferum

UNIT – 3

PHOSPHATE SOLUBILIZERS

Phosphate solubilizing microorganisms

Phosphorus is a major nutrient required for the growth of plants. There are large reserves of phosphorus in soils but very little amount is available to the plant. There are microorganisms in the soil that can solubilize the unavailable phosphorus and make it available to plant. They are called phosphate solubilizing microorganisms (PSM). A group of fungi associates with the roots of higher plants and mobilize the phosphorus from soil to the plant system. Phosphorus solubilizing microorganisms include various bacterial, fungal and actinomycetes forms which help to convert insoluble inorganic phosphate into simple and soluble forms. Members of *Pseudomonas, Micrococcus, Bacillus, Flavobacterium, Penicillum, Fusarium, Sclerotium* and *Aspergillus* are some of the phosphate-solubilizing micro-organisms. They normally grow in a medium containing insoluble tri-calcium phosphate [$Ca_3 (PO_4)_2$], apatite, rock phosphate, $FePO_4$ and $AlPO_4$ as sole source of phosphate.

Occurrence of phosphate solubilizing bacteria

High proportion of PSM is concentrated in the rhizosphere and they are metabolically more active than from other sources. Usually one gram of fertile soil contains 103 to 1010 bacteria. Soil bacteria are in cocci, bacilli or spiral. Bacilli are common in soil, whereas spirilli are very rare in natural environment. The PSB are ubiquitous with variation in forms and population in different soils. Population of PSB depends on different soil properties. Larger populations of PSB are found in agricultural and rangelands soils.

Isolation of phosphate solubilizing microbes

The initial isolation of phosphate solubilizers is made by using Pikovaskaya medium suspended with insoluble-phosphates such as tri-calcium phosphate. The production of clearing zones around the colonies of the organism is an indication of the presence of phosphate-solubilizing organisms. Several rock phosphate dissolving bacteria, fungi, yeast and actinomycetes were isolated from soil samples of rock phosphate deposits and rhizosphere soils of different leguminous crops. The most efficient bacterial isolates were *Pseudomonas striata, Pseudomonas rathonis* and *Bacillus polymyxa* and fungal isolates as *Aspergillus awamori, Penicillium digitatum, Aspergillus niger* and a yeast-*Schwanniomyces occidentalis*. These efficient micro-organisms have consistently their capability to solubilize chemically-fixed soil phosphorus and rock phosphate from different sources. In addition, these microorganisms were found to mineralize organic phosphorus to soluble form due to enzymatic activity.

The efficient cultures have capacity to solubilize insoluble inorganic phosphate such as rock phosphate, tri-calcium phosphate, iron and aluminium phosphates by production of organic acids. They can also mineralize organic phosphatic compounds present in organic manure and soils. Inoculation of PSM to seeds or seedlings increases the grain yield of crops. The inorganic phosphate solubilization by microbes can be attributed to acidification, chelation and exchange reaction in growth medium as well as to the proton transfer during ammonium assimilation.

Mass production of phosphate solubilizing microorganisms

1. A loopful of inoculum is transferred in liquid medium and keeps the flak on rotary shaker for 3-7 days. The content of these flasks called mother culture or starter culture. After sterilization suitable broth is inoculated with the mother culture. Keep the flasks on rotary shaker for 96-120 hours until the viable count per ml reaches to 10 9 /ml cells. Peat or Lignite powder is neutralized by addition of 1% calcium carbonate (CaCO3) and sterilized at 15 lbs pressure for 3-4 hours. The carrier should have high organic matter above 60% and high moisture holding capacity 150 to 200% by weight and provide a nutritive medium for growth of bacteria and prolong their survival in culture.
2. The sterilized and neutralized lignite or peat is mixed with high count broth culture in galvanized trays. After mixing the broth cultures and lignite or peat powder in 1:2 proportion in the galvanized trays then it is kept for curing at room temp (28 °C) for 5 to 10 days. After curing, sieved powder is filled in polythene bag as of 0.5 mm thickness leaving 2/3 space open for aeration of the bacteria. Then the bag is packed by sealing.
3. The viable cells count in the carrier based inoculants should be maintained as per ISI specifications. The inoculants shall be stored by the manufacture in a cool place away from direct heat preferably at a temp of 15 °C for six months.

Effect of PSB on Crop Production

Phosphate rock minerals are often too insoluble to provide sufficient P for crop uptake. Use of PSMs can increase crop yields up to 70 percent. Combined inoculation of arbuscular mycorrhiza and PSB give better uptake of both native P from the soil and P coming from the phosphatic rock Higher crop yields result from solubilization of fixed soil P and applied phosphates by PSB Microorganisms with phosphate solubilizing potential increase the availability of soluble phosphate and enhance the plant growth by improving biological nitrogen fixation enhanced the number of nodules, dry weight of nodules, yield components, grain yield, nutrient availability and uptake in soybean crop. Phosphate solubilizing bacteria enhanced the seedling length, while co-inoculation of PSM and PGPR reduced P application by 50 % without affecting corn yield.

Unit – III

Possible Questions

Two Marks

1. What is the use of phosphorus in soil?
2. What are phosphate solubilizing microorganisms?
3. Mention a few bacterial genera that solubilized phosphorus.
4. What are the sole sources of phosphorus for the growth of microorganisms?
5. What is the indication of the presence of phosphate solubilizing microorganism?
6. Mention a few fungal species that solubilized phosphorus.
7. Which medium is used for the growth of phosphate solubilizing microbes and write the chemical composition of the medium?
8. What is the attribution of inorganic phosphate solubilizing microbes in growth medium?

Eight Marks

1. Write notes on phosphate solubilizing microorganisms.
2. Explain the isolation and mass production of phosphate solubilizing microorganisms.
3. Explain the occurrence and field application of phosphate solubilizing bacteria.
4. Write the mass production and field application of phosphate solubilizing microorganisms.

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UNIT - III	OPTION 1
________ is phosphate solubilizing bacteria	*Bacillus megaterium*
Enzyme involved in phosphate solubilization	Oxidases
Microorganisms make soluble phosphate from insoluble phosphate by produci	Hydrochloric acid
PGPR is	Phosphorous growth prom
____________ is supplied to the soil in the form of chemical fertilizers or org	Phosphorus
Phosphate solubilizing microroganisms grow in the medium containing insolu	Nitrite
The production of clearing zone around the colonies of the organism is an indi	Blue green algae
Quick screening of phosphate solubilizing microroganisms was developed by	1975
________ reported benificial effect of phosphobacteria on berseem and wheat	Sundara Rao
The use of phosphobacteria increased the efficiency of rock phosphate and sup	Neutral
At ______ pH the PSM did not improve the utilization by mycorrhizal plants (	8.1
Singh and Kapoor observed root colonization by VAM in _______	Wheat
___________ bacteria are benificial bacteria capable of solubilizing inorganic	Azospirillum
_____________ is the limiting nutrient for acquatic organisms	Nitrogen
_______% of mined phosphorus is used to make fertilizers	40
Over enrichment of phosphate in both fresh and inshore marine waters can lea	Algal glooms
Phosphorus occurs most abundantly in nature as part of the _________	Phosphate
The plants consumed by the herbivores incorporate the phosphorus into their _	Cytoplasm
_________ is the most common mineral Phosphate weathers from rocks and n	Dolomite
__________ is adsorbed on iron oxides, aluminium hydroxides, clay surface a	Sulphate
__________ gas is normally found to occur under highly reducing conditions	Alginine
Available phosphorus is found in a biogeochemical cycle in the _________ so	Lower
The production and release of oxalic acid by __________ fungi explains their	Filamentous
Microbes that solubilize fixed soil phosphorous are called _________	Phosphorus fixers
The P content in average soil is about ___________ w/w	0.05%
Phosphorus accounts about _________ of the plant dry weight.	0.2-0.4%
Phosphate solubilizing Bacteria (PSB) may also be useful in the phyto-remedi	Heavy metal
The liberation of organic phosphates by bacteria is mediated through the produ	Lipase
The principal mechanism for mineral phosphate solubilization is the productio	Pectinase
In organic acid production mechanisms, __________ seems to be the most fre	Gluconic acid
The acids are produced in the ____ of Gram- negative bacteria by a direct oxid	Outer membrane
Phosphatic fertilizer management in aerobic rice is critical when ______of the	75-90%
The application of ______ acid has been proven to be effective for the solubil	Pectic
Radioactive__________ can be used to evaluate the exchange rates between th	^{24}P b
The excreted ___________ accompanying the decrease in pH acted as a solve	H^+
_______ plays an important role in the development of roots including root ini	Indole acetic acid
Siderophore production by PS bacterial strains has been considered as a potent	Calcium
The application of ________ resistant plant growth-promoting Pseudomonas a	Mercury

Rahnella aquatilis solubilizes P and produces	Oxalic acid
__________produces gluconic acid and solubilizes P	*S. marcescens*
P solubilization mechanisms include _____ formation, chelating metal ions an	Acid
The growth and development of plants by producing or changing the concentra	Oxalic acid
__________ is recommended for detection of phosphate-solubilizing soil micr	Pikovskayas agar
Organic matter derived from dead and decaying plant debris is rich in organic	Carbon
Pikovskayas agar was modified by ____________	Beijerinck
The phosphate solubilizing fungi Aspergillus brasiliensis show ________ grov	Good
Phosphorus is one of the major fundamental macronutrients for plants and is a	Nitrate
When PSB is used with rock phosphate it can save about ____ of the crop requ	90%
The major enzymes involved in biological nitrogen fixation are__________	Nitrogenase and hexokina
Majority of nitrogen fixation occurs by	biological nitrogen fixing
Low grade of ________ was applied at the intial step of filling up of compost	Sulphur
The fixation of the inert atmospheric elemental nitrogen by microorganisms through a reductive process accounts for about______	60%
The digestive fluid of Pachyinlus flavipes had a strong ______________	Antifungal activity
What are the cofactors needed for nitrogen fixation?	Cobalt
The conversion of nitrogen to ammonia or nitrogenous compounds is called as______	Nitrogen assimilation
Symbiotic nitrogen cyanobacteria are present in all except______	Anthoceros
Amount of ATP needed to form 2 moles of ammonia from 1 mole of nitrogen	8
Apart from biological nitrogen fixation by microbes, _________ can fix atmos	Cyclone
______ are genes encoding enzymes involved in the fixation of atmospheric n	*mif*
________ catalyze conversion of atmospheric nitrogen to ammonia	Kinase
Fossil evidence and DNA sequence analysis suggest that this mutalism appear	300-400

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OPTION 2	OPTION 3	OPTION 4	ANSWER KEY
Bacillus anthrax	*Bacillus cereus*	*Bacillus phosphatae*	*Bacillus megaterium*
Reductases	Kinases	Phytases	Phytases
Sulphuric acid	Nitric acid	Organic acids	Organic acids
Plant gibberellin promotin	Plant growth prom	Plant growth promoting bacteria	Plant growth promoti
Iron	Potassium	Zinc	Phosphorus
Nitrate	Phosphate	Sulphate	Phosphate
Phosphate solubilizing mic	Nitrifying bacteria	Nitrogen fixing bacteria	Phosphate solubilizin
1989	1999	1996	1999
Gaind	Nautiyal	Gaur	Sundara Rao
Alkaline	Acid	Neutral to alkaline	Neutral to alkaline
5.2	7.4	6.1	7.4
Rice	Barley	Corn	Wheat
Azotobacter	Rhizobium	Phosphate solubilizing	Phosphate solubilizin
Carbon	Phosphorus	Hydrogen	Phosphorus
60	100	80	80
Azospirillia	Azotobacter	Algal biomass	Algal glooms
Orthophosphate	Inorganic phosphat	Organic phosphate	Orthophosphate
Tissues	Cells	Roots	Tissues
Gypsum	Apatite	Liginite	Apatite
Nitrate	Carbonate	Phosphate	Phosphate
Phosphine	Sulphide	Methionine	Phosphine
Upper	Middle	Both lower and middle	Upper
Mycorrhizal	Dimorphic	Non filamentous	Mycorrhizal
Phosphorous solubilizing r	Nitrogen fixers	Phosphorous solubilizers	Phosphorous solubili
0.06%	0.04%	0.07%	0.05%
0.2-0.6%	0.2-0.8%	0.2-0.5	0.2-0.8%
Carbon	Nitrogen	Ions	Heavy metal
Protease	Maltase	Phytase	Phytase
Phytase	Phosphatase	Protease	Phosphatase
Malic acid	Techoic acid	Succinic acid	Gluconic acid
Cytoplasm	Inner membrane	Periplasm	Periplasm
75-80%	65-90%	75-85%	75-90%
Oxalic	Malic	Organic	Oxalic
^{46}P	^{31}P	^{32}P	^{32}P
C^+	Mg^+	Fe^+	H^+
Auxins	Gibberellins	Amino acids	Indole acetic acid
Iron	Copper	Zinc	Iron
Iron	Cadmium	Nickle	Cadmium

Gibberlic acid	Gluconic acid	Indole acetic acid	Gluconic acid
S. liquefaciens	*S. plymuthica*	*S. rubidaea*	*S. marcescens*
Alkali	Heavy metals	Iron	Acid
Gibberlic acid	Gluconic acid	Indole acetic acid	Indole acetic acid
Nutrient agar	Rose bengal agar	Czepadex agar	Pikovskayas agar
Nitrogen	Sulfur	Phosphorous	Phosphorous
Sundara Rao and Sinha	Schroeder	Dobereiner	Sundara Rao and Sin
Moderate	No growth	Luxuriant	Luxuriant
Nitrite	Phosphate	Succinate	Phosphate
50%	40%	60%	50%
Nitrogenase and hydrogena	Nitrogenase and hy	Nitrogenase and peptidase	Nitrogenase and hydr
Haber Bosch process	Lightning	Volcanisc eruption	biological nitrogen fi
Carbon	Rock phosphate	Nitrogen	Rock Phosphate
70%	90%	50%	70%
Antibacterial actiivty	Antiviral activity	Antimalarial activity	Antibacterial actiivty
Molybdenum	Zinc	Copper	cobalt
Nitrogen fixation	Denitrification	Nitrification	Nitrogen fixation
Azolla	Cycas	Gnetum	Gentum
16	32	64	16
Thunder	Raining	Lightning	Lightning
nif	*sif*	*nod*	*nif*
Hydrogenase	Nitrogenase	Phosphatase	Nitrogenase
400-440	350-400	400-460	400-460

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UNIT – 4

MYCORRHIZAL BIOFERTILIZERS

Mycorrhizae

Mycorrhizae are fungus-root associations, first discovered by Albert Bernhard Frank in 1885. The term "mycorrhizae" comes from the Greek words meaning fungus and roots. These microorganisms contribute to plant functioning in natural environments, agriculture, and reclamation. The roots of about 95% of all kinds of vascular plants are normally involved in symbiotic associations with mycorrhizae. Five mycorrhizal associations have been described. These include both nonseptate and septate fungi. There are endophytic arbuscular mycorrhizae (AM) that form arbuscules and sometimes vesicles septate types associated with orchids and those that form endomycorrhizal relationships with ericoid plants such as blueberries. In the endophytic mycorrhizae, the fungus penetrates the plant cells where it forms characteristic structures, including arbuscules and coils. Vesicles are not consistently observed. In addition, ectendomycorrhizae are formed by basidiomycetes. These have sheaths and intracellular coils. Finally, the **ectomycorrhizae** form a sheath, and the fungus grows between the plant cells, producing the "Hartig net." Such ectomycorrhizae, including *Cennococcum, Pisolithus* and *Amanita,* form irregular structures that are easy to recognize.

Potential benefits of mycorrhizae

- Enhanced water and nutrient uptake
- Reduction of irrigation requirements
- Reduction need for fertilizer
- Increased drought resistance
- Increased pathogen resistance
- Increased plant health and stress tolerance
- Higher transplantation success

Types of mycorrhizae

The classification of mycorrhizal is based on the type of relationship between fungi and plant to the state of communication between root cells with fungus mycelium. Mycorrhizas are commonly divided into ectomycorrhizas and endomycorrhizas. Endomycorrhizal fungi (arbuscular mycorrhizal fungi) form relationships with over 90% of plants (including turf grasses). Ectomycorrhizal fungi form relationships with only about 2% of plants, but some of them are quite common. 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. Ectomycorrhizae is an association that takes place at the surface of the roots. Endomycorrhizal fungi penetrate into the root cortex and form arbuscules within the root cells. They only can reproduce themselves when in presence of a host plants.

Endomycorrhizae

In endomycorrhizae the fungal structure is almost entirely within the host roots. Endomycorrhizas are variable are classified as arbuscular, ericoid, arbutoid, monotropoid, and orchid mycorrhiza.

1. Arbuscular mycorrhiza

Arbuscular mycorrhizas, or AM (formerly known as vesicular-arbuscular mycorrhizas, or VAM), are mycorrhizas whose hyphae enter into the plant cells, producing structures that are either balloon-like (vesicles) or dichotomously branching invaginations (arbuscules). 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. Arbuscular mycorrhizas are found in 85% of all plant families, and occur in many crop species. The hyphae of arbuscular mycorrhizal fungi produce the glycoprotein glomalin, which may be one of the major stores of carbon in the soil.

2. Ericoid mycorrhiza

The ericoid mycorrhiza is a mutualistic symbiosis formed between members of the plant family Ericaceae and several lineages of fungi. The symbiosis represents an important adaptation to acidic and nutrient poor soils and form symbiosis with several crops and ornamental species. Inoculaton eith ercoid mycorrhizae fungi can influence plant growth and nutrient uptake.

3. Arbutoid mycorrhiza

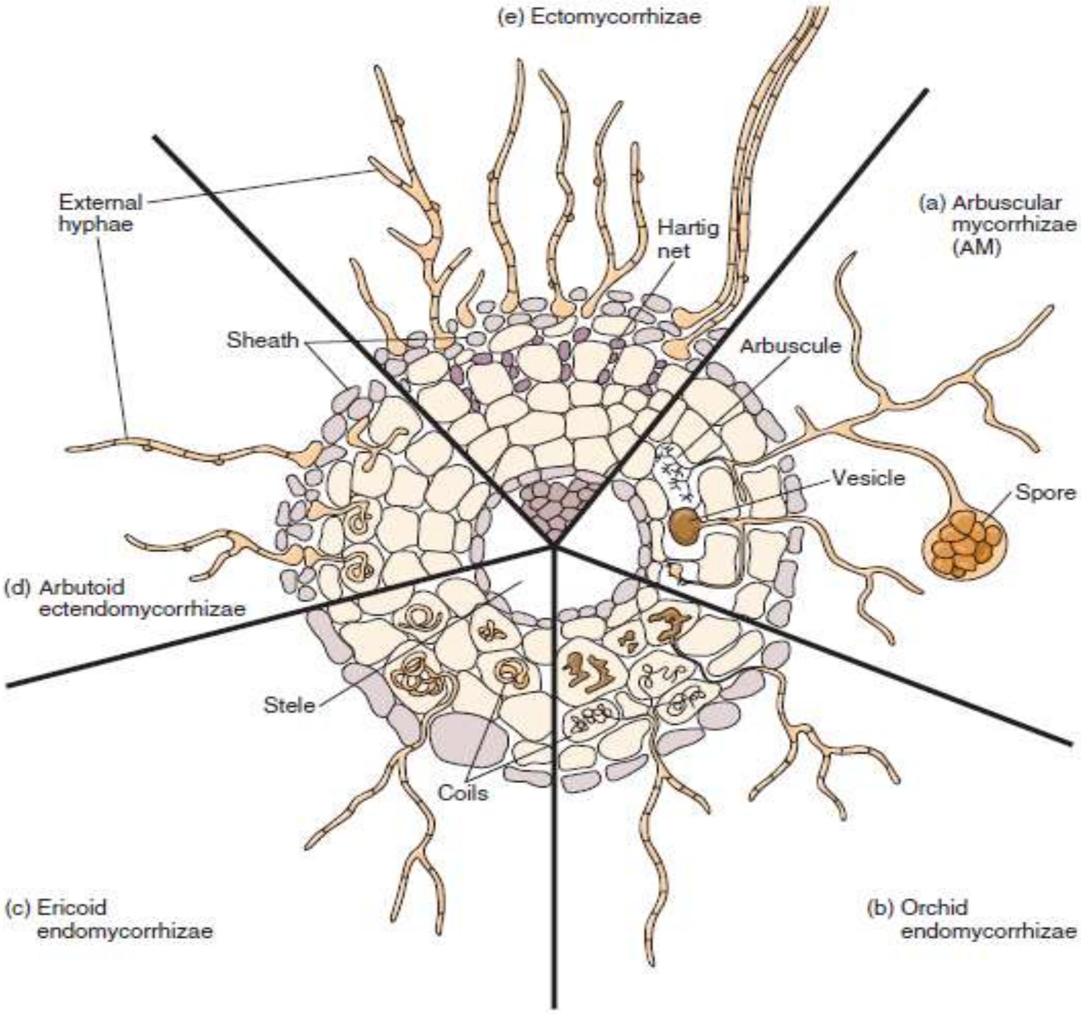
This type of mycorrhiza involves plants of the Ericaceae subfamily Arbutoideae. It is however different from ericoid mycorrhizae and resembles ectomycorrhiza, both functionally and in terms of the fungi involved. The difference to ectomycorrhiza is that some hyphae actually penetrate into the root cells, making this type of mycorrhiza an ectendomycorrhiza.

4. Monotropoid mycorrhiza

This type of of mycorrhiza involves plants of the Ericaceae subfamily Monotropideae. These plants are heterotrophic or mixotrophic and derive carbon from the fungus partner. This is thus a non mutualistic, parasitic type of mycorrhizal symbiosis.

5. Orchid mycorrhiza

All orchids are myco-heterotrophic at some stage during their lifecycle aand form orchid mycorrhiza with a range of basidiomycete fungi. Their hyphae penetrate into the root cells and form typical coils.



Ectomycorrhiza

These fungi are of the class Basimycetes and some are from Ascomycetes and few imperfect funguses and only of type of Zygomycetes called Andogon. These fungus do not enter into root cells and are referred to as Ecto (external). Through the space between the root skin cells the rows of this fungi provide a dense network called the Hartic network for exchange of metabolites with the host plant. In addition by forming a rather thick layer of sheath or a pod on the surface of short and feeder roots, which often by changing the color, the shape of the roots follows frequent branches of two or more. Detection of Ectomycorrhiza is easily done through morphological changes of the root sheath. The plant families, mostly woody plants including the birch, dipterocarp, eucalyptus, oak, pine, and rose families, orchids. An individual tree may have 15 or more different fungal EcM partners at one time. Thousands of ectomycorrhizal fungal species exist, hosted in over 200 genera. 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. Nutrients can be shown to move between different plants through the fungal network. When compared to non-mycorrhizal fine roots, ectomycorrhizae may contain very high concentrations of trace elements, including toxic metals (cadmium, silver) or chlorine.

Mycorrhizal inoculum

Mycorrhizal inoculum is a fungus that forms a symbiotic relationship with the roots of most plants. An **inoculum** is essentially an inoculation, so mycorrhizal inoculum is an inoculation of the roots with a beneficial fungi.

<u>The Importance of Mycorrhizal Fungi</u>

Mycorrhizal fungi form relationships with over 95% of plant species. They surround and even enter the roots of these plants, and provide nutrients such as phosphorus (and even nitrogen) and water to plants in exchange for carbohydrates, usually sugars. In fact, some plants may trade more than 50% of their carbohydrates with these fungi and other microbes in exchange for the vital role soil microorganisms play in the soil including:

- Making nutrients plant ready
- Producing optimized growing conditions
- Significantly improve soil characteristics and quality
- Increasing water availability

In soil that has recently been tilled/worked, compacted, water logged, or treated with chemicals, mycorrhizae will be lacking, unfortunately in this day and age these types of soils are very common. They are not present in imported topsoil or potting soil mix, either, and they cannot be multiplied in compost. In any of these situations, they need to be added back to the soil because they are essential to optimum plant growth and health and should always be used whenever planting or seeding.

Mass production of VAM

The AM fungi are not host specific, any plant species can be infected by an AM fungal species but the degree of AM infection and its effect can differ with different host endophyte combinations.

Cultures of AM fungi on plants growing in disinfected soil have been frequently used technique to increase propagule numbers. A highly susceptible host plant should be used. It should produce abundant roots quickly and tolerate the high-light conditions required for the fungus to reproduce rapidly. Trap plants should be screened to ensure that maximum levels of inoculums were achieved. Large quantities of the inoculum can be produced by pot culture technique. Plants with mycorrhizal associations predominate in most natural eco systems, so inoculum of mycorrhizal fungi is present in most soils. The quantity of inoculum of AM fungi were compatible with a host plant in soils can be measured by bioassay experiments. In these experiments, seedlings were grown in intact soil cores or mixed soil samples for sufficient time to allow mycorrhizas to form, and then roots were sampled, processed and assessed to measure mycorrhiza formation investigated high level of root colonization in drought stressed plants. Attempts have been made to use the genetic variability in fungal efficiency and host response to select AM fungal isolates to improve plant production. Variation in the effect of AM colonization has also been linked with genotype of host plant.

Unit – IV
Possible Questions

Two Marks

1. What are mycorrhizae?
2. What is mycorrhizal inoculum?
3. Write any two importances of mycorrhizal fungi.
4. What is arbuscular mycorrhiza?
5. What is heterokaryosis?
6. What are the types of endomycorrhiza?
7. On which basis mycorrhizae are classified?

Eight Marks

1. What is mycorrhizal inoculum and write the importance of mycorrhizal fungi?
2. Explain the types of mycorrhizae.
3. What are mycorrhizae? Write a note on their interaction with plants.
4. Write a short note on ectomycorrhizae and endomycorrhizae.

UNIT - IV	OPTION 1
The symbiotic relationship between funig and higher plants are called	Lichen
The advantage of fungus in this association is ______	Food
In mycorrhiza, the fungus may form colonies___________	Extracellularly & Intracellu
The advantage of plants in this association is	Food
The ectomycorrhizas are commonly formed in _____	Herbaceous plants
The endomycorrhizas are also called as __________	Hartig nets
The ectomycorrhizas form an intracellular network in root cortex calle	Arbuscular
The characteristic feature of VAM is it penetrates plant cell wall and f	Spores intracellularly
The major advantages of a plant with VAM is _________	Increased N2 absorption
The fungal partner in ectomycorrhiza belongs to the class_______	Basidiomycetes
The endomycorrhizal association is present in ______	10% of plant families
The endomycorrhizas are also called as __________	Hartig nets
The endomycorrhizae are found in _______	Grains
The ectomycorrhizal association are found in ______	10% of plant families
Mycorrhizae play an important role in __________	Soil biology
Mycorrhizae are present in _______ % of plant families	92
Mycorrhizae are divided into _________ types	Three
___________ mycorrhizae form a special category	Orchid
Arbuscular mycorrhizae are mycotthizae whose hyphae enter into the	Oval
The fungal hyphae do not penetrate the __________ in mycorrhizae	Outer membrane
Fossil evidence and DNA sequence analysis suggest that this mutalism	300-400
The hyphae of arbuscular mycorrhizal fungi produce the glycoprotein	Carbon
Ectomycorrhizae are typically formed between the roots of around __	20
Ectomycorrhizae are mostly found in __________ region of the plants	Root
Thousands of ectomycorrhizal fungal fungal species exist, hosted in o	100
Ectomycorrhizae covering the ______ tip consist of a hyphal sheath o	Stem
The ectomycorrhizal fungus Laccaria bicolor has been found to kill sp	Nitrogen
Association of fungi with the roots of plants have been known since th	19
The symbiosis was described by _____	Albert Bernhard Frank
Who introduced the term mycorhizae?	Albert Bernhard Frank
The term mycorrhizae refers to the role of the fungi in the plant _____	Leaf
_________ forest has indicated that mycorrhizal fungi and plants have a relationship that may be	
The mycorrhizal mutualistic association provides rhe fungus with rela	Carbohydrates
Immobilization occurs in soil with high clay content or with a strongly	Acidic
Many plants are able to obtain ______ without using soil as a source	Sulphur
_________ has been shown to be move from birch tree into fir trees th	Carbon
___________ mycorrhizae are foung in inhospitable environments	Arbuscular
The fungi involved in symbiotic relationship are __________	Glomeromycota
_______ mycorrhizae are found in the plant genera Arctostaphylos an	Eriocoid

____________ fungi produce no chlorophyll	Monotropoid
Monotropoid mycorrhizae are most commonly found in ___________	Boreal
The life cycle of _____ mycorrhizae go through a period of time wher	Ardutoid
The hyphae penetrate the cells of the embryo from hyphael coils calle	Root hairs
Arbuscular mycorrhizae belong to the phylum __________	Zygomycota
________ fungi help to capture nutrients such as phosphorous and mi	Arbutoid
____________ forest large amounts of phosphate and other nutrients	Coniferous
The fungal hyphae of Arbutoid mycorrhizae form a structure known a	Hartig net
_______ tree root system is used to prevent the rain from washing ph	Inga
The red pigment present in the root nodule is __________	Leg haemoglobin
Nitrogen fixation in rice occurs due to presence of ___________	Nostoc
The medium for the growth of Rhizobium is __________	Yeast extract mannitol aga
Except Rhizobium, which on of the following bacteria forms nitrogen fixing nodules in plants	Actinorhiza
The root nodule of legumes contain pink pigment which has high affi	nod haemoglobin
Azolla as biofertilizer increase the yield of rice fields by	10%
Which one is green manure/biofertilizer?	Sesbania

EDUCATION
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OPTION 2	OPTION 3	OPTION 4	ANSWER KEY
Mycorrhiza	Helotism	Mutualism	Mycorrhiza
Protection	Mineral absorption	Water	Food
Intracellularly	Depends on conditions		Extracellularly & Intr
Protection	Increased mineral absorption and disease protection		Increased mineral abs
Woody plants	All plants	Grasses	Woody plants
Mat forming mycorrh	Vesicular arbuscular my	Intracellular mycorrhizas	Vesicular arbuscular
Vescicles	Hartig net	Haustoria	Hartig net
Vesicles and dichotor	Haustoria	Massive spore forming structures intracellularly	
Increased P absorptio	Increased K absorption	Increased Mn absorption	Increased P absorptio
Ascomycetes	Zygomycees	Every three groups	Every three groups
40% of plant families	85% of plant families	Less than 5% of plant families	85% of plant families
Mat forming mycorrh	Vesicular arbuscular my	Intracellular mycorrhizas	Vesicular arbuscular
Paddy	Rice	Woody plants and grasses	Woody plants and gra
40% of plant families	85% of plant families	Less than 5% of plant families	10% of plant families
Food Microbiology	Environment	Agriculture	Soil biology
88	96	90	92
Four	Two	Five	Two
Ericoid	Arbuscular	Monotropoid	Monotropoid
Balloon	Mucoid	Spherical	Balloon
Cytoplasm	Protoplast	Periplasm	Protoplast
400-440	350-400	400-460	400-460
Nitrogen	Metals	pH	Carbon
5	10	30	10
Wood	Flower	Stem	Wood
200	500	300	200
Leaf	Fruit	Root	Root
Carbon	Phosphorous	Sulfur	Nitrogen
17	18	16	19
Gaind	Nautiyal	Franciszek Kamieriski	Franciszek Kamierisk
Gaind	Nautiyal	Gaur	Albert Bernhard Fran
Stem	Root	Rhizosphere	Rhizosphere
Coniferous	Evergreen	Tidal	Boreal
Proteins	Lipids	Fats	Carbohydrates
Basic	Low acidic	Neutral	Basic
Phosphate	Nitrogen	Carbon	Phosphate
Nitrogen	Phosphorous	Sulphur	Carbon
Eriocoid	Ardutoid	Monotropoid	Eriocoid
Zygomycota	Ascomycota	Deutromycota	Ascomycota
Monotropoid	Arbuscular	Arbutoid	Arbutoid

Arbutoid	Eriocoid	Arbuscular	Monotropoid
Evergreen	Coniferous	Tidal	Coniferous
Orchid	Monotropoid	Ericoid	Orchid
Hartig net	Fungal peg	Pelotons	Pelotons
Glomeromycota	Ascomycota	Deutromycota	Glomeromycota
Monotropoid	Eriocoid	Arbucsular	Arbucsular
Dystrophic	Boreal	Tidal	Dystrophic
Root hairs	Pelotons	Fungal peg	Hartig net
Mango	Neem	Palm	Inga
Haemoglobin	Iron	Protein	Leg haemoglobin
Azolla	Anabena	Rhizobium	Anabena
Rose bengal agar	Nutrient agar	Malt extract agar	
			Yeast extract mannit
Burholderia	Micrococcus	Pseudomonas	
			Burholderia
leghaemoglobin	haemoglobin	bacterial haemoglobin	leghaemoglobin
20%	30%	50%	50%
Rice	Oat	Maize	Sesbania

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UNIT – 5

BIOPESTICIDES

Biopesticides

Biopesticides is a broad term and includes bioinsecticides, biofungicides, bioherbicides and bionematicides. Microorganisms belonging to different groups like bacteria, fungi and viruses are used as biopesticides (which can be used to kill a susceptible insect). Biopesticides are an important group of pesticides that can reduce pesticide risks. They are derived from animals, plants and microorganisms such as bacteria and viruses. The advantages of biopesticides are:

- They are inherently less harmful than chemical pesticides.
- They, in general, have a narrow target range and a very specific mode of action.
- They are often effective in small quantities. Also, they decompose quickly and do not leave problematic residues.
- They are safer to humans and the environment than conventional pesticides.

Bacterial - *Bacillus thuringiensis*

Bacteria belonging to genus *Bacillus* are potent against many insect pests. They suppress pests by producing a toxin specific to the pest; causing a disease; preventing establishment of other microorganisms through competition; or other modes of action. An example of a bacterial pesticide is *Bacillus thuringiensis*, or "Bt".

Bacillus thuringiensis (Bt) is a ubiquitous gram-positive, spore forming bacterium which produces parasporal crystals during sporulation (stationary phase of its growth cycle). *Bacillus thuringiensis* is a naturally occurring soil bacterium that is toxic to the larvae of several species of insects but not toxic to non-target organisms. It is primarily a pathogen of lepidopterpous pests that are some of the most damaging. These include American bollworm in cotton and stem borers in rice. These crystals are predominantly comprised of d-endotoxins or insecticidal crystal proteins (ICPs), known to possess insecticidal activity when ingested by certain insects. *Bacillus thuringiensis* can be applied to plant foliage or incorporated into the genetic material of crops. *Bacillus thuringiensis*, as discovered, is toxic to the caterpillars (larvae) of moths and butterflies. Several strains of Bt have been developed and now strains are available that control fly larvae. These can be used in controlling mosquitoes and blackflies.

The mode of action of Bt involves the following stages:

- Ingestion of sporulated Bt and ICP by an insect larva.
- Solubilization of the crystalline ICP in the midgut: When Bt crystals are ingested by insects, the crystal proteins are dissolved from the crystals. The pH in the gut of lepidopteran larvae varies between 9 and 12 and the lepidopteranspecific crystal bodies can only be solubilized above pH9.5. On getting solubilized in the midgut, the crystalline bodies release the protein called dendotoxins.
- Activation of the ICP by midgut proteases: The crystalline protoxins are inactive, until they are hydrolysed by the gut proteases. The proteases cleave amino acids from both C-terminus and N-terminus of the protoxin and thus form the active toxin.
- Binding of the activated ICP to specific receptors in the midgut cell membrane: Brush border membrane vesicles (BBMVs) are the primary binding site for several insect species. The active toxins initially bind reversibly to the specific receptors located on the apical brush border membrane of the columnar cells.
- Insertion of the toxin in the cell membrane and formation of pores and channels in the gut cell membrane, followed by destruction of the epithelial cells: After binding to the receptor, the toxin inserts irreversibly into the plasma membrane of the cell. The formation of toxin induced pores in the columnar cell of apical membranes allows rapid fluxes of ions. The disruption of the gut integerity leads to the death of the insect through starvation or septicemia.
- Subsequent Bt spore germination and septicemia may enhance mortality.

For biopesticide applications, the Bt protein is usually used in a formulation containing the spores and crystalline inclusions that are released upon lysis of Bt cells during growth. The molecular potency of the toxin is 300 times greater than synthetic pyrethroids, and the toxin breaks down quickly when exposed to ultraviolet light/sunlight.

Viruses

Viruses that are pathogenic for specific insects include nuclear polyhedrosis viruses (NPVs), granulosis viruses (GVs), and cytoplasmic polyhedrosis viruses (CPVs). Currently over 125 types of NPVs are known, of which approximately 90% affect the *Lepidoptera*—butterflies and moths. Approximately 50 GVs are known, and they, too, primarily affect butterflies and moths. CPVs are the least hostspecific viruses, affecting about 200 different types of insects. An important commercial viral pesticide is marketed under the trade name Elcar for control of the cotton bollworm *Heliothis zea*. One of the most exciting advances involves the use of baculoviruses that have been genetically modified to produce a potent scorpion toxin active against insect larvae. After ingestion by the larvae, viruses are dissolved in the midgut and are released. Because the recombinant baculovirus produces this insect selective neurotoxin, it acts more rapidly than the parent virus, and leaf damage by insects is markedly decreased.

Insect Viruses

Members of at least seven virus families (*Baculoviridae, Iridoviridae, Poxviridae, Reoviridae, Parvoviridae, Picornaviridae*, and *Rhabdoviridae*) are known to infect insects and reproduce or even use them as the primary host. Of these, probably the three most important are the *Baculoviridae, Reoviridae,* and *Iridoviridae.*

The *Iridoviridae* are icosahedral viruses with lipid in their capsids and a linear double-stranded DNA genome. They are responsible for the iridescent virus diseases of the crane fly and some beetles. The group's name comes from the observation that larvae of infected insects can have an iridescent coloration due to the presence of crystallized virions in their fat bodies. Many insect virus infections are accompanied by the formation of inclusion bodies within the infected cells. Granulosis viruses form granular protein inclusions, usually in the cytoplasm. Nuclear polyhedrosis and cytoplasmic polyhedrosis virus infections produce polyhedral inclusion bodies in the nucleus or the cytoplasm of affected cells. Although all three types of viruses generate inclusion bodies, they belong to two distinctly different families. The cytoplasmic polyhedrosis viruses are reo-viruses; they are icosahedral with double shells and have double-stranded RNA genomes. Nuclear polyhedrosis viruses and granulosis viruses are baculoviruses—rod-shaped, enveloped viruses of helical symmetry and with double-stranded DNA. The inclusion bodies, both polyhedral and granular, are protein in nature and enclose one or more virions. Insect larvae are infected when they feed on leaves contaminated with inclusion bodies. Polyhedral bodies protect the virions against heat, low pH, and many chemicals; the viruses can remain viable in the soil for years. However, when exposed to alkaline insect gut contents, the inclusion bodies dissolve to liberate the virions, which then infect midgut cells. Some viruses remain in the midgut while others spread throughout the insect. Just as with bacterial and vertebrate viruses, insect viruses can persist in a latent state within the host for generations while producing no disease symptoms. A reappearance of the disease may be induced by chemicals, thermal shock, or even a change in the insect's diet.

Baculoviruses have received the most attention for at least three reasons. First, they attack only invertebrates and have considerable host specificity; this means that they should be fairly safe for nontarget organisms. Second, because they are encased in protective inclusion bodies, these viruses have a good shelf life and better viability when dispersed in the environment. Finally, they are well suited for commercial production since they often reach extremely high concentrations in larval tissue (as high as 1010 viruses per larva). The granulosis virus of the codling moth also is useful. Usually inclusion bodies are sprayed on foliage consumed by the target insects. More sensitive viruses are administered by releasing infected insects to spread the disease. As in the case of other pesticides, it is possible that resistance to these agents may develop in the future.

Unit – V

Possible Questions

Two Marks

1. Define pesticide.
2. Write the advantages of pesticides?
3. What are the types of pesticides based on microorganisms?
4. Mention the stages in mode of action of *Bacillus thuringiensis.*
5. What are the types of viral pesticides?
6. Give examples of bacterial pesticides.

Eight Marks

1. Briefly describe how the *Bacillus thuringiensis* toxin kills insects.
2. What types of viruses are being used to attempt to control insects?
3. What two important bacteria have been used as bioinsecticides?
4. What is biopesticides? Write the mode of action of *Bacillus thuringiensis*?
5. Give a brief note on *Bacillus thuringiensis*

UNIT - V

______ is organic matter, mostly derived from animal waste/feces
______ is the used for seed treatment of groundnut
_______ are best phosphate mobilizers
_______ is a biocontrol agent
________ are rich in beneficial microorganisms that enrich the nutrient quality of soil
________ is a best biofertilizer used in paddy fields
________ is a form of agriculture that relies on techniques such as crop rotation, green n
Fossil evidence and DNA sequence analysis suggest that this mutalism appeared ______
________ is the biological oxidation of ammonia
_________ can be used with crops like wheat, maize,mustard, cotton, potato and other v
_________ is a plant growth promoting bacteria found naturally in soil
A carrier used in preparation of biofertilizers
A fertilizer consisting of growing plants that are ploughed back into the soil
Chemoautotrophic involved in nitrification
Cyanobacteria are
Denitrification is a microbially facilitated process of
Denitrifying bacteria
Foliar spray is
Indole acetic acid and gibberelins are
Liquid extract of composting by earthworms
Majority of atmospheric nitrogen is obtained from
Phyllosphere refers to
Rhizobacteria are bacteria growing in & around ______ of plants
VAM is
Which are important nutrients for plant growth in soil?
Which bacteria is used as biofertilizer in sugarcane crop?
Which forms symbiotic relation with higher plants?
Expect Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants?
Rhizobium has symbiotic association with_______
Which of the following is not the biofertilisers producing bacteria?
Which of the following is capable of oxidising sulfur to sulfates?
Azolla is used as biofertilizer as it has _______
The most quickly available source of nitrogen to plants are______
Most effective pesticide is _______
Which is true for DDT
Which is major component of bordeaux mixture?

Which one is correctly matched
IPM stands for
Which is major component of bordeaux mixture?
Insecticides generally attack
Organisms associated with sorghum and cotton which provide nutrition to them are

Azolla as biofertilizer, increase the yield of rice fields by ________
Denitrification is __________
Which of the following soil microorganism is involved in the reduction of sulfates to
Which one of the following structure is formed in plant roots by mycorrhizae
Except Rhizobium, which one of the following bacteria forms nitrogen fixing nodules ir
Which one of the following genes is responsible for nod factor in bacteria
In which one of the following relationship one partner benefits but the other is neither hurt nor helpless
The proteinaceous compounds ae converted to ammonia in the presence of which one of the following bacteria
In soil, which one of the following bacterial genera is responsible for degradation of cellulose
Which one of the following compound is known as the most resisant to microbial degradation during organic matter decomposition
Soil microorganisms influence above ground ecosystems by contributing to except which one of the following
Mycorrizha is a symbiotic association between a fungus and the roots of a vascular plant
Denitrification is done only by microorganisms, usually by which one of the following
The plant disease control agents inclde to which one of the following microorganism, ex
In plants, the strains of which one of the following bacterium initiates to the formation o
In 1888, a dutch microbiologist Beijerinck succeeded in isolating which one of the follo
Ammonia produced in the bacteriod needs to be transported to the plants through which
Bacillus thuringiensis produce _________

ːADEMY OF HIGHER EDUCATION
MENT OF MICROBIOLOGY
ꓥND BIOFERTILIZERS (16MBU404A)

OPTION 1	OPTION 2	OPTION 3	OPTION 4
Biomanure	Fertilizer	Potash	NPK
Azospirillum	*Azotobacter*	*Rhizobium*	*Nostoc*
Mycorrhizae	*Bacillus*	*Citrobacter*	*Candida*
Bacillus polymyxa	*Azospirillum*	*Trichoderma viridc*	*Aspergillus flavus*
Biofertilizers	Humus	NPK	Vermicompost
Bradyrhizobium	*Azospirillum*	*Anabaena*	*Frankia*
Terrestial farming	Hill farming	Inorganic farming	Organic farming
300-400	400-440	350-400	400-460
Oxidation	Nitrification	Denitrification	Reduction
Anabaena	*Azotobacter*	*Rhizobium*	*Mycorrhizae*
Pseudomonas aerug	*Staphylococcus aur*	*Pseudomonas fluor*	*Aspergillus fumigatus*
Rubber	Peat	Plastic	Soil
Green manure	Vermicompost	Biomanure	Organic fertilizer
Alcaligenes	*Fusarium*	*Nitrosomonas*	*Arthrobacter*
Photoheterotrophs	Chemotrophs	Prototrophs	Photoautotrophs
Nitrate degradation	Nitrate assimilation	Nitrate oxidation	Nitrate reduction
Thiobacillusdenitrifi	*Bacillus*	*Aspergillus*	*Micrococcus denitrificans*
Spraying on roots	Spraying on Stem	Spraying on leaves	Spraying on Flowers
Hormones of bacteri	Hormones that retai	Plant growth hormo	Weedicides
Vermiwash	Germiwash	Wormiwash	Liquidwash
Fossil fuel	Hospital waste	Domestic waste	Industrial waste
Surface of roots	Surface of leaves	Surface of Stem	Surface of flowers
Leaf	Root	Stem	Fruit
Ventricular arbuscul	Vesicular augument	Vesicular arbuscula	Vesicular arbuscular mycobacteriu
Nitrogen	Phosphorous	NPK	Potassium
Beijerinckia	*Acetobacter diazotr*	*Bacillus*	*Pseudomonas*
Aspergillus fumigatu	*Bradyrhizobium*	*Pseudomonas fluor*	*Mycorrhizae*
Actinorhiza	Burholderia	Micrococcus	Pseudomonas
Legumes	non-legume crop	sugarcane	paddy
Nostoc	Anabena	Both a and	Clostridium
Thiobacillus	Desulfotomaculum	Rhodospirillium	Rhodomicrobium
Rizobium	Cyanobacteria	Mycorrhiza	Large quantity of
amide fertilizers	ammonia	nitrate fertilizers	ammonia nitrate
carbamates	organophosphates	organochlorines	phosphates
not a pollutant	an antibiotic	an antiseptic agent	a non degradable
copper sulphate	sodium chloride	calcium chloride	magnesium

carbamates- integrated plant	organophosphates- integrated plant	carbamates- integrated plant	organochloride- integrated pest
copper sulphate	sodium chloride	calcium chloride	magnesium
respiratory system	muscular system	nervus system	circulatory system
Azospirillum, Azoto	Azotobacter, Azospirillum	Anabena, Rhizobium	Rhizobium, Azotobacter
10%	20%	30%	50%
reduction of nitrate	reduction of nitrate	both a and b	reduction of
Thiobacillus	Desulfotomaculum	Rhodospirillium	Rhodomicrobium
Arbuscles	Hartig net	Haustoria	Rhizomorph
Actinorhiza	Burholderia	Micrococcus	Pseudomonas
fix gene	gag gene	nif gene	nol gene
Amensalism	Commensalism	Parasitization	Predation
Ammonifying bacteria	Denitrifying bacteria	Nitrifying bacteria	Putrefying bacteria
Escherichia	Pseudomonas	Salmonella	Staphylococcus
cellulose	chitin	hemicellulose	lignin
plant nutrition and health	soil fertility	soil structure	soil texture
Crick	Fisher	Frank	Funk
Facultative anaerobe	obligate aerobe	phototrophic aerob	Microaerophilic
Ampelomyces quisq	Bacillus subtilis	Trichoderme sp.	Bacillus anthrax
Agrobacterium	Rhizobium	Pseudomonas	Ralstonia
Bradyrhizobium jap	Rhizobium legumin	Sinorhizobium mel	Azolla
lipid membrane	periplasmic membr	symbiosome memb	plasma membrane
Insecticidal protein	Nematocidal protei	Funigicidal Protein	Bactericidal protein

ANSWER KEY

Biomanure
Rhizobium
Mycorrhizae
Trichoderma viridae
Biofertilizers
Both b and c
Organic farming
400-460
Nitrification
Azotobacter
Pseudomonas fluorescens
Peat
Green manure
Nitrosomonas
Photoautotrophs
Nitrate reduction
Both I & IV
Spraying on leaves
Plant growth hormones
Vermiwash
Both a and c
Surface of leaves
Root
Vesicular arbuscular mycorrhizae
NPK
Acetobacter diazotrophicus
Mycorrhizae
Burholde
ria
legumes
Clostridi
Thiobacil
Cyanobac
amide
carbamat
a non
sodium

organochl
integrated
sodium
muscular system

Azotobacter-Azospirillum
10%
Both a and b
Desulfotomaculum
Hartig net
Burholderia
nol gene
Commen
salism
Ammonif
ying
Pseudom
onas

lignin

soil texture
Frank
Facultative anaerobes
Trichoderma sp.
Agrobacterium
Rhizobium leguminosarum
symbiosome membrane
Insecticidal protein

Reg. No.__________________

[16MBP404A]

KARPAGAM ACADEMY OF HIGHER EDUCATON

Eachanari Post, Coimbatore, Tamil Nadu, India – 641 021

(For candidates admitted from 2009, onwards)

MICROBIOLOGY

B. Sc., DEGREE INTERNAL EXAMINATION, January - 2018

BIOFERTILIZERS AND BIOPESTICIDES

Time: 2 hours **Maximum: 50 marks**

Date:

Part - A (20 X 1 = 20 Marks)

1. A free-living anaerobic photosynthetic bacterium________
a) *Anabaena azollae* b) *Clostridium thermocellum*
c) *Rhodospirillum rubrum* d) *Klebsiella pneumonia*

2. Chemicals produced by the *Rhizobia* called ______ cause the colonized root hairs to curl
a) Pod factors b) Nod factors
c) Sod factors d) Mod factors

3. *Frankia* is a__________
a) Bacteria b) Actinomycete
c) Fungi d) Algae

4. In biological nitrogen fixation, ____ moles of NH_3 are produced from one mole of N_2 gas
a) 2 b) 4
c) 6 d) 8

5. ______ is aerobic and free-living nitrogen nitrogen fixers
a) *Frankia* & *Azospirillum* b) *Clostridium*& *Desulfovibrio*
c) *Beijerinckia* & *Klebsiella* d) *Rhizobium*& *Anabaena*

6. Nitrogenase enzyme consists of
a) Iron protein b) Molybdenum-iron protein
c) Iron protein and a molybdenum-iron protein d) Hemoglobin

7. Symbiotic nitrogen *Cyanobacteria* are present in all except______
a) *Anthoceros* b) *Azolla*
c) *Cycas* d) *Gnetum*

8. All the following are free living nitrogen fixers except________
a) *Rhizobium* b) *Azotobacter*
c) *Rhodospirillum* d) *Clostridium*

9. *Anabena* is a nitrogen fixer present in the root pockets of_______
a) *Marselia* b) *Salvinia*
c) *Pistia* d) *Azolla*

10. All of the following are examples of negative symbiosis________
a) Amensalism b) Competition
c) Commensalism d) Parasitism

11. Which one of the following is nonleguminous?
a) *Casuarina* b) *Bacillus*
c) *Sesbania* d) *Penicillium*

12. Which one is green manure/biofertilizer?
a) Sesbania b) Rice
c) Oat d) Maize

13. Beijerinck discovered aerobic bacteria capable of fixing molecular nitrogen in the year _____

a) 1906 b) 1901

c) 1899 d) 1910

14. *Azotobacter* is _________ shaped bacterium

a) Rod b) Cocci

c) Sprillium d) Comma

15. *Azotobacter chroococcum* grows in ________ soil

a) Acidic b) Neutral

c) Basic d) Neutral and alkaline

16. Homologous selection of a pigmented strain of *Azotobacter* established better on ______

a) Stem b) Leaf

c) Flower d) Roots

17. The optimum pH for the growth of *Azotobacter* is _________

a) 7.2-7.6 b) 7

c) 6 d) 4

18. *Azotobacter* species require __________humidity

a) High b) Low

c) Extreme d) Moderate

19. The multiple action of __________ contributes to better germination percentage of seeds

a) *Azospirillium* b) *Azotobacter*

c) *Azolla* d) *Anabena*

20. *Azotobacter* has ability to produce ________ compounds

a) Antifungal b) Antibacterial

c) Antimalarial d) Antiviral

Part - B Answer all the questions (3 X 2 = 6 Marks)

21. What are free living nitrogen fixers?
22. What are the two microbial conversions that take place in biofertilizer?
23. Define biofertilizer.

Part - C Answer all the questions (3 X 8 = 24 Marks)

24. a) Give an elaborate note on the mass multiplication, field application of *Rhizobium*.

(or)

b) Write about the characteristics and uses of Alder and Casurina.

25. a) Give briefly about *Azotobacter* as a non symbiotic microorganism.

(or)

b) Describe the mass multiplication and field application of *Cyanobacteria*.

26. a) Write in detail the isolation, characterization and mass production of *Azotobacter*.

(or)

b) Describe the mass multiplication and field application of *Azolla*.