Semester – IV

18MBU404A **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

COURSE OBJECTIVES

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

COURSE OUTCOME

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

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, Almus, Casurina plants, non-leguminous crop symbiosis. Cyanobacteria, *Azolla* – Isolation, characterization, mass multiplication, their role in rice cultivation, crop response and field application.Soil nutrients and plant growth

Unit II

Free living *Azospirillum*, *Azotobacter* – isolation, characteristics, mass production and field application. Nitrogen cycle. Zinc solubilizer and potash solubilizing microbes

Unit III

Phosphate potash and zinc solubilizing microbes – Isolation, characterization, mass production, field application. Role of phosphate and zinc in plant growth and yield

Unit IV

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

Unit V

General account of microbes used as bio-insecticides and their advantages over synthetic pesticides, bio nematicide, *Bacillus thuringiensis, Pseudomonas, Bacillus, Streptomyces*- 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.

LECTURE PLAN

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	R1: 10-18 R2: 166-172
2	1	Advantages of biofertilizers over chemical fertilizers	R2: 172
3	1	Symbiotic nitrogen fixers - Rhizobium isolation, characteristics, mass production and field application, legume/pulses plants	R2: 166-173
4	1	Frankia isolation and characteristics, Almus and Casurina, non-leguminous crop symbiosis.	W1, W2
5	1	Cyanobacteria – isolation, characteristics, mass production and field application	R2: 151-165
6	1	Azolla - isolation, characteristics, mass production and their role in rice cultivation, crop response and field application, Soil nutrients and plant growth, Unit Revision	R2:160-163 R2: 15-18, 381
		Total No. of Hours Planned For Unit I=6	

REFERENCES

1. K. V. B. R. Tilak, K.K. Pal & R. Day. 1985. Microbes for sustainable agriculture. I. K. International Publishing Pvt. Ltd., New Delhi
2. Subba Rao, NS. 1995. Soil Microbiology, Fourth Edition, Oxford & IBH Publishing Co. Pvt Ltd., New Delhi

WEBSITES

W1: https//en.m.wikipedia.org//wiki/Frankia
W2: https://en.m.wikipedia.org/wiki/Alnus

LECTURE PLAN

S. No	Lecture Duration Period	Topics to be covered	Support material/Page Nos
		UNIT - II	
S. No	**Duration**	**Topic**	**Support material/Page Nos**
1	1	Free living *Azospirillum*, *Azotobacter*	R2: 166-173
2	1	Azotobacter – isolation, characteristics, mass production, field application	R2: 130-131
3	1	Azospirillum - isolation, characteristics, mass production, field application	R2: 131-135
4	1	Nitrogen cycle	R3: 81-84
5	1	Zinc solubilizer	J1: 51-60
6	1	Potash solubilizing microbes, Unit Revision	J2: 1-41
		Total No. of Hours Planned For Unit II=6	

REFERENCES

1. K. V. B. R. Tilak, K.K. Pal & R. Day. 1985. Microbes for sustainable agriculture. I. K. International Publishing Pvt. Ltd., New Delhi
2. Subba Rao, NS. 1995. Soil Microbiology, Fourth Edition, Oxford & IBH Publishing Co. Pvt Ltd., New Delhi
3. Shukla, R.S.& Chandel, P.S. 1972. Plant Ecology and Soil Science, First Edition. S. Chand & Company Ltd., New Delhi
4. Rengaswami, G & Bagyaraj, D.J. 1993. Agricultural Microbiology, Second Edition. Prentice-Hall of India Pvt. Ltd., New Delhi.

WEBSITES

W1: https//en.m.wikipedia.org//wiki/Frankia
W2: https://en.m.wikipedia.org/wiki/Alnus
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

JOURNALS

J1: Mumtaz, M.Z., Ahmad, M., Jamil, M & Hussain, T (2017). Zinc solubilizing *Bacillus spp.* potential candidate for biofortification in Maize. Microbial Research. 202: 51-60
J2: Chandra, K & Greep, S. (2006). Potash mobilizing bacteria. Regional centre of organic farming press, Bangalore.

J3: Mishra, R.K., Bohra, A., Kamaal, N., Kumar, K., Gandhi, K., Sujayanand, G.K, Saabale, P. R., Naik, S.J., Sarma, B.K., Kumar, D., Mishra, M., Srivastava, D.K., Singh, N.P. 2018. Utilization of biopesticides as sustainable solutions for management of pests in legume crops: achievements and prospects. Egyptian Journal of Biological Pest Control. 28: 3

LECTURE PLAN

S. No	Lecture Duration Period	Topics to be covered	Support material/Page Nos
		UNIT - III	
S. No	**Duration**	**Topic**	**Support material/Page Nos**
1	1	Phosphate, potash and zinc solubilizing microbes	R1: 57-58
2	1	Phosphate solubilizing microbes – isolation, characteristics, mass production, field application	R1: 57-60
3	1	Potash solubilizing microbes – isolation, characteristics, mass production, field application	J2: 1-42
4	1	Zinc solubilizing microbes – isolation, characteristics, mass production, field application	J1:51-60
5	1	Role of phosphate in plant growth and yield, Unit Revision	R2: 293-298
6	1	Role of zinc in plant growth and yield, Unit Revision	J1: 51-60
Total No. of Hours Planned For Unit III=6			

REFERENCES

1. K. V. B. R. Tilak, K.K. Pal & R. Day. 1985. Microbes for sustainable agriculture. I. K. International Publishing Pvt. Ltd., New Delhi
2. Subba Rao, NS. 1995. Soil Microbiology, Fourth Edition, Oxford & IBH Publishing Co. Pvt Ltd., New Delhi
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J2: Chandra, K & Greep, S. (2006). Potash mobilizing bacteria. Regional centre of organic farming press, Bangalore.
J3: Mishra, R.K., Bohra, A., Kamaal, N., Kumar, K., Gandhi, K., Sujayanand, G.K, Saabale, P. R., Naik, S.J., Sarma, B.K., Kumar, D., Mishra, M., Srivastava, D.K., Singh, N.P. 2018. Utilization of biopesticides as sustainable solutions for management of pests in legume crops: achievements and prospects. Egyptian Journal of Biological Pest Control. 28: 3.

LECTURE PLAN

S. No	Lecture Duration Period	Topics to be covered	Support material/Page Nos
		UNIT – IV	
S. No	**Duration**	**Topic**	**Reference Support material/Page Nos**
1	1	Introduction of mycorrhizae	R1: 85
2	1	Importance of mycorrhizal inoculums	R1: 85-87
3	1	Types of mycorrhizae and associated plants	R1: 89
4	1	Mass production of VAM	R1: 84-85
5	1	Field application of ectomycorrhizae and VAM	R1: 84-86
6	1	Entamopathogenic fungi, Unit Revision	R4: 283-306
Total No. of Hours Planned For Unit IV=6			

REFERENCES

1. K. V. B. R. Tilak, K.K. Pal & R. Day. 1985. Microbes for sustainable agriculture. I. K. International Publishing Pvt. Ltd., New Delhi
2. Subba Rao, NS. 1995. Soil Microbiology, Fourth Edition, Oxford & IBH Publishing Co. Pvt Ltd., New Delhi
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sustainable solutions for management of pests in legume crops: achievements and prospects. Egyptian Journal of Biological Pest Control. 28: 3.

LECTURE PLAN

S. No	Lecture Duration Period	Topics to be covered	Support material/Page Nos
		UNIT - V	
S. No	**Duration**	**Topic**	**Support material/Page Nos**
1	1	General account of microbes used as bioinsecticides	W4, R2: 376-380
2	1	Advantages of bioinsecticides over synthetic pesticides	W4
3	1	*Bacillus thuringiensis* – introduction	W5
4	1	*Pseudomonas, Bacillus* – production, field applications	J3: 1-11
5	1	*Streptomyces* – production, field applications	J3: 1-11
6	1	Virus, Cultivation, Field application, Unit Revision	J3: 1-11; W4
Total No. of Hours Planned For Unit V=6			

REFERENCES

1. K. V. B. R. Tilak, K.K. Pal & R. Day. 1985. Microbes for sustainable agriculture. I. K. International Publishing Pvt. Ltd., New Delhi
2. Subba Rao, NS. 1995. Soil Microbiology, Fourth Edition, Oxford & IBH Publishing Co. Pvt Ltd., New Delhi
3. Shukla, R.S.& Chandel, P.S. 1972. Plant Ecology and Soil Science, First Edition. S. Chand & Company Ltd., New Delhi
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Unit I

Biofertilizers

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, Alnus, Casurina plants, non-leguminous crop symbiosis. Cyanobacteria, Azolla – Isolation, characterization, mass multiplication, their role in rice cultivation, crop response and field application. Soil nutrients and plant growth

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 ℃ 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.

Soil nutrients and Plant growth

Soil is a major source of nutrients needed by plants for growth. The three main nutrients are nitrogen (N), phosphorus (P) and potassium (K). Together they make up the trio known as NPK. Other important nutrients are calcium, magnesium and sulfur. Plants also need small quantities of iron, manganese, zinc, copper, boron and molybdenum, known as trace elements because only traces are needed by the plant. The role these nutrients play in plant growth is complex, and this document provides only a brief outline.

Macronutrients

Nitrogen (N)

Nitrogen is a key element in plant growth. It is found in all plant cells, in plant proteins and hormones, and in chlorophyll.

Atmospheric nitrogen is a source of soil nitrogen. Some plants such as legumes fix atmospheric nitrogen in their roots; otherwise fertiliser factories use nitrogen from the air to make ammonium sulfate, ammonium nitrate and urea. When applied to soil, nitrogen is converted to mineral form, nitrate, so that plants can take it up.

Soils high in organic matter such as chocolate soils are generally higher in nitrogen than podzolic soils. Nitrate is easily leached out of soil by heavy rain, resulting in soil acidification. You need to apply nitrogen in small amounts often so that plants use all of it, or in organic form such as composted manure, so that leaching is reduced.

Phosphorus (P)

Phosphorus helps transfer energy from sunlight to plants, stimulates early root and plant growth, and hastens maturity.

Very few Australian soils have enough phosphorus for sustained crop and pasture production and the North Coast is no exception. The most common phosphorus source on the North Coast is superphosphate, made from rock phosphate and sulfuric acid. All manures contain phosphorus; manure from grain-fed animals is a particularly rich source.

Potassium (K)

Potassium increases vigour and disease resistance of plants, helps form and move starches, sugars and oils in plants, and can improve fruit quality.

Potassium is low or deficient on many of the sandier soils of the North Coast. Also, heavy potassium removal can occur on soils used for intensive grazing and intensive horticultural crops (such as bananas and custard apples).Muriate of potash and sulfate of potash are the most common sources of potassium.

Sulfur

Sulfur is a structural component of some amino acids (including cystein and methionine) and vitamins, and is essential for chloroplast growth and function; it is found in the iron-sulfur complexes of the electron transport chains in photosynthesis. It is needed for N_2 fixation by legumes, and the conversion of nitrate into amino acids and then into protein.In plants, sulfur cannot be mobilized from older leaves for new growth, so deficiency symptoms are seen in the youngest tissues first.Symptoms of deficiency include yellowing of leaves and stunted growth.

Calcium

Calcium regulates transport of other nutrients into the plant and is also involved in the activation of certain plant enzymes. Calcium deficiency results in stunting. This nutrient is involved in photosynthesis and plant structure. Blossom end rot is also a result of inadequate calcium.

Another common symptom of calcium deficiency in leaves is the curling of the leaf towards the veins or center of the leaf. Many times this can also have a blackened appearance Calcium has been found to have a positive effect in combating salinity in soils. It has been shown to ameliorate the negative effects that salinity has such as reduced water usage of plants.[24] Calcium in plants occurs chiefly in the leaves, with lower concentrations in seeds, fruits, and roots. A major function is as a constituent of cell walls. When coupled with certain acidic compounds of the jelly-like pectins of the middle lamella, calcium forms an insoluble salt. It is also intimately involved in meristems, and is particularly important in root development, with roles in cell division, cell elongation, and the detoxification of hydrogen ions. Other functions attributed to calcium are; the neutralization of organic acids; inhibition of some potassium-activated ions; and a role in nitrogen absorption. A notable feature of calcium-deficient plants is a defective root system.[12] Roots are usually affected before above-ground parts.

Magnesium

The outstanding role of magnesium in plant nutrition is as a constituent of the chlorophyll molecule. As a carrier, it is also involved in numerous enzyme reactions as an effective activator, in which it is closely associated with energy-supplying phosphorus compounds. Magnesium is very mobile in plants, and, like potassium, when deficient is translocated from older to younger tissues, so that signs of deficiency appear first on the oldest tissues and then spread progressively to younger tissues.

Micro-nutrients

Plants are able sufficiently to accumulate most trace elements. Some plants are sensitive indicators of the chemical environment in which they grow (Dunn 1991), and some plants have barrier mechanisms that exclude or limit the uptake of a particular element or ion species, e.g., alder twigs commonly accumulate molybdenum but not arsenic, whereas the reverse is true of spruce bark (Dunn 1991). Otherwise, a plant can integrate the geochemical signature of the soil mass permeated by its root system together with the contained groundwaters. Sampling is facilitated by the tendency of many elements to accumulate in tissues at the plant's extremities.

Iron

Iron is necessary for photosynthesis and is present as an enzyme cofactor in plants. Iron deficiency can result in interveinal chlorosis and necrosis. Iron is not a structural part of chlorophyll but very much essential for its synthesis. Copper deficiency can be responsible for promoting an iron deficiency. It helps in the electron transport of plant.

Molybdenum

Molybdenum is a cofactor to enzymes important in building amino acids and is involved in nitrogen metabolism. Molybdenum is part of the nitrate reductase enzyme (needed for the reduction of nitrate) and the nitrogenase enzyme (required for biological nitrogen fixation). Reduced productivity as a result

of molybdenum deficiency is usually associated with the reduced activity of one or more of these enzymes.

Boron

Boron is absorbed by plants in the form of the anion BO^{3-} . It is available to plants in moderately soluble mineral forms of Ca, Mg and Na borates and the highly soluble form of organic compounds. It is available to plants over a range of pH, from 5.0 to 7.5. It is mobile in the soil, hence, it is prone to leaching. Leaching removes substantial amounts of boron in sandy soil, but little in fine silt or clay soil. Boron's fixation to those minerals at high pH can render boron unavailable, while low pH frees the fixed boron, leaving it prone to leaching in wet climates. It precipitates with other minerals in the form of borax in which form it was first used over 400 years ago as a soil supplement. Decomposition of organic material causes boron to be deposited in the topmost soil layer. When soil dries it can cause a precipitous drop in the availability of boron to plants as the plants cannot draw nutrients from that desiccated layer. Hence, boron deficiency diseases appear in dry weather.

Boron has many functions within a plant: it affects flowering and fruiting, pollen germination, cell division, and active salt absorption. The metabolism of amino acids and proteins, carbohydrates, calcium, and water are strongly affected by boron. Many of those listed functions may be embodied by its function in moving the highly polar sugars through cell membranes by reducing their polarity and hence the energy needed to pass the sugar. If sugar cannot pass to the fastest growing parts rapidly enough, those parts die.

Boron is not relocatable in the plant via the phloem. It must be supplied to the growing parts via the xylem. Foliar sprays affect only those parts sprayed, which may be insufficient for the fastest growing parts, and is very temporary.

Boron is essential for the proper forming and strengthening of cell walls. Lack of boron results in short thick cells producing stunted fruiting bodies and roots. Calcium to boron ratio must be maintained in a narrow range for normal plant growth. For alfalfa, that calcium to boron ratio must be from 80:1 to 600:1. Boron deficiency appears at 800:1 and higher. Boron levels within plants differ with plant species and range from 2.3 mg/kg for barley to 94.7 mg/kg for poppy. Lack of boron causes failure of calcium metabolism which produces hollow heart in beets and peanuts.

Inadequate amounts of boron affect many agricultural crops, legume forage crops most strongly. Of the micronutrients, boron deficiencies are second most common after zinc. Deficiency results in the death of the terminal growing points and stunted growth.

Boron supplements derive from dry lake bed deposits such as those in Death Valley, USA, in the form of sodium tetraborate (borax), from which less soluble calcium borate is made. Foliar sprays are used on fruit crop trees in soils of high alkalinity. Boron is often applied to fields as a contaminant in other soil amendments but is not generally adequate to make up the rate of loss by cropping. The rates of application of borate to produce an adequate alfalfa crop range from 15 pounds per acre for a sandy-silt, acidic soil of low organic matter, to 60 pounds per acre for a soil with high organic matter, high cation exchange capacity and high pH.

Boron concentration in soil water solution higher than one ppm is toxic to most plants. Toxic concentrations within plants are 10 to 50 ppm for small grains and 200 ppm in boron-tolerant crops

such as sugar beets, rutabaga, cucumbers, and conifers. Toxic soil conditions are generally limited to arid regions or can be caused by underground borax deposits in contact with water or volcanic gases dissolved in percolating water. Application rates should be limited to a few pounds per acre in a test plot to determine if boron is needed generally. Otherwise, testing for boron levels in plant material is required to determine remedies. Excess boron can be removed by irrigation and assisted by application of elemental sulfur to lower the pH and increase boron solubility.

Boron deficiencies can be detected by analysis of plant material to apply a correction before the obvious symptoms appear, after which it is too late to prevent crop loss. Strawberries deficient in boron will produce lumpy fruit; apricots will not blossom or, if they do, will not fruit or will drop their fruit depending on the level of boron deficit. Broadcast of boron supplements is effective and long term; a foliar spray is immediate but must be repeated.

Copper

Copper is important for photosynthesis. Symptoms for copper deficiency include chlorosis. It is involved in many enzyme processes; necessary for proper photosynthesis; involved in the manufacture of lignin (cell walls) and involved in grain production. It is also hard to find in some soil conditions.

Manganese

Manganese is necessary for photosynthesis,[22] including the building of chloroplasts. Manganese deficiency may result in coloration abnormalities, such as discolored spots on the foliage.

Sodium

Sodium is involved in the regeneration of phosphoenolpyruvate in CAM and C4 plants. Sodium can potentially replace potassium's regulation of stomatal opening and closing.

Essentiality of sodium:

- Essential for C4 plants rather C3
- Substitution of K by Na: Plants can be classified into four groups:

    1. Group A—a high proportion of K can be replaced by Na and stimulate the growth, which cannot be achieved by the application of K
    2. Group B—specific growth responses to Na are observed but they are much less distinct
    3. Group C—Only minor substitution is possible and Na has no effect
    4. Group D—No substitution occurs

- Stimulate the growth—increase leaf area and stomata. Improves the water balance
- Na functions in metabolism

    1. C4 metabolism
    2. Impair the conversion of pyruvate to phosphoenol-pyruvate
    3. Reduce the photosystem II activity and ultrastructural changes in mesophyll chloroplast

- Replacing K functions

1. Internal osmoticum
2. Stomatal function
3. Photosynthesis
4. Counteraction in long distance transport
5. Enzyme activation

- Improves the crop quality e.g. improves the taste of carrots by increasing sucrose

Zinc

Zinc is required in a large number of enzymes and plays an essential role in DNA transcription. A typical symptom of zinc deficiency is the stunted growth of leaves, commonly known as "little leaf" and is caused by the oxidative degradation of the growth hormone auxin.

Nickel

In higher plants, nickel is absorbed by plants in the form of Ni^{2+} ion. Nickel is essential for activation of urease, an enzyme involved with nitrogen metabolism that is required to process urea. Without nickel, toxic levels of urea accumulate, leading to the formation of necrotic lesions. In lower plants, nickel activates several enzymes involved in a variety of processes, and can substitute for zinc and iron as a cofactor in some enzymes.

Chlorine

Chlorine, as compounded chloride, is necessary for osmosis and ionic balance; it also plays a role in photosynthesis.

Cobalt

Cobalt has proven to be beneficial to at least some plants although it does not appear to be essential for most species. It has, however, been shown to be essential for nitrogen fixation by the nitrogen-fixing bacteria associated with legumes and other plants.

Aluminium

Aluminum is one of the few elements capable of making soil more acidic. This is achieved by aluminum taking hydroxide ions out of water, leaving hydrogen ions behind. As a result, the soil is more acidic, which makes it unlivable for many plants. Another consequence of aluminum in soils is aluminum toxicity, which inhibits root growth.

- Tea has a high tolerance for aluminum (Al) toxicity and the growth is stimulated by Al application. The possible reason is the prevention of Cu, Mn or P toxicity effects.
- There have been reports that Al may serve as a fungicide against certain types of root rot.

Silicon

Silicon is not considered an essential element for plant growth and development. It is always found in abundance in the environment and hence if needed it is available. It is found in the structures of plants and improves the health of plants.

In plants, silicon has been shown in experiments to strengthen cell walls, improve plant strength, health, and productivity. There have been studies showing evidence of silicon

improving drought and frost resistance, decreasing lodging potential and boosting the plant's natural pest and disease fighting systems. Silicon has also been shown to improve plant vigor and physiology by improving root mass and density, and increasing above ground plant biomass and crop yields.[32] Silicon is currently under consideration by the Association of American Plant Food Control Officials (AAPFCO) for elevation to the status of a "plant beneficial substance".

Vanadium

Vanadium may be required by some plants, but at very low concentrations. It may also be substituting for molybdenum.

Selenium

Selenium is probably not essential for flowering plants, but it can be beneficial; it can stimulate plant growth, improve tolerance of oxidative stress, and increase resistance to pathogens and herbivory.

Selenium is, however, an essential mineral element for animal (including human) nutrition and selenium deficiencies are known to occur when food or animal feed is grown on selenium-deficient soils. The use of inorganic selenium fertilizers can increase selenium concentrations in edible crops and animal diets thereby improving animal health.

UNIT-V

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 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.

PART-C (6 MARKS)

1. Describe the advantage of biofertilizers.
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. Describe the isolation, mass cultivation and field application of *Azolla.*
5. Write the importance of major and minor soil nutrients for plant growth?

UNIT – 2

NON-SYMBIOTIC NITROGEN FIXERS

Free living *Azospirillum*, *Azotobacter* – isolation, characteristics, mass production and field application. Nitrogen cycle. Zinc solubilizer and potash solubilizing microbes

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 °C and 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℃ 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.

Nitrogen cycle

The **nitrogen cycle** is the biogeochemical cycle by which nitrogen is converted into multiple chemical forms as it circulates among atmosphere, terrestrial, and marine ecosystems. The conversion of nitrogen can be carried out through both biological and physical processes. Important processes in the nitrogen cycle include fixation, ammonification, nitrification, and denitrification. The majority of Earth's atmosphere (78%) is atmosphere nitrogen,[16] making it the largest source of nitrogen. However, atmospheric nitrogen has limited availability for biological use, leading to a scarcity of usable nitrogen in many types of ecosystems.

The nitrogen cycle is of particular interest to ecologists because nitrogen availability can affect the rate of key ecosystem processes, including primary production and decomposition. Human activities such as fossil fuel combustion, use of artificial nitrogen fertilizers, and release of nitrogen in wastewater have dramatically altered the global nitrogen cycle.[17][18][19] Human modification of the global nitrogen cycle can negatively affect the natural environment system and also human health.

Processes

Nitrogen is present in the environment in a wide variety of chemical forms including organic nitrogen, ammonium (NH^+_4), nitrite (NO^-_2), nitrate (NO^-_3), nitrous oxide (N_2O), nitric oxide (NO) or inorganic nitrogen gas (N_2). Organic nitrogen may be in the form of a living organism, humus or in the intermediate products of organic matter decomposition. The processes in the nitrogen cycle is to transform nitrogen from one form to another. Many of those processes are carried out by microbes, either in their effort to harvest energy or to accumulate nitrogen in a form needed for their growth. For example, the nitrogenous wastes in animal urine are broken down by nitrifying bacteria in the soil to be used by plants. The diagram alongside shows how these processes fit together to form the nitrogen cycle.

Nitrogen fixation

The conversion of nitrogen gas (N_2) into nitrates and nitrites through atmospheric, industrial and biological processes is called nitrogen fixation. Atmospheric nitrogen must be processed, or "fixed", into a usable form to be taken up by plants. Between 5 and 10 billion kg per year are fixed by lightning strikes, but most fixation is done by free-living or symbiotic bacteria known as diazotrophs. These bacteria have the nitrogenase enzyme that combines gaseous nitrogen with hydrogen to produce ammonia, which is converted by the bacteria into other organic compounds. Most biological nitrogen fixation occurs by the activity of Mo-nitrogenase, found in a wide variety of bacteria and some Archaea. Mo-nitrogenase is a complex two-component enzyme that has multiple metal-containing prosthetic groups. An example of free-living bacteria is *Azotobacter*. Symbiotic nitrogen-fixing bacteria such as *Rhizobium* usually live in the root nodules of legumes (such as peas, alfalfa, and locust trees). Here they form a mutualistic relationship with the plant, producing ammonia in exchange for carbohydrates. Because of this relationship, legumes will often increase the nitrogen content of nitrogen-poor soils. A few non-legumes can also form such symbioses. Today, about 30% of the total fixed nitrogen is produced industrially using the Haber-Bosch process, which uses high temperatures and pressures to convert nitrogen gas and a hydrogen source (natural gas or petroleum) into ammonia.

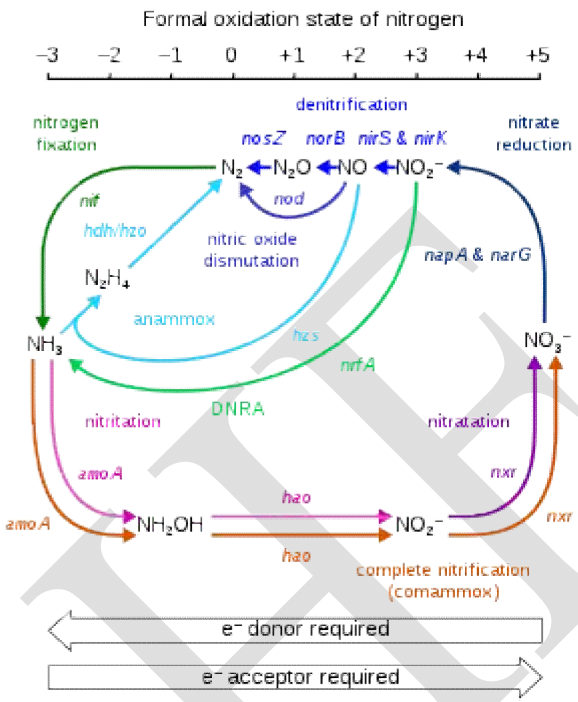
Assimilation

Plants can absorb nitrate or ammonium from the soil by their root hairs. If nitrate is absorbed, it is first reduced to nitrite ions and then ammonium ions for incorporation into amino acids, nucleic acids, and chlorophyll. In plants that have a symbiotic relationship with rhizobia, some nitrogen is assimilated in the form of ammonium ions directly from the nodules. It is now known that there is a more complex cycling of amino acids between *Rhizobia* bacteroids and plants. The plant provides amino acids to the bacteroids so ammonia assimilation is not required and the bacteroids pass amino acids (with the newly fixed nitrogen) back to the plant, thus forming an interdependent relationship. While many animals, fungi, and other heterotrophic organisms obtain nitrogen by ingestion of amino acids, nucleotides, and other small organic molecules, other heterotrophs (including many bacteria) are able to utilize inorganic compounds, such as ammonium as sole N sources. Utilization of various N sources is carefully regulated in all organisms.

Ammonification

When a plant or animal dies or an animal expels waste, the initial form of nitrogen is organic. Bacteria or fungi convert the organic nitrogen within the remains back into ammonium (NH^+_4), a process called ammonification or mineralization. Enzymes involved are:

- GS: Gln Synthetase (Cytosolic & Plastic)
- GOGAT: Glu 2-oxoglutarate aminotransferase (Ferredoxin & NADH-dependent)
- GDH: Glu Dehydrogenase:
  - Minor Role in ammonium assimilation.
  - Important in amino acid catabolism.



A schematic representation of the microbial nitrogen cycle.ANAMMOX is anaerobic ammonium oxidation, DNRA is dissimilatory nitrate reduction to ammonium, and COMMAMOX is complete ammonium oxidation.

Nitrification

The conversion of ammonium to nitrate is performed primarily by soil-living bacteria and other nitrifying bacteria. In the primary stage of nitrification, the oxidation of ammonium (NH^+_4) is performed by bacteria such as the *Nitrosomonas* species, which converts ammonia to nitrites (NO^-_2). Other bacterial species such as *Nitrobacter*, are responsible for the oxidation of the nitrites (NO^-_2) into nitrates (NO^-_3). It is important for the ammonia (NH3) to be converted to nitrates or nitrites because ammonia gas is toxic to plants.

Due to their very high solubility and because soils are highly unable to retain anions, nitrates can enter groundwater. Elevated nitrate in groundwater is a concern for drinking water use because nitrate can interfere with blood-oxygen levels in infants and cause methemoglobinemia or blue-baby syndrome.[28] Where groundwater recharges stream flow, nitrate-enriched groundwater can contribute to eutrophication, a process that leads to high algal population and growth, especially blue-green algal populations. While not directly toxic to fish life, like ammonia, nitrate can have indirect effects on fish if it contributes to this eutrophication. Nitrogen has contributed to severe eutrophication problems in some water bodies. Since 2006, the application of nitrogen fertilizer has been increasingly controlled in

Britain and the United States. This is occurring along the same lines as control of phosphorus fertilizer, restriction of which is normally considered essential to the recovery of eutrophied waterbodies.

Denitrification

Denitrification is the reduction of nitrates back into nitrogen gas (N_2), completing the nitrogen cycle. This process is performed by bacterial species such as *Pseudomonas* and *Paracoccus*, under anaerobic conditions. They use the nitrate as an electron acceptor in the place of oxygen during respiration. These facultatively (meaning optionally) anaerobic bacteria can also live in aerobic conditions. Denitrification happens in anaerobic conditions e.g. waterlogged soils. The denitrifying bacteria use nitrates in the soil to carry out respiration and consequently produce nitrogen gas, which is inert and unavailable to plants.

Dissimilatory nitrate reduction to ammonium

Dissimilatory nitrate reduction to ammonium (DNRA), or nitrate/nitrite ammonification, is an anaerobic respiration process. Microbes which undertake DNRA oxidise organic matter and use nitrate as an electron acceptor, reducing it to nitrite, then ammonium ($NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$). Both denitrifying and nitrate ammonification bacteria will be competing for nitrate in the environment, although DNRA acts to conserve bioavailable nitrogen as soluble ammonium rather than producing dinitrogen gas.

Anaerobic ammonia oxidation

In this biological process, nitrite and ammonia are converted directly into molecular nitrogen (N_2) gas. This process makes up a major proportion of nitrogen conversion in the oceans. The balanced formula for this "anammox" chemical reaction is:

$NH^+_4 + NO^-_2 \rightarrow N_2 + 2H_2O$ ($\Delta G° = -357$ kJ·mol^{-1})

Other processes

Though nitrogen fixation is the primary source of plant-available nitrogen in most ecosystems, in areas with nitrogen-rich bedrock, the breakdown of this rock also serves as a nitrogen source.

Zinc solubilizer

Generally, all macroelements are applied through high-analysis fertilizers. But micronutrients are neglected, not directly involved in yield expansion, and zinc (Zn) is one of them. Zinc (Zn) is a key micronutrient, required for all living forms including plants, humans, and microorganisms for their development. Humans and other living organisms require zinc in their lives in little amounts for proper physiological functions. Zinc is a crucial micronutrient for plants which plays various important functions in their life cycle. The deficiency of zinc in the soil is one of the very common micronutrient deficiencies and results in decreased crop production. Majority of the agricultural soil is either zinc deficient or contains zinc in a fixed form which is unavailable to plants, as a result reflecting zinc deficiency in plants and soils. Therefore, to solve the above problem, there is a requirement for alternative and eco-friendly technology such as plant growth-promoting

rhizobacteria (PGPR) and organic farming practices to enhance zinc solubilization and its availability to plants. Zinc-solubilizing bacteria (Zn-SB) are promising bacteria to use for sustainable agriculture. Zn-SB have various plant growth-promoting (PGP) properties such as Zn solubilization, P solubilization, K solubilization, nitrogen fixation, and production of phytohormones like kinetin, indole-3-acetic acid (IAA), and gibberellic acid, besides production of 1-aminocyclopropane-1-carboxylate (ACC) deaminase and siderophores, hydrogen cyanide, and ammonia. Zn-SB secrete different organic acids that solubilize the fixed form of zinc to available form, which enhances plant growth promotion, yield, and fertility status of the soil.

Potash solubilizing microbes

Potassium (K) is one of the major macronutrients which play an important role in plant growth and development. Total soil potassium reserves are generally large; however, major portion of it exists in insoluble K minerals and very little potassium becomes available to plants. There are certain microorganisms which use a number of biological processes to make potassium available from unavailable forms. These potassium-solubilizing bacteria (KSB) can be used as a promising approach to increase K availability in soils, thus playing an important role for crop establishment under K-limited soils. Owing to naturally available source of potassium in soil and high price of synthetic potassium fertilizers, the importance of KSB is increasing day by day. The use of chemical fertilizers can be decreased by using KSB in agriculture that can lead to sustainable agriculture. A number of workers have demonstrated the role of KSB in crop improvement.

Unit – II

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 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.

PART-C (6 MARKS)

1. Give brief note on nitrogen cycle
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 – 3

PHOSPHATE SOLUBILIZERS

Phosphate potash and zinc solubilizing microbes – Isolation, characterization, mass production, field application. Role of phosphate and zinc in plant growth and yield

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.

Potassium solubilizing microbes

Potassium (K) is considered as an essential nutrient and a major constituent within all living cells. Naturally, soils contain K in larger amounts than any other nutrients; however most of the K is unavailable for plant uptake. Application of chemical fertilizers has a considerably negative impact on environmental sustainability. It is known that potassium solubilizing bacteria (KSB) can solubilize K-bearing minerals and convert the insoluble K to soluble forms of K available to plant uptake. Many bacteria such as *Acidothiobacillus ferrooxidans, Paenibacillus* spp., *Bacillus mucilaginosus, B. edaphicus,* and *B. circulans* have capacity to solubilize K minerals (e.g., biotite, feldspar, illite, muscovite, orthoclase, and mica). KSB are usually present in all soils, although their number, diversity and ability for K solubilization vary depending upon the soil and climatic conditions. KSB can dissolve silicate minerals and release K through the production of organic and inorganic acids, acidolysis, polysaccharides, complexolysis, chelation, and exchange reactions. Hence, the production and management of biological fertilizers containing KSB can be an effective alternative to chemical fertilizers.

Feeding a growing world population, as projected to reach 9 billion by 2050, adopting more efficient and sustainable production methods, responding to increased concerns about managing the natural resources, and adapting to climate change and drought conditions in several developing regions (notably in Europe, Central Asia and the Horn of Africa) are some of the significant challenges that agriculture will face in the 21st century.

In order to feed the increasing world population, agriculture must be intensive and sustainable in the future. However, it is well known that the food production by agriculture cannot be generally sustained unless the nutrients removed from soil as a result of increased crop production are replaced. Many agricultural soils lack a sufficient amount of one or more of essential plant nutrients so that plant growth is suboptimal. To obviate this problem and obtain higher plant yields, farmers have become increasingly dependent on chemical sources of fertilizers. While the chemical fertilizers helped plant grow, they did not improve the properties of soil. It is well known that the constant use of chemical fertilizers, mainly phosphorous, nitrogenous, and potassic fertilizers have harmful effects on the environment.

After nitrogen (N) and phosphorus (P), potassium (K) is the most important plant nutrient that has a key role in the growth, metabolism and development of plants. In addition to increasing plant resistance to diseases, pests, and abiotic stresses, K is required to activate over 80 different enzymes responsible for plant and animal processes such as energy metabolism, starch synthesis, nitrate reduction, photosynthesis, and sugar degradation. K is the seventh most abundant element in Earth's crust. Total K content in soils ranges between 0.04 and 3% K. Although K is present as an abundant element in soil, only 1 to 2 % of this element is available to plants. The rest are bound with other minerals and therefore are unavailable to plants.

K is present in several forms in the soil, including mineral K, non-exchangeable K, exchangeable K, and solution K. Interrelationships of various forms of soil K are shown in Figure 1. Depending on soil type, from 90 to 98% of soil K is mineral K and most of this K is unavailable for plant uptake. Minerals containing K are feldspar (orthoclase and microcline) and mica (biotite and muscovite). The non-exchangeable form of K makes up approximately 1 to 10 % of soil K and is trapped between the layers or sheets of certain kinds of clay minerals (Sparks, 1987). Solution K is the form of K that directly and readily is taken up by plants and microbes in soil. In addition, this form is most subject to leaching in soils. The concentration of soil solution K varies from 2 to 5 mg l^{-1} for normal agricultural soils.

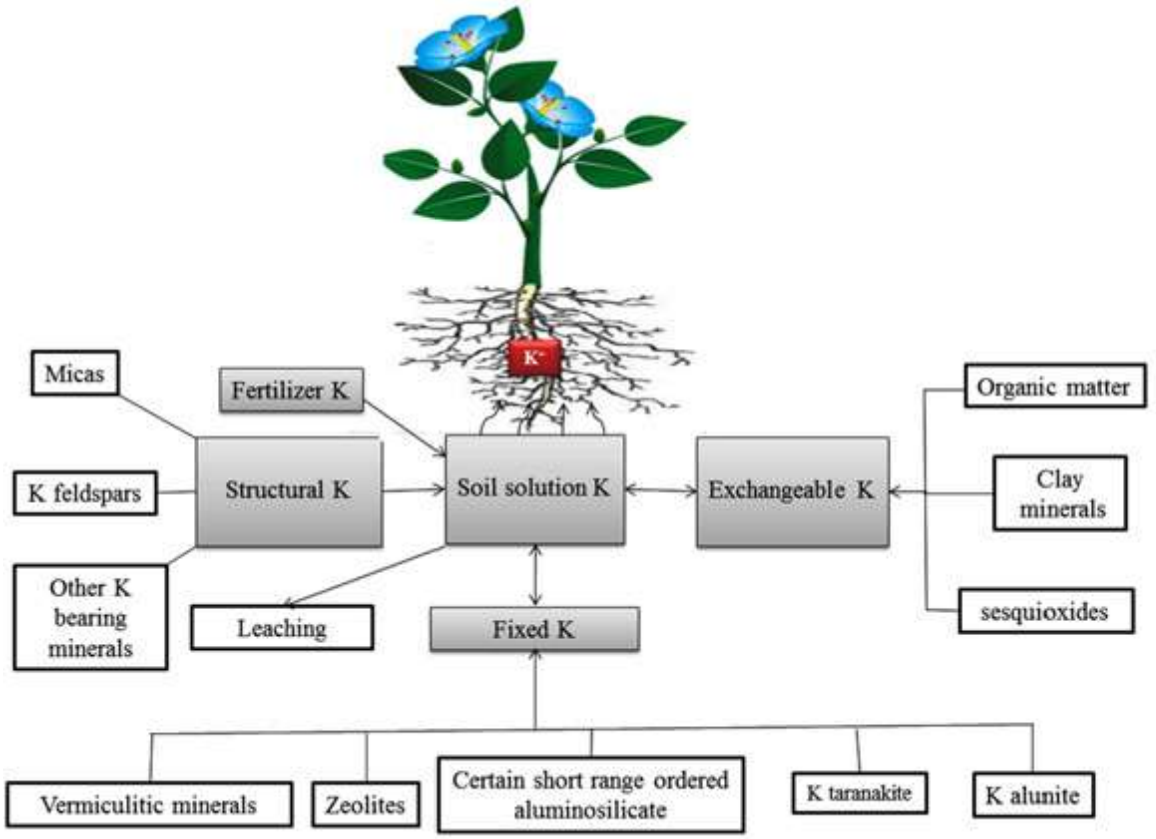


Figure 1. Interrelationships of various forms of soil K (Sparks and Huang, 1985).

Due to being the major amounts of K in the soil as a fixed form (non-available to plant indirectly), imbalanced fertilizer utilization, great increase of crop yield (depleting soil solution K), and the depletion of K in the soil system as a result of not being added crop residue to the soil by farmers, K deficiency has been reported in most of the crop plants. Since cost of K-fertilizers (the price of potash $470 per ton since 2011) is increasing every year and also use of these fertilizers has harmful effects on the environment, it is necessary to find an alternative indigenous source of K and maintain K level in soils for sustainable crop production.

It is proven that microbial soil community is able to influence soil fertility through soil processes viz. decomposition, mineralization, and storage / release of nutrients. It was reported that some beneficial soil microorganisms, a wide range of saprophytic bacteria, fungal strains and actinomycetes, could solubilize the insoluble K to soluble forms of K by various mechanisms including production of inorganic and organic acids, acidolysis, polysaccharides, complexolysis, chelation, polysaccharides, and exchange reactions.

Among these microorganisms, K solubilizing bacteria (KSB) have attracted the attention of agriculturists as soil inoculum to promote the plant growth and yield. The KSB are effective in releasing K from inorganic and insoluble pools of total soil K through solubilization. It had been reported that inoculation with KSB produced beneficial effect on growth of different plants.

The above studies show that KSB can provide an alternative technology to make K available for uptake by plants. Thus, identification of efficient bacterial strains capable of solubilizing K minerals can quickly conserve our existing resources and avoid environmental pollution hazards caused by heavy application of K-fertilizers. Hence, in this review, we elaborated the studies of KSB including isolation and mechanisms of solubilizing K-bearing minerals to develop efficient bacterial inoculants for solubilization of K in soil, which is one of the aims of achieving sustainable agriculture.

Bacteria-soil-plant interactions

Soil is a complex mixture of minerals, water, air, organic matter, billions of organisms, and the changes taking place in its composition (biogeochemical transformations). Soil fertility refers to the capacity of the soil to supply essential plant nutrients such as N, P, K and micronutrients, which are often not available in free form or are in limited quantities in the soil. This is where root-associated beneficial microbes are important partners.

It is known that microorganisms can make nutrients available to plant by different mechanisms. In the soil, it is possible to find various types of microorganisms such as bacteria, fungi, actinomycetes, protozoa, and algae which bacteria are by far the most common (i.e., ~ 95%). There is an estimated 60,000 different type of bacteria that reside in the soil, most of which have yet to be even named, and each has its own particular roles and capabilities. The number and diversity of bacteria are influenced by the soil conditions such as organic carbon, temperature, moisture, electrical conductivity and other chemicals as well as by the number and types of plants found in those soils. Therefore, soil-grown plants are immersed in a sea of microorganisms especially bacteria.

Recent studies show that most plant species require microbial associations for survival. In addition, plants possess the ability to select their own root microflora from the surrounding soil. In other words, each particular plant species has a characteristic group of associated microbes. The establishment of beneficial plant–microbial interactions needs a mutual recognition and a considerable orchestration of the responses at both the plant and the microbial side. By exuding chemicals or signals,plants can effectively communicate with the rhizosphere microorganisms, while their associated microbes may establish an efficient associative symbiosis with plants by triggering host functional signals (e.g., microbial chemotaxis and colonization).

The interactions established between bacteria and plant may be beneficial (e.g., plant growth promoting rhizobacteria, PGPRs), harmful (e.g. pathogens), or neutral for the plant, and sometimes the impact of a bacterium may vary as the soil conditions change. The bacteria that provide some benefits to plants are: (i) those that form nodules on host plant roots (symbiotic relationship) and fix nitrogen; (ii) those that are endophytic and colonize the internal plant tissues without pathogenic effects in host; (iii) those that have ability to competitively colonize the rhizosphere and plant root surface; and (iv) those that are free living in the soil.

In agriculture, beneficial bacteria are defined as any bacteria that colonize the roots of plants following inoculation onto seed and improve plant growth by increasing seed emergence, plant weight, and crop yields. Despite the limited understanding of soil bacteria-plant interactions, a number of these bacteria are used commercially as adjuncts to agricultural practice. These bacteria include *Burkholderia cepacia*, *Delfitia acidovorans*, *Paenibacillus macerans*, *Pantoea agglomerans*, *Pseudomonas* spp., *P. aureofaciens*, *P. chlororaphis*, *P. fluorescens*, *P. solanacearum*, *Bacillus* spp., *B. mucilaginous*, *B. pumilus*, *B. subtilis*, *B. amyloliquefaciens*, *B. fimus*, *B. licheniformis*, *B. megaterium*, *Agrobacterium radiobacter*, *Azospirillum brasilense*, *A. lipoferum*, *Azotobacter chroococcum*, *P. syringae*, *Serratia entomophilia*, *Streptomyces* spp., *S. griseoviridis*, and *S. lydicus*.

According to definition mentioned above, KSB are also known as plant growth promoting bacteria (PGPRs). In general, PGPRs help the plant growth by two mechanisms: (i) direct action mechanisms by either providing plants with resources/nutrients (e.g., N, P, Fe and other essential minerals) or regulating plant hormone levels (cytokinins, gibberellins, indole-3-acetic acid, and ethylene); and (ii) indirect action mechanisms by decreasing the deleterious effects of various pathogens on the growth and yield of plants as bio-control agents. Direct and indirect mechanisms of PGPRs in promoting plant growth and in providing K to plant are shown in Figure 2.

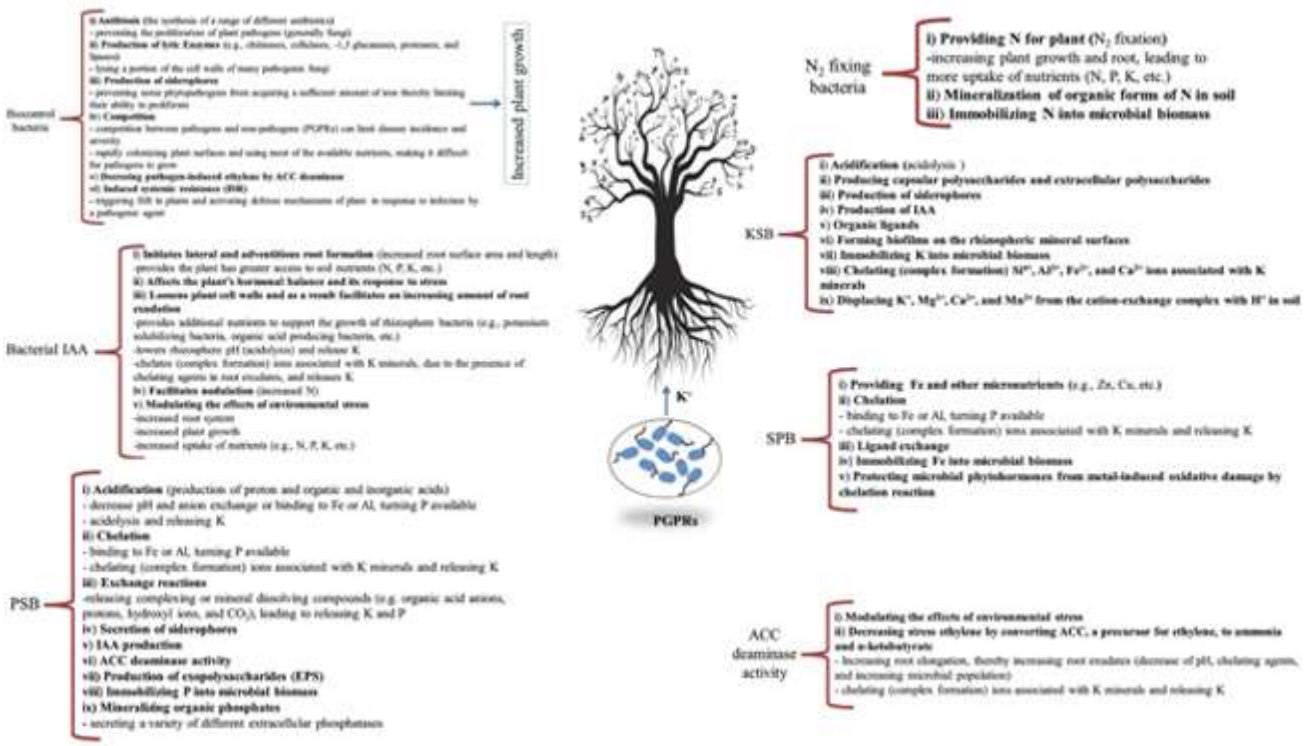


Figure 2. Direct and indirect mechanisms of PGPRs in promoting plant growth and in providing K to plant. IAA, indole-3-acetic acid; PSB, phosphate solubilizing bacteria; ACC, 1-aminocyclopropane-1-carboxylate; KSB, potassium solubilizing bacteria; and SPB, siderophore producing bacteria.

Potassium solubilizing bacteria (KSB) and their screening

K solubilization is done by a wide range of saprophytic bacteria, fungal strains and actinomycetes *(*Ahmad*et al.,* 2016*;* Bakhshandeh *et al.,* 2017*; Gundala et al., 2013; Meena et al.,* 2014). There are strong evidences that soil bacteria are capable of transforming soil K to the forms available to plant effectively (Meena *et al.*, 2015a; Meena *et al.,* 2014; Meena *et al.,* 2016). There is considerable population of KSB in soil and in plant rhizosphere. These include both aerobic and anaerobic isolates that the most frequently KSB in soil are aerobic. A considerably higher concentration of KSB is commonly found in the rhizosphere in comparison with non-rhizosphere soil (Padma and Sukumar, 2015). Solubilization of K by KSB from insoluble and fixed forms is an import aspect regarding K availability in soils. The ability to solubilize the silicate rocks by *B. mucilaginosus*, *B. circulanscan*, *B. edaphicus*, *Burkholderia, A. ferrooxidans*, *Arthrobacter* sp., *Enterobacter hormaechei*, *Paenibacillus mucilaginosus*, *P. frequentans*, *Cladosporium*, *Aminobacter, Sphingomonas, Burkholderia*, and *Paenibacillus glucanolyticus* has been reported (Meena *et al.,* 2016). Among the soil bacterial communities, *B. mucilaginosus, B. edaphicus* and *B. circulanscan* have been described as effective K solubilizers

(Meena *et al.*, 2015a; Meena *et al.,* 2014; Meena *et al.,* 2016). KSB are usually present in all soils and have been isolated from rhizosphere soil, non-rhizosphere soil, paddy soil (Bakhshandeh *et al.,* 2017) and saline soil (Bhattacharya *et al.*, 2016).

KSB are isolated by serial dilution plate method using modified Aleksandrov medium including 5.0 g glucose; 0.5 g $MgSO_4.7H_2O$; 0.1 g $CaCO_3$; 0.006 g $FeCl_3$; 2.0 g $Ca_3(PO_4)_2$; 3.0 g potassium aluminium silicate; and 20.0 g agar in 1 l of deionized sterile water. The pH of this medium is adjusted to 7.2 by adding 1 N NaOH. The plates are incubated at 28 ± 2°C in biological oxygen demand incubator for 3-4 days. The colonies exhibiting clear zones are selected and diameter of the solubilization zone is calculated in mm and the values are reported as mean ± standard deviation for each sample. Recently, Rajawat *et al.* (2016) suggested a modified plate assay for rapid screening of KSB. This assay is based on improved visualization of halo zone formation around the colonies on agar plates, through inclusion of an acid-base indicator dye (bromothymol blue, BTB), to modify the Aleksandrov medium. This assay is also time-saving, more sensitive, and beneficial in comparison to the Aleksandrov plate assay.Comparison of K solubilization on the Aleksandrov agar plate and modified agar medium plate suggested by Rajawat *et al.* (2016) is shown in Figure 3.

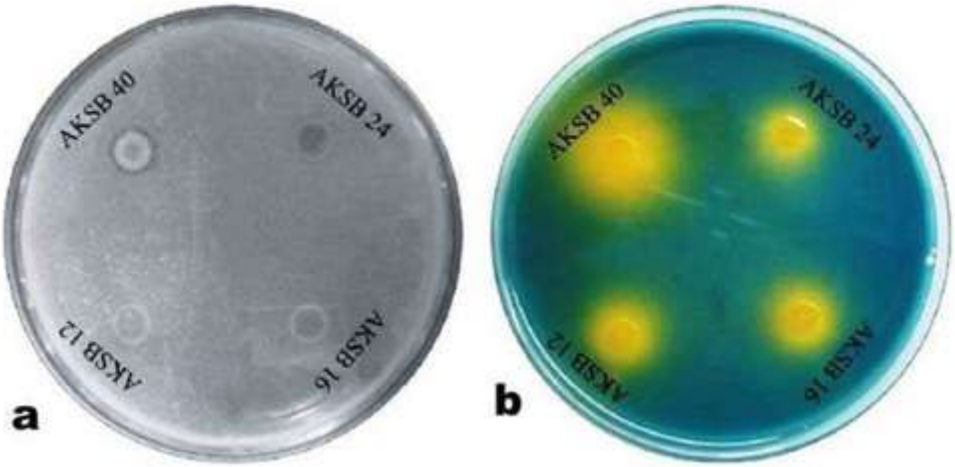


Figure 3. Comparison of K solubilization on the Aleksandrov agar plate (a) and modified agar medium plate (b) after 72 h of incubation (Rajawat *et al.,* 2016).

Quantitative estimation of K solubilization is performed by flame photometry or atomic absorption spectrophotometer wherein culture broth is centrifuged and supernatant is used for precipitation of cobalt nitrite. Standard curve for quantification of K is prepared using various concentrations of KCl solution (Hu *et al.,* 2006). In this assay, mica is usually used as a source of insoluble form of K, although other K sources were also used in screening KSB (e.g., insoluble magnesium trisilicate, muscovite, illite powder, montmorillonite, kaolinite, potassium-feldspar, biotite, waste mica, bentonite, wood ash, and potassium aluminium silicate) (Meena *et al.,* 2016). In addition, the amount of K solubilization in different culture media (e.g., different pH, temperature, K source, and

carbon source) is different. For example, the amount of K solubilization by *B. edaphicus* in the liquid media was more and a better growth was detected on illite than feldspar (Uroz *et al.*, 2009). Sugumaran and Janarthanam (2007) reported that the *B. mucilaginosus* released the 4.29 mg l^{-1} K in media supplemented with muscovite mica. The amount of K solubilization by KSB at pH 6.5–8.0 was recorded 4.90 mg l^{-1} (Badr *et al*., 2006).Bagyalakshmi *et al*. (2012) studied the ability of K solubilization by *Bacillus* sp., *Burkholderia* sp., and *Pseudomonas* sp. at different temperatures and carbon sources from tea (*Camellia sinensis*). Among the various types of carbon sources like glucose, fructose, sucrose and starch, the best carbon source for solubilization of K was found to be glucose at 35 ºC temperature.

In general, the microbial solubilization of K is strongly influenced by pH, oxygen, the bacterial strains used, and kind of K bearing minerals; in fact, moderate alkalinity favors the solubilization of silicate (Sheng and Huang, 2001). These studies show that optimal conditions for K solubilization by KSB need to be determined in the future.

Action mechanisms of KSB in solubilizing K

Currently there is little information available on the mechanisms which by KSB can solubilize K-bearing minerals and release K for improving the growth and yield of plant. It is generally believed that microorganisms contribute to the release of K^+ from K-bearing minerals by several mechanisms. Released H^+ can directly dissolve the mineral K as a result of slow releases of exchangeable K, readily available exchangeable K. As occurs in the case of P solubilization, the major mechanism of K mineral solubilization is by production the organic and inorganic acids and production of protons (acidolysis mechanism) (Maurya *et al.,* 2014; Meena *et al.,* 2014; Meena *et al.*, 2015b; Parmar and Sindhu, 2013; Sheng *et al.*, 2003; Sheng *et al.*, 2008; Uroz *et al.,* 2009), which are able to convert the insoluble K (mica, muscovite, and biotite feldspar) to soluble forms of K, easily taking up by the plant (Hu *et al*., 2006; Meena e*t al.*, 2014; Mo and Lian, 2011).

The types of various organic acids such as oxalic acid, tartaric acids, gluconic acid, 2-ketogluconic acid, citric acid, malic acid, succinic acid, lactic acid, propionic acid, glycolic acid, malonic acid, fumaric acid, etc. have been reported in KSB, which are effective in releasing K from K-bearing minerals (Hu *et al.*, 2006; Keshavarz Zarjani *et al.,* 2013; Krishnamurthy, 1989; Liu *et al*., 2012; Prajapati *et al.,* 2012; Prajapati *et al.,* 2013; Saiyad *et al.,* 2015; Sheng and He, 2006). It has also been known that the type of the organic acid produced by KSB may be different. Among the different organic acids involved in the solubilization of insoluble K, tarteric acid, citric acid, succinic acid, α-ketogluconic acid, and oxalic acid are the most prominent acids released by KSB (Meena et al., 2014).

Microbial decomposition of organic materials also produces ammonia and hydrogen sulfide that can be oxidized in the soil to form the strong acids such as nitric acid (HNO_3) and sulfuric acid (H_2SO_4). Hydrogen ions displace K^+, Mg^{2+}, Ca^{2+}, and Mn^{2+} from the cation-exchange complex in a soil (Huang *et al.*, 2013). In addition to decreasing soil pH, organic acids produced by KSB can release of K ions from the mineral K by chelating (complex formation) Si^{4+}, Al^{3+}, Fe^{2+}, and

Ca^{2+} ions associated with K minerals (Meena *et al*., 2014; Römheld and Kirkby, 2010; Štyriaková *et al*., 2003). For example, it was reported that KSB weathered phlogopite via aluminum chelation and acidic dissolution of the crystal network (Abou-el-Seoud and Abdel-Megeed, 2012; Leyval and Berthelin, 1989; Uroz *et al.,* 2009).In addition, The *B. altitudinis* strain could accelerate weathering of potash feldspar, change mineral surface morphology, and induce the formation of new mineral complex. This strain dissolved potash feldspar and significantly released more Si, Al, and Fe elements by producing more organic acids (Huang *et al.,* 2013).

Microorganisms including KSB can have a considerable role in proving K to plant by storing K in their biomass (a significant quantity of fixed K), which is potentially available to plants (Jones *et al.,* 2003). It has been reported that the production of various extracellular polymers (primarily proteins and polysaccharides) can also be led to release of K from K-bearing minerals for plant uptake (Liu *et al.,* 2006; Shelobolina *et al.,* 2012; Sheng and He, 2006). These substances serve as attachment structures to mineral or rock surface. For example, a study by Welch and Vandevivere (1994) suggested that naturally occurring polymers can affect the mineral dissolution. Solution containing fresh microbial EPS (exopolysaccharides) increases the dissolution rate of feldspars probably by forming complexes with framework ions in solution. KSB also synthesize biofilms, which create controllable microenvironments around microbial cells for weathering (Meena *et al.,* 2014). Biofilm formation on aluminosilicate increases the residence time of water as compared to the residence time at the bare rock or mineral surface and enhances the mineral weathering.

It was accepted that the microbial biofilms not only accelerated the weathering process but also regulated denudation losses by acting as a protective layer covering the mineral-water-hyphal/root hair interface in the mycorrhizosphere and rhizosphere of vascular plants. Besides, biofilm formation on mineral surface promoted the corrosion of potassium-rich shale and the release of K, Si and Al in the bacteria-mineral contact model (Man *et al.*, 2014). In addition, it is known that the release of organic acids from the plant roots can be effective in enhancing mobilization of mineral K (Wang *et al.,* 2000). Therefore, it can be suggested that other PGPRs (e.g., IAA-producing bacteria) can also have a role in providing K for plant by increasing root exudates (Figure 2) (Etesami *et al.,* 2015). In general, the most important mechanisms known in K mineral solubilization by KSB are "(i) by lowering the pH; (ii) by enhancing chelation of the cations bound to K; and (iii) acidolysis of the surrounding area of microorganism (Meena *et al.,* 2014)".

Effect of KSB on plant growth and yield

With the introduction of high yielding crop varieties and the progressive intensification of agriculture, the soils are getting exhausted in K stock at a faster rate. Inoculation of seeds and seedlings of different plants with KSB generally showed significant enhancement of germination percentage, seedling vigor, plant growth, yield, and K uptake by plants under greenhouse and field conditions (Anjanadevi *et al.*, 2016; Awasthi *et al.,* 2011; Lynn *et al.,* 2013; Meena *et al.*, 2015a; Meena *et al.*, 2014; Subhashini and Kumar, 2014; Zhang *et al.*, 2013; Zhang and Kong, 2014). For example, Lin *et al.* (2002) observed 125 % increase in biomass, whereas K and P uptake were more than 150 % in tomato plant due to inoculation of silicate-dissolving bacteria *B. mucilaginosus* strain

RCBC13 as compared to un-inoculated plants. Parmar (2010) showed that inoculation of K-solubilizing isolate HWP47 in wheat (*Triticum aestivum* L.) var. WH711 caused 51.46 % increase in root dry weight (RDW) in soil at 60 days after sowing in pots. Similarly, 44.28 % increase in shoot dry weight (SDW) was found in HWP47 inoculated plants. Addition of rock material along with inoculation of HWP47 isolate showed 22.35 % increase in RDW and 73.68 % increase in SDW. Isolates HWP15 and HWP47 also caused significant K uptake in the shoot tissues. Similarly, Badar *et al.* (2006) reported that application of KSB with K- and P-bearing minerals on sorghum enhanced dry matter yield by 48 %, 65 %, and 58 %; phosphorus uptake by 71 %, 110 %, and 116 %; and K uptake by 41 %, 93 %, and 79 % in clay, sandy, and calcareous soils, respectively.

As reported by previous researchers, inoculation with KSB also exerted beneficial effects on growth of cotton and rape (Sheng, 2005), eggplant (Han and Lee, 2005), pepper and cucumber (Han and Lee, 2006; Sangeeth *et al.*, 2012), peanut (Youssef *et al.,* 2010), maize (Abou-el-Seoud and Abdel-Megeed, 2012; Leaungvutiviroj *et al.,* 2010; Singh *et al.,* 2010), sorghum (Badr *et al.,* 2006), wheat (Sheng and He, 2006), Sudan grass (Basak and Biswas, 2012; Basak and Biswas, 2010),sorghum (Badr *et al.,* 2006),tea (Bagyalakshmi *et al.*, 2012), Okra (Prajapati *et al.*, 2013), potato (Abdel-Salam and Shams, 2012), and tomato (Lynn *et al.*, 2013). These studies indicate that the use of KSB as bio-fertilizers for agriculture improvement can reduce the use of agrochemicals and support ecofriendly crop production (Archana *et al.,* 2013; Archana *et al.,* 2012; Prajapati *et al.,* 2013).

Potentialities and challenges of KSB in industry

KSB can accelerate weathering reactions of K minerals; especially when they are in direct contact with mineral surfaces by different action mechanisms. Attempts have been made to use of K mobilizing bacteria for solubilizing K from different K bearing minerals (Meena *et al.,* 2016) and hence to improve plant nutrition. Although KSB could be an alternative and viable technology to solubilize insoluble K into soluble form, their application in agricultural practice is still prevented by several factors. For example, (i) lack of awareness about bio-fertilizers amongst the farmers; (ii) slow influence of the K bio-fertilizer on crop yield; (iii) less interest in scientific community on the development of K bio-fertilizer technologies; (iv) culture collection banks not yet developed for KSB due to the loss of efficient strains developed by scientists; and (v) and deficiency in technology in respect to carrier suitability and product formulations are some of the major limitations of the industry, which need to be improved in the near future.

Zinc solubilizing microbes

DEFICIENCIES in plants due to the imbalanced supply of micronutrients are turning out to be an alarming condition in today's agricultural world. Among the micronutrient deficiencies, zinc (Zn) deficiency appears to be present unanimously. Zinc is a central component of several enzymes that drive and boost the rate of many important metabolic reactions of the plants. Thus Zn deficiency will result in the cessation of physiological and biochemical functions of plants leading to abnormal growth and adverse effect on the yield of crops. Zn deficiency has become a serious problem affecting nearly half of the world's population. This is

actually due to low Zn content of the crops grown in Zn-deficient soils. In India, about 50% of the soils are deficient in zinc and this remains the most important nutritional disorder affecting majority of the crop production. The reasons for the zinc deficiency are increased application of chemical fertilizers, intensive agriculture and poor irrigation system that leads to the reduction of zinc content in the Indian soils. Zinc deficiency is expected to increase from 42% to 63% by 2025 due to continuous depletion of soil fertility Though marked response of crops to zinc application has been noticed, zinc deficiency is a major nutritional constraint for successful crop production in Tamil Nadu . It is estimated that about 53% of the soils in Tamil Nadu are deficient in zinc.

Exogenous application of Zn to counter its deficiency in plants in the form of zinc sulphate also gets transformed into different unavailable forms like Zn(OH) and Zn(OH2) at pH of 7.7 and 9.1 (ref. 6); ZnCO3 in calcium-rich alkali soils, Zn(PO3)4 in nearneutral to alkali soils of high P application7 , and gets accumulated in the soil. Though there is plenty of zinc in the soil to support crop growth, the crops exhibit deficiency due to the presence of the unavailable fractions. This necessitates a system that releases essential quantity of zinc from the unavailable state in which it is retained in the soil to the plants for good growth. Numerous bacteria, especially those associated with the rhizosphere have the ability to transform unavailable form of a metal into available form through solubilization mechanism . The secretion of organic acids appears to be the functional mechanism involved in metal solubilization . Gluconic acid is considered to be the major organic acid involved in the solubilization of insoluble minerals . Organic acids secreted by micro-flora increase soil Zn availability by sequestering cations and by reducing rhizospheric pH. Therefore, isolation and identification of such bacteria is an eco-friendly approach to eradicate zinc deficiency in plants. The greatest challenge for researchers is the identification of bacterial taxa from the soil resources. Various phenotypic and genotypic methodologies are being used to identify and characterize bacteria present in the soil community.

Although phenotypic methods play a significant role in the identification, molecular methods are found to be more reliable and authenticated for identification and to study genetic diversity of bacterial isolates. Each bacterial species has at least one copy of the 16S rRNA gene containing highly conserved regions together with hyper variable regions. The use of 16S rRNA gene sequences to identify new strains of bacteria is gaining momentum in recent years. Most of the zinc-deficient plants exhibit low levels of auxin such as indole-3-acetic acid (IAA) because Zn plays an essential role in the biosynthesis of IAA. Many researchers have observed that Zn is required for the synthesis of tryptophan, which in turn is the precursor for the synthesis of IAA. In the absence of IAA, plant growth is stunted. This is because auxin forms a central regulator in many biological functions of plants such as cell division, elongation and differentiation to tropic responses, fruit development and senescence13. Many bacteria isolated from the rhizosphere have the capability to synthesize IAA in vitro in the presence or absence of physiological precursors, mainly tryptophan14. The application of such plant growth promoting rhizobacteria will resolve auxin deficiency in plants.

Mass production and field application of Zn solubilizing microbes

Soil samples were collected from agricultural fields. The field areas that were deficient in zinc and were cultivated with plants that require zinc as vital nutrients for growth were taken as the criteria for soil sample collection. Samples were randomly collected ten times from the rhizosphere of young plants at a depth of 6–15 cm using soil augur. The soil samples that were collected from each location were air-dried, crushed and passed through 2 mm sieve before being mixed into a single composite C for further study.°sample. Then the soil samples were stored at 5

Enumeration of zinc solubilizing bacteria (ZSB) present in the soil samples was done by adopting plate count method. Addition of different insoluble sources of zinc (ZnO, ZnCO3 and Zn (PO3)4) at 0.1% in the modified Bunt and Rovira agar medium helped in the enumeration of bacteria. The plates were incubated for three days at C in an incubator. The colonies that exhibited the°30 clearing zone were considered as zinc solubilizers. The clear-zone forming organisms were counted, isolated and purified. The 35 most predominant and morphologically distinct bacterial colonies were selected for qualitative assay and were designated ZSB-1, ZSB-2 … ZSB-35. Qualitative estimation of zinc solubilizing potential of the isolates In the qualitative stu dy, all the 35 bacterial isolates were tested for solubilization efficiency by means of plate assay using modified Bunt and Rovira agar medium containing 0.1% of ZnO, ZnCO3 and Zn (PO3)4 as insoluble C for 48 h. By°source. The plates were incubated at 30 measuring the diameter of the clear zone and colony growth, Zn solubilization efficiency was tested15 Solubilization diameter Solubilization efficiency = 100. Diameter of colony growth × Based on the results of the plate assay, five isolates (ZSB-1, ZSB-10, ZSB-13, ZSB-22 and ZSB-23) which showed the best solubilization of zinc were identified using molecular marker 16S rRNA. They were subjected to further experimental studies such as quantitative estimation (broth assay), influence of the isolates on pH of the medium and production of gluconic acid as well as IAA.

The bacterial isolates were screened to find out the amount of zinc solubilized in the broth by growing them in 100 ml Erlenmeyer flasks containing 50 ml of Bunt and Rovira broth supplemented with 0.1% ZnO, ZnCO3 and Zn(PO3)4. Appropriate uninoculated controls were maintained. All the treatments were replicated. The bacterial cultures were withdrawn after the sixth, eighth and C for the estimation of soluble Zn. The bacterial cultures were centrifuged at°tenth day of incubation at 30 15,000 rpm for 20 min and the supernatant was passed m membrane filter so as to obtain the culture filtrate containing only the soluble forms of metal21µthrough 0.2 . Then the sample was fed to an atomic absorption spectrometer (Shimadzu 7000AA) to find the concentration of available zinc present in the sample. The minimum detection limit of zinc in the instrument is 0.2 mg/kg and the linearity over the concentration range is 0.9952 (fit factor).

UNIT-IV

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 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?

PART-C (6 MARKS)

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

Unit IV

Mycorrhizal fertilizers

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

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.

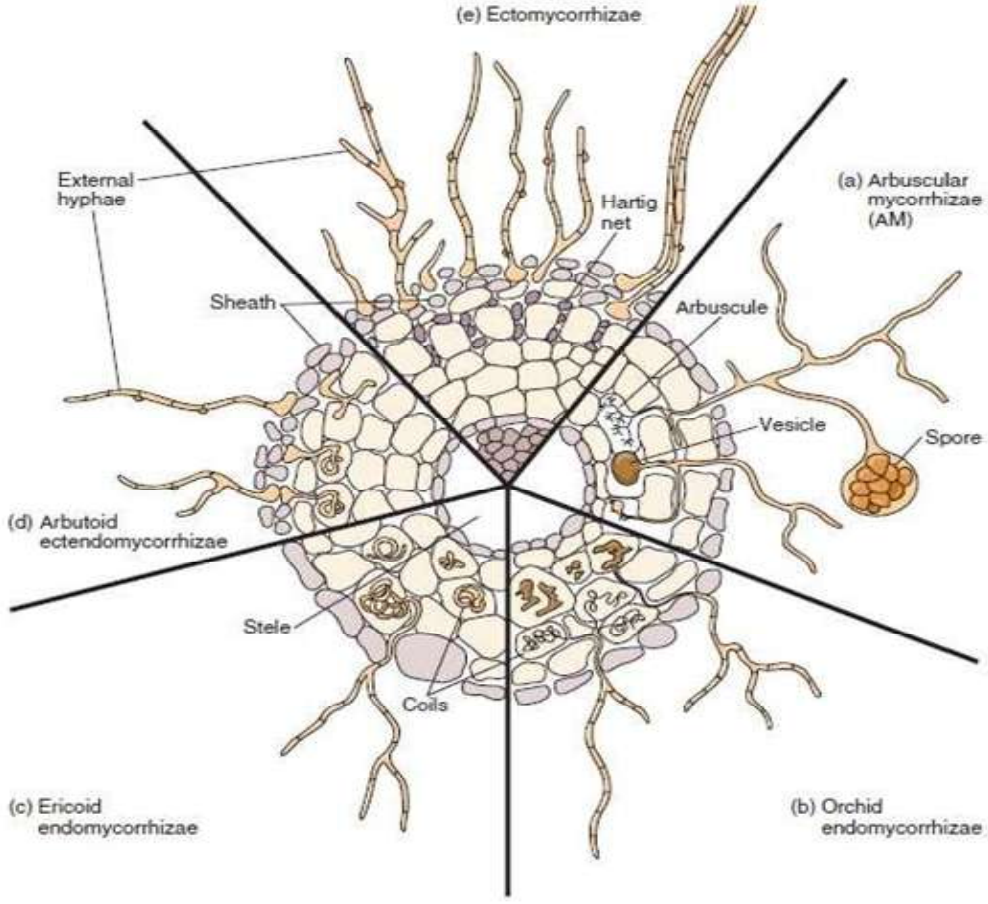
2. Ericoid mycorrhiza

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



Ectomycorrhi

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.

The Importance of Mycorrhizal Fungi

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.

Entomopathogenic fungus

An entomopathogenic fungus is a fungus that can act as a parasite of insects and kills or seriously disables them. These fungi usually attach to the external body surface of insects in the form of microscopic spores (usually asexual, mitosporic spores also called conidia). Under the right conditions of temperature and (usually high) humidity, these spores germinate, grow as hyphae and colonize the insect's cuticle; which they bore through by way of enzymatic hydrolysis, reaching the insects' body cavity (hemocoel). Then, the fungal cells proliferate in the host body cavity, usually as walled hyphae or in the form of wall-less protoplasts (depending on the fungus involved). After some time the insect is usually killed (sometimes by fungal toxins), and new propagules (spores) are formed in or on the insect if environmental conditions are again right. High humidity is usually required for sporulation.

The entomopathogenic fungi include taxa from several of the main fungal groups and do not form a monophyletic group. Many common and/or important entomopathogenic fungi are in the order Hypocreales of the Ascomycota: the asexual (anamorph) phases *Beauveria*, *Isaria* (was *Paecilomyces*), *Hirsutella*, *Metarhizium*, *Nomuraea* and the sexual (teleomorph) state *Cordyceps*; others (*Entomophthora*, *Zoophthora*, *Pandora*, *Entomophaga*) belong in the order Entomophthorales of the Zygomycota.

Related fungi attack and kill other invertebrates (e.g. nematodes).

Pest control

Since they are considered natural mortality agents and environmentally safe, there is worldwide interest in the use and manipulation of entomopathogenic fungi for biological control of insects and other arthropod pests. In particular, the asexual phases of Ascomycota (*Beauveria* spp., *Isaria* spp., *Lecanicillium* spp., *Metarhizium* spp., *Purpureocillium* spp. and others) are under intense scrutiny due to traits favouring their use as biological insecticides.

Production

Most entomopathogenic fungi can be grown on artificial media. However, some require extremely complex media; others, like *Beauveria bassiana* and exploitable species in the genus *Metarhizium*, can be grown on starch-rich substrates like cereal grains (rice, wheat).

Virulence

The Entomophthorales are often reported as causing high levels of mortality (epizootics) in nature. These fungi are highly virulent. The anamorphic Ascomycota (*Metarhizium*, *Beauveria* etc.) are reported as causing epizootics less frequently in nature.

Also important for pesticide development are their properties regarding specificity (host range), storage, formulation, and application.

UNIT-IV

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)
PART-B (2 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?

PART-C (6 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 detailed notes on ectomycorrhizae and endomycorrhizae.
5. Explain the role of VAM symposis in crop plants

Unit 5
Biopesticides

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

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.

Bio-nematicides

Agricultural crops are severely damaged by root-knot nematodes causing extensive financial losses globally. Historically, agrochemicals have been the preferred method to combat these pests; however, threats to humans and the environment posed by these agrochemicals led to the need for developing new biocontrol agents. Importantly, the latter should adhere to biosafety regulations while being highly effective. Root-knot nematodes live in soil and thus the use of rhizobacteria such as *Bacillus* for biocontrol development have shown potential. Although various *Bacillus* species have been tested in this capacity, little is known about their secondary metabolites and the mechanisms of action responsible for their nematicidal activity. If these secondary metabolites can be qualitatively and quantitatively characterised, metabolic features could be synthetically engineered and used to combat root-knot nematodes. Although there is great potential for bionematicides, the commercialisation and development of such products can be difficult. This review summarises the importance of *Bacillus* species as natural antagonists of root-knot nematodes through the production of secondary metabolites. It provides an overview of the significance of

root-knot nematodes in agriculture and the advances of chemical nematicides in recent years.

Pseudomonas, Bacillus, Streptomyces

Biological control agents (BCAs) and their formulations for pest management in legumes Considerable efforts have been made to manage important pests of legumes by incorporating BCA-colonized natural substrates into the rhizosphere. Bacterial endophytes like Bacillus, Paenibacillus, and Pseudomonas show antifungal activity against major pathogens like Rhizoctonia solani (Kuhn.), Rhizoctonia bataticola (Taub.) Butler, Fusarium udum Butler., F. oxysporum f. sp. cireri (Padwick) Snyd. & Hans., and Sclerotium rolfsii Sacc. infecting pulse crops (Senthilkumar et al., 2009).

Among the most pronounced antagonistic fungi, Trichoderma species have been extensively investigated as potential BCAs in pulse-based ecosystem. These are demonstrated to be effective against wilt and root rot (root, collar, and stem) diseases conditioned by different Fusarium spp., R. solani, R. bataticola, Sclerotium rolfsii, Sclerotinia sclerotiorum (Lib.) de Bary, Phytophthora drechsleri f. sp. Cajani Tucker, and Pythium spp. in different pulses and other field crops (Chaudhary et al., 2004). The fungi belonging tohyphomycetes such as Metarhizium anisopliae, Beauvaria bassiana, Nomuraea, Verticillium, and Paecilomyces have been employed as biopesticides in legume crops. In soybean, the application of Paecilomyces lilacinus, Pochonia chlamydosporia, Aspergillus nidulans var. dentatus, and T. harzianum at 2 g/kg soil can effectively reduce nematode (Rotylenchus reniform) population along with promoting plant growth (Gurjar et al., 2012; Singh and Prasad, 2014). Apart from controlling diseases in pulses, BCAs are also reported to enhance nodulation. Several isolates of Trichoderma spp. have been characterized and evaluated against different fungal pathogens of pulse crops (Dubey et al., 2006; 2007; 2009; 2011; 2012; 2013; Jamali et al., 2004 Chaudhary et al., 2004; Mishra et al., 2015). A list of BCAs that are employed to manage different pests of pulses is given in Table 3. Besides, B. thuringiensis is one of the most promising biopesticides used worldwide for managing many lepidopterous pests. According to Roh et al. (2007), more than 100 Bt-based biopesticide formulations have been developed. Nuclear polyhedrosis viruses (NPVs) are specific biopesticides widely used in cotton, chickpea, pigeon pea, maize, groundnut, tomato, sorghum, sunflower, vegetables, and other crops (Pawar and Thombre, 1992).

Researchers at Indian Institute of Pulses Research (IIPR), India, has identified several native potential strains of Trichoderma spp. (T. harzianum, T. asperellum, T. longibrachiatum, and T. reesei) and plant growth-promoting rhizobacteria (PGPRs) isolated from rhizospheres in major pulse-growing areas in India and evaluated these for their antagonistic potential against a variety of pathogens (Fig. 2a, b). Accordingly, mass production technology has been developed and popularized among the pulse-growing farmers in different agro-ecosystems (Chaudhary et al., 2004; Mishra et al., 2015, 2016).

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.

POSSIBLE QUESTIONS

UNIT-V

PART-A (20 MARKS)

(Q.NO 1 TO 20 Online Examination)

PART-B (2 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.

PART-C (6 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*

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc Microbiology **COURSE NAME: Biofertilizers and Biopesticides**

COURSE CODE: 18MBU404A **Unit 1** **BATCH-2018-2021**

S.No	UNIT - I	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER KEY
1	_____ are aerobic and free-living nitrogen nitrogen fixers	*Frankia* & *Azospirillum*	*Clostridium*& *Desulfovibrio*	*Beijerinckia* & *Klebsiella*	*Rhizobium*& *Anabaena*	*Beijerinckia* & *Klebsiella*
2	_____ are genes encoding enzymes involved in the fixation of atmospheric nitrogen	*mif*	*nif*	*sif*	*nod*	*nif*
3	_____ catalyze conversion of atmospheric nitrogen to ammonia	Kinase	Hydrogenase	Nitrogenase	Phosphatase	Nitrogenase
4	_____ is a typical example of symbiotic nitrogen fixation seen in paddy fields	Azolla-*Anabaena*	Alder-*Frankia*	Legume-*Rhizobium*	Higher plants-*Mycorrhizae*	Azolla-*Anabaena*
5	_____ recycles the H_2 produced during N_2 fixation, thereby minimizing the loss of energy	Reductase	Catalase	Nitrogenase	Hydrogenase	Hydrogenase
6	A free-living anaerobic photosynthetic bacterium	*Anabaena azollae*	*Clostridium thermocellum*	*Rhodospirillum rubrum*	*Klebsiella pneumoniae*	*Rhodospirillum rubrum*
7	A free-living soil bacteria that is involved in nitrogen fixation	*Alcaligenes*	*Acetobacter*	*Pseudomonas*	*Azotobacter*	*Azotobacter*
8	Amount of ATP needed to form 2 moles of ammonia from 1 mole of nitrogen gas during biological nitrogen fixation	8	16	32	64	16
9	Apart from biological nitrogen fixation by microbes, _____ can fix atmospheric nitrogen	Cyclone	Thunder	Raining	Lightning	Lightning
10	Bacteria that forms root nodules in legume plants	*Rhizobium*	*Azotobacter*	*Azospirillum*	Cyanobacteria	*Rhizobium*
11	Biological nitrogen fixation was discovered by	Winogradsky	Beijerinck	Pasteur	Koch	Beijerinck
12	Chemicals produced by the Rhizobia called _____ that cause the colonized root hairs to curl	Pod factors	Nod factors	Sod factors	Mod factors	Nod factors
13	Example of associative nitrogen fixation	Legume-*Rhizobium*	Rice-*Azospirillum*	Higher plants-Mycorrhizae	Azolla-*Anabaena*	Rice-*Azospirillum*
14	*Frankia* is a	Bacteria	Actinomycete	Fungi	Algae	Actinomycete
15	Group of irregularly shaped bacteria in root nodules are called as	Bacteroids	Asteroids	Mesteroids	Histeroids	Bacteroids

16	In biological nitrogen fixation, ____ moles of ammonia are produced from one mole of nitrogen gas	2	4	6	8	2
17	In Cyanobacteria, nitrogen fixation occurs in terminally differentiated cells known as	Cyanocysts	Nitrocycts	Heterocysts	Homocysts	Heterocysts
18	In root nodules, _____ bind and regulate the levels of oxygen in the nodule	Teghemoglobin	Peghemoglobin	Leghemoglobin	Hemoglobin	Leghemoglobin
19	Legume plants belongs to	*Solanaceae*	*Rosaceae*	*Astraceae*	*Fabaceae*	*Fabaceae*
20	Most abundant gas in atmosphere	Nitrogen	Oxygen	Carbon dioxide	Hydrogen	Nitrogen
21	Nitrogenase enzyme consists of	Iron protein	Molybdenum-iron protein	Iron protein and a molybdenum-iron protein	Hemoglobin	Iron protein and a molybdenum-iron protein
22	Rhizobia are attracted to _____ released by the host legume's roots	Flavonoids	Enzymes	Toxins	Chemicals	Flavonoids
23	The enzyme nitrogenase is inhibited by _____	CO_2	Sulfur	Hydrogen	Oxygen	Oxygen
24	Which is not true about *Anabaena* and *Nostoc*	Filamentous	Nitrogen fixing	Cyanobacteria	Symbiotic	Symbiotic
25	The majority of hydrogenases in prokaryotes are _____ containing enzymes	Nickel	Copper	Molybdenum	Sulfur	Nickel
26	With associative nitrogen fixation, which one of the following genera is associated?	Azotobacter	Escherichia	Rhizobium	Anabena	Azotobacter
27	The conversion of nitrogen to ammonia or nitrogenous compounds is called as_____	Nitrogen assimilation	Nitrogen fixation	Denitrification	Nitrification	Nitrogen fixation
28	Symbiotic nitrogen cyanobacteria are present in all except_____	Anthoceros	Azolla	Cycas	Gnetum	Gentum
29	All the following are free living nitrogen fixers except_____	Rhizobium	Azotobacter	Rhodospirillum	Clostridium	Rhizobium
30	Anabena is a nitrogen fixer present in the root pockets of_____	Marselia	Salvinia	Pistia	Azolla	Azolla
31	Splitting of dinitrogen molecule into free nitrogen atom in biological nitrogen fixation is carried out by _____	hydrogenase	nitrogenase	dinitrogenase	nitrate reductase	nitrogenase
32	Which of the following aid plants in the acquisition of nitrogen from nitrogen gas of the atmosphere?	Bacteria	Algae	Nematodes	Moulds	Bacteria
33	A major plant macronutrient found in nucleic acids and proteins is _____	calcium	nitrogen	sulphur	iron	nitrogen

34	Organisms capable of converting nitrogen to nitrate are_____	yeast	bacteria	roundworms	moulds	bacteria
35	The conversion of amino acids to ammonium by soil decomposers is called_____	ammonification	mineralization	deamination	both a and b	both a and b
36	To fix one molecule of nitrogen _____	6 ATP molecules are required	12 Atp molecules are required	16 ATP molecules are required	20 ATP molecules are required	16 ATP molecules are required
37	Conversion of nitrite to nitrate is carried out by _____	Nitrosomonas	Nitrosococcus	Nitrobacter	Clostridium	Nitrobacter
38	All of the following are examples of negative symbiosis_____	amensalism	competition	commensalism	parasitism	competition
39	The reservior for nitrogen is _____	the atmosphere	rocks	ammonia	nitrates	the atmosphere
40	Degree of compost maturity can be assesed by _____	infrared tehnique	germination test	both a and b	MPN test	both a and b
41	Which one of the following nacterium peodices nodule in alfalfa	Bradyrhizobium sp.	Rhizobium aggregatum	Rhizobium leguminosarum	Rhizobium melliloti	Rhizobium melliloti
42	In non leguminous plant, nodules are formed by which one of the following	Anabaena	Frankia	Ralstonia sp.	Sinorhizobium	Frankia
43	Which one of the following component is the limiting and critical for soil	Carbon	Nitrogen	Oxygen	Phosphorous	Phosphorous
44	Which of the following is a classical example of a rhizobial species having biovars	Rhizobium borbori	Rhizobium leguminosarum	Rhizobium lupini	Rhizobium vignae	Rhizobium leguminosarum
45	Which of the following compound is known as the most resistant to microbial degradation during organic matter decomposition	Cellulose	chitin	Hemicellulose	Lignin	Lignin
46	Which of the following forms symbiotism in soyabean crops	Azotobacter paspali	Bradyrhizobium	Nostoc	Rhizobium	Bradyrhizobium
47	In1888, a Dutch microbiologist Beijerinck succeeded in isolating which one of the following bacterial strain from root nodules?	Bradyrhizobium japonicum	Rhizobium leguminosarum	Sinorhizobium meliloti	Nostoc	Rhizobium leguminosarum
48	Ammonia produced in the bacteriod needs to be transported to the plant through which one of the following membrane_____	Lipid membrane	Periplasmic membrane	Symbiosome membrane	Cytoplasmic membrane	Symbiosome membrane
49	Which one of the following is the first species of rhizobia, identified in 1889	Rhizobium borbori	Rhizobium leguminosarum	Rhizobium leucaenae	Rhizobium lupine	Rhizobium leguminosarum

50	The fixation of the inert atmospheric elemental nitrogen by microorganisms through a reductive process accounts for about_____	60%	70%	90%	50%	70%
51	Which one of the following is nonleguminous	Casuarina	Bacillus	Sesbania	Penicillium	Casuarina
52	Nif gene is associated with _____	Rhizobium bacteriod	Arthrobacter	Myrica	Bacillus	Rhizobium bacteriod
53	What are the cofactors needed for nitrogen fixation?	Cobalt	Molybdenum	Zinc	Copper	cobalt
54	Anabena is a nitrogen fixer present in the root pockets of_____	Marselia	Salvinia	Pistia	Azolla	Azolla
55	The conversion of amino acids to ammonium by soil decomposers is called_____	ammonification	mineralization	deamination	both a and b	both a and b
56	Which is not true about *Anabaena* and *Nostoc*	Filamentous	Nitrogen fixing	Cyanobacteria	Symbiotic	Symbiotic
57	Symbiotic nitrogen cyanobacteria are present in all except_____	Anthoceros	Azolla	Cycas	Gnetum	Gentum
58	Which of the following forms symbiotism in soyabean crops	Azotobacter paspali	Bradyrhizobium	Nostoc	Rhizobium	Bradyrhizobium
59	To fix one molecule of nitrogen _____	6 ATP molecules are required	12 Atp molecules are required	16 ATP molecules are required	20 ATP molecules are required	16 ATP molecules are required
60	Which one of the following is nonleguminous	Casuarina	Bacillus	Sesbania	Penicillium	Casuarina

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc Microbiology **COURSE NAME: Biofertilizers and Biopesticides**

COURSE CODE: 18MBU404A Unit 2 **BATCH-2018-2021**

S.No	UNIT - II	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER KEY
1	Nitrogen fixing bacteria colonizing graminaceous plants can be classified into _____ categories	5	3	2	6	3
2	Beijerinck discovered an aerobic bacteria capable of fixing molecular nitrogen in the year _____	1906	1901	1899	1910	1901
3	Subramoney and Abraham isolated Azotobacter chroococcum strains from _____ soil	Red soil	Red loamy soil	Black soil	Clay	Red loamy soil
4	Azotobacter chroococcum grows in _____ soil	Acidic	Neutral	Basic	Neutral and alkaline	Neutral and alkaline
5	Azotobacter are _____ shaped bacterium	Rod	Cocci	Sprillium	Comma	Rod
6	The incubation period for the isolation of Azotobacter is_____	3 Days	5 Days	1 Day	7 Days	3 Days
7	Aged cultures of Azotobacter chrococcum form an insoluble _____ colored pigment	Yellow	Red	Black brown	Black	Black brown
8	The melanin formed by Azotobacter is due to the presence of _____ enzyme	Tyrosinase	Maltase	Trypticase	Pectinase	Tyrosinase
9	The optimal temperature for Azotobacter are between _____	25 - 30 °C	27 - 30 °C	25 - 35 °C	25 - 40 °C	25 - 30 °C
10	Inoculation with Azotobacter was found to increase vitamin ____ in tomatoes	A	B12	C	E	C
11	The coinoculation of Azotobacter with other bioinoculant like Rhizobium enhance the growth of _____	Legumes	Fruits	Pulses	Seeds	Legumes
12	Seed inoculation of Azotobacter chrococcum increases the yield of field crops by about _____ percentage	10	30	50	20	10
13	Low grade of _____ was applied at the intial step of filling up of compost pit	Sulphur	Carbon	Rock phosphate	Nitrogen	Rock Phosphate
14	Homologous selection of a pigmented strain of Azotobacter established better on _____ part of the crop	Stem	Leaf	Flower	Roots	Roots

15	Azotobacter has ability to produce _____ compounds	Antifungal	Antibacterial	Antimalarial	Antiviral	Antifungal
16	The optimum pH for the growth of Azotobacter is _____	7.2-7.6	7	6	4	7.2-7.6
17	Azotobacter are _____ bacterium	Anaerobic	Facultative anaerobes	Aerobic	Obligate	Aerobic
18	Azotobacter species require _____humidity	High	Low	Extreme	Moderate	High
19	_____ species is being used as inoculum for seed bacterization of agricultural crops	Azotobacter chrococcum	Azotobacter paspali	Azotobacter vinelandii	Azotobacter beijerinckii	Azotobacter chrococcum
20	The multiple action of _____ contributes to better germination percentage of seeds	Azospirillium	Azotobacter	Azolla	Anabena	Azotobacter
21	_____ species is specific to the rhizosphere of Paspalum notatum	Azotobacter chrococcum	Azotobacter beijerinckii	Azotobacter vinelandii	Azotobacter paspali	Azotobacter paspali
22	Example of associative nitrogen fixation	Legume-*Rhizobium*	Rice-*Azospirillum*	Higher plants-Mycorrhizae	Azolla-*Anabaena*	Rice-*Azospirillum*
23	Azospirillium are _____ shaped bacterium	Vibriod	Rod	Cocci	Bacilli	Vibriod
24	The root piece for the isolation of Azospirillium are surface sterilized in _____% of alcohol	80	50	70	100	70
25	Who reported nitrogen fixing bacterium under the name spirillum?	Beijerinck	Schroeder	Dobereiner	Tilak	Beijerinck
26	Azospirillum grows free with nitrogen as microaerophilic and when supplied with nitrogen as _____	Anaerobic	Aerobic	Facultative anaerbic	Microaerophilic	Aerobic
27	The strains of some Azospirillum fail to grow in the absence of particular nitrogen source _____ with glucose	Peptone	Sodium nitrate	Yeast extract	Ammonium chloride	Yeast extract
28	In which year Azospirillum was described	1954	1966	1984	1983	1983
29	Which one of the following a-proteobacteria is known as nitrogen fixers	Acetobacter	Azospirillum	Gluconobacter	Rhodospirillum	Azospirillum
30	Azospirillum amazonense peoduce _____ colored colonies in potato dextrose agar	White	Pink	Red	Black	White
31	Azosprillium irakense was found in association with _____ roots	Wheat	Rice	Grass	Barley	Rice
32	Azospirillum rugosum a new species isolated from _____ soil	Sewage contaminated	Water	Oil contaminated	Waste contaminated	Oil contaminated
33	_____ is used as carrier for Azospirillum	Farmyard manure	Lignite	Charcoal	Press mud	Farmyard manure

34	The ratio of carrier used in Azospirillum is _____	2:01	1:10	1:01	2:02	1:01
35	_____ is used in the mass multiplication of Azospirillum	NH4Cl	NH4So4	H2NH4Cl	Na2Cl	NH4Cl
36	Which of the following are nitrogen fixing bacteria associated with roots of C4 plants like maize sugarcane?	Azospirillum	Clostridium	Azotobacter	Bacillus polymyxa	
37	Which of the following is nitrogen fixing bacterium living in association with sugarcane?	Acetobacter	Azotobacter	Frankia	Azospirillum	Azotobacter
38	All are free living nitrogen fixers except_____	Azospirillum	Clostridium	Azotobacter	Bacillus polmyxa	Azospirillum
39	Anbena a nitrogen fixer is present in the root pockets of _____	Salvinia	Marselia	Azolla	Pistia	Azolla
40	Organism associated with sorghum and cotton which provide nutrition to them are	Azospirillum, Azotobacter	Azotobacter, Acetobacter	Anabena, Rhizobium	Rhizobium, Azotobacter	Azospirillum, Azotobacter
41	To fix one molecule of nitrogen	6 ATP molecules are required	12 ATP molecules are required	16 ATP molecules are required	20 ATP molecules are required	16 ATP molecules are required
42	The major enzymes involved in biological nitrogen fixation are_____	Nitrogenase and hexokinase	Nitrogenase and hydrogenase	Nitrogenase and hydrolyase	Nitrogenase and peptidase	Nitrogenase and hydrogenase
43	Majority of nitrogen fixation occurs by	biological nitrogen fixing organisms	Haber Bosch process	Lightning	Volcanisc eruption	biological nitrogen fixing organisms
44	_____ species of Azospirillum has pectinolytic activites	Azospirillum irakense	Azospirillum amazonense	Azospirillum doebereinerae	Azospirillum zeae	Azospirillum irakense
45	Which Azospirillum species are acid tolerant?	Azospirillum oryzae	Azospirillum irakense	Azospirillum amazonense	Azospirillum zeae	Azospirillum amazonense
46	Pure culture of Azospirillum brasilense synthesized auxin from _____	Tryptophan	Tyrosin	Alanine	Methionine	Tryptophan
47	Azospirillum brasilense produced _____ in defined media amended with malate, gluconate or fructose	Minerals	Proteins	Vitamins	Carbohydrates	Vitamins
48	The ability of Azospirillum to synthesize siderophores may contribute to improved _____ nutrition of plants	Magnesium	Sulphur	Carbon	Iron	Iron
49	Azospirilla are capable of living in soils or as _____	Ectophytes	Endophytes	Mesophytes	Pteridophytes	Endophytes

50	Azospirillum could fall prey to certain macrofauna like _____	Earthworms	Snail	Snake	Frog	Earthworms
51	The digestive fluid of Pachyinlus flavipes had a strong _____	Antifungal activity	Antibacterial actiivty	Antiviral activity	Antimalarial activity	Antibacterial actiivty
52	Salt tolerance among species of Azospirillum was the lowest in	A. zeae	A. irakense	A. amazonense	A. doebereinerae	A. amazonense
53	Strains of _____ exhibit higher tolerance to salt content	A. doebereinerae	A. halopraeferens	A. irakense	A. zeae	A. halopraeferens
54	Azospirillum are highly sensitive to _____	Minerals	Salts	Heavy metals	Sugars	Heavy metals
55	Soil salinity plays vital role in diversity of native strains of _____	A. braslense and A. lipoferum	A. zeae and A. halopraeferens	A. amazonense and A. zeae	A. amazonense and A. doebereinerae	A. braslense and A. lipoferum
56	Homologous selection of a pigmented strain of Azotobacter established better on _____ part of the crop	Stem	Leaf	Flower	Roots	Roots
57	Azospirillum amazonense peoduce _____ colored colonies in potato dextrose agar	White	Pink	Red	Black	White
58	Which of the following is nitrogen fixing bacterium living in association with sugarcane?	Acetobacter	Azotobacter	Frankia	Azospirillum	Azotobacter
59	Azospirillum could fall prey to certain macrofauna like _____	Earthworms	Snail	Snake	Frog	Earthworms
60	In which year Azospirillum was described	1954	1966	1984	1983	1983

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc Microbiology **COURSE NAME: Biofertilizers and Biopesticides**

COURSE CODE: 18MBU404A Unit 3 **BATCH-2018-2021**

S.No	UNIT - III	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER KEY
1	_____ is phosphate solubilizing bacteria	*Bacillus megaterium*	*Bacillus anthrax*	*Bacillus cereus*	*Bacillus phosphatae*	*Bacillus megaterium*
2	Enzyme involved in phosphate solubilization	Oxidases	Reductases	Kinases	Phytases	Phytases
3	Microorganisms make soluble phosphate from insoluble phosphate by producing _____	Hydrochloric acid	Sulphuric acid	Nitric acid	Organic acids	Organic acids
4	PGPR is	Phosphorous growth promoting bacteria	Plant gibberellin promoting bacteria	Plant growth promoting biomass	Plant growth promoting bacteria	Plant growth promoting bacteria
5	_____ is supplied to the soil in the form of chemical fertilizers or organic sources as decomposed plants and animal materials	Phosphorus	Iron	Potassium	Zinc	Phosphorus
6	Phosphate solubilizing microroganisms grow in the medium containing insolubkle tri calcium phoaphate as a sole source of _____	Nitrite	Nitrate	Phosphate	Sulphate	Phosphate
7	The production of clearing zone around the colonies of the organism is an indication of _____	Blue green algae	Phosphate solubilizing microbes	Nitrifying bacteria	Nitrogen fixing bacteria	Phosphate solubilizing microbes
8	Quick screening of phosphate solubilizing microroganisms was developed by Nautiyal in the year _____	1975	1989	1999	1996	1999
9	_____ reported benificial effect of phosphobacteria on berseem and wheat	Sundara Rao	Gaind	Nautiyal	Gaur	Sundara Rao
10	The use of phosphobacteria increased the efficiency of rock phosphate and superphosphate applied to _____ soil	Neutral	Alkaline	Acid	Neutral to alkaline	Neutral to alkaline
11	At _____ pH the PSM did not improve the utilization by mycorrhizal plants of a labelled source of insoluble phosphate	8.1	5.2	7.4	6.1	7.4

12	Singh and Kapoor observed root colonization by VAM in _____	Wheat	Rice	Barley	Corn	Wheat
13	_____ bacteria are benificial bacteria capable of solubilizing inorganic phosphorus from insoluble compounds	Azospirillum	Azotobacter	Rhizobium	Phosphate solubilizing	Phosphate solubilizing
14	_____ is the limiting nutrient for acquatic organisms	Nitrogen	Carbon	Phosphorus	Hydrogen	Phosphorus
15	_____% of mined phosphorus is used to make fertilizers	40	60	100	80	80
16	Over enrichment of phosphate in both fresh and inshore marine waters can lead to massive _____	Algal glooms	Azospirillia	Azotobacter	Algal biomass	Algal glooms
17	Phosphorus occurs most abundantly in nature as part of the _____	Phosphate	Orthophosphate	Inorganic phosphate	Organic phosphate	Orthophosphate
18	The plants consumed by the herbivores incorporate the phosphorus into their _____	Cytoplasm	Tissues	Cells	Roots	Tissues
19	_____ is the most common mineral Phosphate weathers from rocks and minerals	Dolomite	Gypsum	Apatite	Liginite	Apatite
20	_____ is adsorbed on iron oxides, aluminium hydroxides, clay surface and organic matter particles and become incorporated	Sulphate	Nitrate	Carbonate	Phosphate	Phosphate
21	_____ gas is normally found to occur under highly reducing conditions	Alginine	Phosphine	Sulphide	Methionine	Phosphine
22	Available phosphorus is found in a biogeochemical cycle in the _____ soil	Lower	Upper	Middle	Both lower and middle	Upper
23	The production and release of oxalic acid by _____ fungi explains their inportance in maintaining and supplying phosphorus to plants	Filamentous	Mycorrhizal	Dimorphic	Non filamentous	Mycorrhizal
24	Microbes that solubilize fixed soil phosphorous are called _____	Phosphorus fixers	Phosphorous solubilizing microorganisms	Nitrogen fixers	Phosphorous solubilizers	Phosphorous solubilizing microorganisms
25	The P content in average soil is about _____ w/w	0.05%	0.06%	0.04%	0.07%	0.05%
26	Phosphorus accounts about _____ of the plant dry weight.	0.2-0.4%	0.2-0.6%	0.2-0.8%	0.2-0.5	0.2-0.8%
27	Phosphate solubilizing Bacteria (PSB) may also be useful in the phyto-remediation of _____impacted soil	Heavy metal	Carbon	Nitrogen	Ions	Heavy metal

28	The liberation of organic phosphates by bacteria is mediated through the production of _____ enzyme	Lipase	Protease	Maltase	Phytase	Phytase
29	The principal mechanism for mineral phosphate solubilization is the production of organic acids and acid	Pectinase	Phytase	Phosphatase	Protease	Phosphatase
30	In organic acid production mechanisms, _____ seems to be the most frequent agent of inorganic phosphate solubilization	Gluconic acid	Malic acid	Techoic acid	Succinic acid	Gluconic acid
31	The acids are produced in the ____ of Gram- negative bacteria by a direct oxidation pathway of glucose	Outer membrane	Cytoplasm	Inner membrane	Periplasm	Periplasm
32	Phosphatic fertilizer management in aerobic rice is critical when _____of the applied phosphate fertilizers are precipitated by Fe, Al and Ca in the soils	75-90%	75-80%	65-90%	75-85%	75-90%
33	The application of _____ acid has been proven to be effective for the solubilization of P	Pectic	Oxalic	Malic	Organic	Oxalic
34	Radioactive_____ can be used to evaluate the exchange rates between the P in the soil solution and solid phases of the soil	^{24}P b	^{46}P	^{31}P	^{32}P	^{32}P
35	The excreted _____ accompanying the decrease in pH acted as a solvent agent for P solubilization.	H^+	C^+	Mg^+	Fe^+	H^+
36	_____ plays an important role in the development of roots including root initiation, cell enlargement and cell division	Indole acetic acid	Auxins	Gibberellins	Amino acids	Indole acetic acid
37	Siderophore production by PS bacterial strains has been considered as a potential way to improve plant growth under_____ stress conditions	Calcium	Iron	Copper	Zinc	Iron
38	The application of _____ resistant plant growth-promoting Pseudomonas aeruginosa exhibiting P solubilization	Mercury	Iron	Cadmium	Nickle	Cadmium
39	Rahnella aquatilis solubilizes P and produces	Oxalic acid	Gibberlic acid	Gluconic acid	Indole acetic acid	Gluconic acid
40	_____produces gluconic acid and solubilizes P	*S. marcescens*	*S. liquefaciens*	*S. plymuthica*	*S. rubidaea*	*S. marcescens*
41	P solubilization mechanisms include _____ formation, chelating metal ions and exchange reactions.	Acid	Alkali	Heavy metals	Iron	Acid
42	The growth and development of plants by producing or changing the concentration of the plant hormones such as	Oxalic acid	Gibberlic acid	Gluconic acid	Indole acetic acid	Indole acetic acid

43	_____ is recommended for detection of phosphate-solubilizing soil microorganisms	Pikovskayas agar	Nutrient agar	Rose bengal agar	Czepadex agar	Pikovskayas agar
44	Organic matter derived from dead and decaying plant debris is rich in organic sources of _____	Carbon	Nitrogen	Sulfur	Phosphorous	Phosphorous
45	Pikovskayas agar was modified by _____	Beijerinck	Sundara Rao and Sinha	Schroeder	Dobereiner	Sundara Rao and Sinha
46	The phosphate solubilizing fungi Aspergillus brasiliensis show _____ growth when cultured in Pikovskayas Agar	Good	Moderate	No growth	Luxuriant	Luxuriant
47	Phosphorus is one of the major fundamental macronutrients for plants and is applied to soil as_____biofertilizer.	Nitrate	Nitrite	Phosphate	Succinate	Phosphate
48	When PSB is used with rock phosphate it can save about ____ of the crop requirement of phosphatic fertilizer	90%	50%	40%	60%	50%
49	The major enzymes involved in biological nitrogen fixation are_____	Nitrogenase and hexokinase	Nitrogenase and hydrogenase	Nitrogenase and hydrolyase	Nitrogenase and peptidase	Nitrogenase and hydrogenase
50	Majority of nitrogen fixation occurs by	biological nitrogen fixing organisms	Haber Bosch process	Lightning	Volcanisc eruption	biological nitrogen fixing organisms
51	Low grade of _____ was applied at the intial step of filling up of compost pit	Sulphur	Carbon	Rock phosphate	Nitrogen	Rock Phosphate
52	The fixation of the inert atmospheric elemental nitrogen by microorganisms through a reductive process accounts for about_____	60%	70%	90%	50%	70%
53	The digestive fluid of Pachyinlus flavipes had a strong _____	Antifungal activity	Antibacterial actiivty	Antiviral activity	Antimalarial activity	Antibacterial actiivty
54	What are the cofactors needed for nitrogen fixation?	Cobalt	Molybdenum	Zinc	Copper	cobalt
55	The conversion of nitrogen to ammonia or nitrogenous compounds is called as_____	Nitrogen assimilation	Nitrogen fixation	Denitrification	Nitrification	Nitrogen fixation
56	Symbiotic nitrogen cyanobacteria are present in all except_____	Anthoceros	Azolla	Cycas	Gnetum	Gentum
57	Amount of ATP needed to form 2 moles of ammonia from 1 mole of nitrogen gas during biological nitrogen fixation	8	16	32	64	16
58	Apart from biological nitrogen fixation by microbes,	Cyclone	Thunder	Raining	Lightning	Lightning

	_____ can fix atmospheric nitrogen					
59	_____ are genes encoding enzymes involved in the fixation of atmospheric nitrogen	*mif*	*nif*	*sif*	*nod*	*nif*
60	_____ catalyze conversion of atmospheric nitrogen to ammonia	Kinase	Hydrogenase	Nitrogenase	Phosphatase	Nitrogenase
61	Fossil evidence and DNA sequence analysis suggest that this mutalism appeared _____ million years ago	300-400	400-440	350-400	400-460	400-460

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc Microbiology **COURSE NAME: Biofertilizers and Biopesticides**

COURSE CODE: 18MBU404A Unit 4 **BATCH-2018-2021**

S.No	UNIT - IV	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER KEY
1	The symbiotic relationship between funig and higher plants are called _____	Lichen	Mycorrhiza	Helotism	Mutualism	Mycorrhiza
2	The advantage of fungus in this association is _____	Food	Protection	Mineral absorption	Water	Food
3	In mycorrhiza, the fungus may form colonies_____	Extracellularly & Intracellularly	Intracellularly	Depends on conditions		Extracellularly & Intracellularly
4	The advantage of plants in this association is	Food	Protection	Increased mineral absorption and disease protection		Increased mineral absorption and disease protection
5	The ectomycorrhizas are commonly formed in _____	Herbaceous plants	Woody plants	All plants	Grasses	Woody plants
6	The endomycorrhizas are also called as _____	Hartig nets	Mat forming mycorrhizas	Vesicular arbuscular mycorrhiza	Intracellular mycorrhizas	Vesicular arbuscular mycorrhiza
7	The ectomycorrhizas form an intracellular network in root cortex called _____	Arbuscular	Vescicles	Hartig net	Haustoria	Hartig net
8	The characteristic feature of VAM is it penetrates plant cell wall and form_____	Spores intracellularly	Vesicles and dichotomously branched invaginations called arbuscules	Haustoria	Massive spore forming structures intracellularly	Vesicles and dichotomously branched invaginations called arbuscules
9	The major advantages of a plant with VAM is _____	Increased N2 absorption	Increased P absorption	Increased K absorption	Increased Mn absorption	Increased P absorption
10	The fungal partner in ectomycorrhiza belongs to the class_____	Basidiomycetes	Ascomycetes	Zygomycees	Every three groups	Every three groups

11	The endomycorrhizal association is present in _____	10% of plant families	40% of plant families	85% of plant families	Less than 5% of plant families	85% of plant families
12	The endomycorrhizas are also called as _____	Hartig nets	Mat forming mycorrhizas	Vesicular arbuscular mycorrhiza	Intracellular mycorrhizas	Vesicular arbuscular mycorrhiza
13	The endomycorrhizae are found in _____	Grains	Paddy	Rice	Woody plants and grasses	Woody plants and grasses
14	The ectomycorrhizal association are found in _____	10% of plant families	40% of plant families	85% of plant families	Less than 5% of plant families	10% of plant families
15	Mycorrhizae play an important role in _____	Soil biology	Food Microbiology	Environment	Agriculture	Soil biology
16	Mycorrhizae are present in _____ % of plant families	92	88	96	90	92
17	Mycorrhizae are divided into _____ types	Three	Four	Two	Five	Two
18	_____ mycorrhizae form a special category	Orchid	Ericoid	Arbuscular	Monotropoid	Monotropoid
19	Arbuscular mycorrhizae are mycotthizae whose hyphae enter into the cells producing structures that are _____ like	Oval	Balloon	Mucoid	Spherical	Balloon
20	The fungal hyphae do not penetrate the _____ in mycorrhizae	Outer membrane	Cytoplasm	Protoplast	Periplasm	Protoplast
21	Fossil evidence and DNA sequence analysis suggest that this mutalism appeared _____ million years ago	300-400	400-440	350-400	400-460	400-460
22	The hyphae of arbuscular mycorrhizal fungi produce the glycoprotein are the major stores of _____ in the soil	Carbon	Nitrogen	Metals	pH	Carbon
23	Ectomycorrhizae are typically formed between the roots of around _____% of plant families	20	5	10	30	10
24	Ectomycorrhizae are mostly found in _____ region of the plants of eucalyptus, oak and pine	Root	Wood	Flower	Stem	Wood
25	Thousands of ectomycorrhizal fungal fungal species exist, hosted in over _____ genera	100	200	500	300	200
26	Ectomycorrhizae covering the _____ tip consist of a hyphal sheath or mantel	Stem	Leaf	Fruit	Root	Root
27	The ectomycorrhizal fungus Laccaria bicolor has been	Nitrogen	Carbon	Phosphorous	Sulfur	Nitrogen

	found to kill spring tails to obtain _____					
28	Association of fungi with the roots of plants have been known since the mid of _____ century	19	17	18	16	19
29	The symbiosis was described by _____	Albert Bernhard Frank	Gaind	Nautiyal	Franciszek Kamieriski	Franciszek Kamieriski
30	Who introduced the term mycorhizae?	Albert Bernhard Frank	Gaind	Nautiyal	Gaur	Albert Bernhard Frank
31	The term mycorrhizae refers to the role of the fungi in the plant _____	Leaf	Stem	Root	Rhizosphere	Rhizosphere
32	_____ forest has indicated that mycorrhizal fungi and plants have a relationship that may be more complex than simply mutualistic		Coniferous	Evergreen	Tidal	Boreal
33	The mycorrhizal mutualistic association provides rhe fungus with relatively constant and direct access to _____	Carbohydrates	Proteins	Lipids	Fats	Carbohydrates
34	Immobilization occurs in soil with high clay content or with a strongly _____ pH	Acidic	Basic	Low acidic	Neutral	Basic
35	Many plants are able to obtain _____ without using soil as a source	Sulphur	Phosphate	Nitrogen	Carbon	Phosphate
36	_____ has been shown to be move from birch tree into fir trees thereby promoting succession in ecosystem	Carbon	Nitrogen	Phosphorous	Sulphur	Carbon
37	_____ mycorrhizae are foung in inhospitable environments	Arbuscular	Eriocoid	Ardutoid	Monotropoid	Eriocoid
38	The fungi involved in symbiotic relationship are _____	Glomeromycota	Zygomycota	Ascomycota	Deutromycota	Ascomycota
39	_____ mycorrhizae are found in the plant genera Arctostaphylos and Arbutus	Eriocoid	Monotropoid	Arbuscular	Arbutoid	Arbutoid
40	_____ fungi produce no chlorophyll	Monotropoid	Arbutoid	Eriocoid	Arbuscular	Monotropoid
41	Monotropoid mycorrhizae are most commonly found in _____ forest	Boreal	Evergreen	Coniferous	Tidal	Coniferous
42	The life cycle of _____ mycorrhizae go through a period of time where they are not photosynthetic	Ardutoid	Orchid	Monotropoid	Ericoid	Orchid
43	The hyphae penetrate the cells of the embryo from hyphael coils called _____ within the cells of orchid mycorrhizae	Root hairs	Hartig net	Fungal peg	Pelotons	Pelotons
44	Arbuscular mycorrhizae belong to the phylum _____	Zygomycota	Glomeromycota	Ascomycota	Deutromycota	Glomeromycota
45	_____ fungi help to capture nutrients such as	Arbutoid	Monotropoid	Eriocoid	Arbucsular	Arbucsular

	phosphorous and micronutrients from the soil					
46	_____ forest large amounts of phosphate and other nutrients are taken up by mycorrhizal hyphae	Coniferous	Dystrophic	Boreal	Tidal	Dystrophic
47	The fungal hyphae of Arbutoid mycorrhizae form a structure known as _____	Hartig net	Root hairs	Pelotons	Fungal peg	Hartig net
48	_____ tree root system is used to prevent the rain from washing phosphorous out of the soil	Inga	Mango	Neem	Palm	Inga
49	The red pigment present in the root nodule is _____	Leg haemoglobin	Haemoglobin	Iron	Protein	Leg haemoglobin
50	Nitrogen fixation in rice occurs due to presence of _____	Nostoc	Azolla	Anabena	Rhizobium	Anabena
51	The medium for the growth of Rhizobium is _____	Yeast extract mannitol agar	Rose bengal agar	Nutrient agar	Malt extract agar	Yeast extract mannitol agar
52	Except Rhizobium, which on of the following bacteria forms nitrogen fixing nodules in plants	Actinorhiza	Burholderia	Micrococcus	Pseudomonas	Burholderia
53	The root nodule of legumes contain pink pigment which has high affinity for oxygen is	nod haemoglobin	leghaemoglobin	haemoglobin	bacterial haemoglobin	leghaemoglobin
54	Azolla as biofertilizer increase the yield of rice fields by	10%	20%	30%	50%	50%
55	Which one is green manure/biofertilizer?	Sesbania	Rice	Oat	Maize	Sesbania
56	Monotropoid mycorrhizae are most commonly found in _____ forest	Boreal	Evergreen	Coniferous	Tidal	Coniferous
57	Who introduced the term mycorhizae?	Albert Bernhard Frank	Gaind	Nautiyal	Gaur	Albert Bernhard Frank
58	The hyphae of arbuscular mycorrhizal fungi produce the glycoprotein are the major stores of _____ in the soil	Carbon	Nitrogen	Metals	pH	Carbon
59	The ectomycorrhizal association are found in _____	10% of plant families	40% of plant families	85% of plant families	Less than 5% of plant families	10% of plant families
60	_____ has been shown to be move from birch tree into fir trees thereby promoting succession in ecosystem	Carbon	Nitrogen	Phosphorous	Sulphur	Carbon

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS: II B.Sc Microbiology **COURSE NAME: Biofertilizers and Biopesticides**

COURSE CODE: 18MBU404A **Unit 5** **BATCH-2018-2021**

S.No	UNIT - V	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER KEY
1	_____ is organic matter, mostly derived from animal waste/feces	Biomanure	Fertilizer	Potash	NPK	Biomanure
2	_____ is the used for seed treatment of groundnut	*Azospirillum*	*Azotobacter*	*Rhizobium*	*Nostoc*	*Rhizobium*
3	_____ are best phosphate mobilizers	*Mycorrhizae*	*Bacillus*	*Citrobacter*	*Candida*	*Mycorrhizae*
4	_____ is a biocontrol agent	*Bacillus polymyxa*	*Azospirillum*	*Trichoderma viridae*	*Aspergillus flavus*	*Trichoderma viridae*
5	_____ are rich in beneficial microorganisms that enrich the nutrient quality of soil	Biofertilizers	Humus	NPK	Vermicompost	Biofertilizers
6	_____ is a best biofertilizer used in paddy fields	*Bradyrhizobium*	*Azospirillum*	*Anabaena*	*Frankia*	Both b and c
7	_____ is a form of agriculture that relies on techniques such as crop rotation, green manure, compost, and biological pest control.	Terrestial farming	Hill farming	Inorganic farming	Organic farming	Organic farming
8	Fossil evidence and DNA sequence analysis suggest that this mutalism appeared _____ million years ago	300-400	400-440	350-400	400-460	400-460
9	_____ is the biological oxidation of ammonia	Oxidation	Nitrification	Denitrification	Reduction	Nitrification
10	_____ can be used with crops like wheat, maize,mustard, cotton, potato and other vegetable crops	*Anabaena*	*Azotobacter*	*Rhizobium*	*Mycorrhizae*	*Azotobacter*
11	_____ is a plant growth promoting bacteria found naturally in soil	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Pseudomonas fluorescens*	*Aspergillus fumigatus*	*Pseudomonas fluorescens*
12	A carrier used in preparation of biofertilizers	Rubber	Peat	Plastic	Soil	Peat
13	A fertilizer consisting of growing plants that are ploughed back into the soil	Green manure	Vermicompost	Biomanure	Organic fertilizer	Green manure
14	Chemoautotrophic involved in nitrification	*Alcaligenes*	*Fusarium*	*Nitrosomonas*	*Arthrobacter*	*Nitrosomonas*
15	Cyanobacteria are	Photoheterotroph	Chemotrophs	Prototrophs	Photoautotro	Photoautotrophs

		s			phs	
16	Denitrification is a microbially facilitated process of	Nitrate degradation	Nitrate assimilation	Nitrate oxidation	Nitrate reduction	Nitrate reduction
17	Denitrifying bacteria	*Thiobacillusdenitrificans*	*Bacillus*	*Aspergillus*	*Micrococcus denitrificans*	Both I & IV
18	Foliar spray is	Spraying on roots	Spraying on Stem	Spraying on leaves	Spraying on Flowers	Spraying on leaves
19	Indole acetic acid and gibberelins are	Hormones of bacteria	Hormones that retard plant growth	Plant growth hormones	Weedicides	Plant growth hormones
20	Liquid extract of composting by earthworms	Vermiwash	Germiwash	Wormiwash	Liquidwash	Vermiwash
21	Majority of atmospheric nitrogen is obtained from	Fossil fuel	Hospital waste	Domestic waste	Industrial waste	Both a and c
22	Phyllosphere refers to	Surface of roots	Surface of leaves	Surface of Stem	Surface of flowers	Surface of leaves
23	Rhizobacteria are bacteria growing in & around _____ of plants	Leaf	Root	Stem	Fruit	Root
24	VAM is	Ventricular arbuscular mycorrhizae	Vesicular augumenting mycorrhizae	Vesicular arbuscular mycorrhizae	Vesicular arbuscular mycobacterium	Vesicular arbuscular mycorrhizae
25	Which are important nutrients for plant growth in soil?	Nitrogen	Phosphorous	NPK	Potassium	NPK
26	Which bacteria is used as biofertilizer in sugarcane crop?	*Beijerinckia*	*Acetobacter diazotrophicus*	*Bacillus*	*Pseudomonas*	*Acetobacter diazotrophicus*
27	Which forms symbiotic relation with higher plants?	*Aspergillus fumigatus*	*Bradyrhizobium*	*Pseudomonas fluorescens*	*Mycorrhizae*	*Mycorrhizae*
28	Expect Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants?	Actinorhiza	Burholderia	Micrococcus	Pseudomonas	Burholderia
29	Rhizobium has symbiotic association with_____	Legumes	non-legume crop	sugarcane	paddy	legumes
30	Which of the following is not the biofertilisers producing bacteria?	Nostoc	Anabena	Both a and	Clostridium	Clostridium
31	Which of the following is capable of oxidising sulfur to	Thiobacillus	Desulfotomac	Rhodospirilli	Rhodomicro	Thiobacillus

	sulfates?	thioxidans	ulum	um	bium	thiooxidans
32	Azolla is used as biofertilizer as it has _____	Rizobium	Cyanobacteria	Mycorrhiza	Large quantity of humus	Cyanobacterium
33	The most quickly available source of nitrogen to plants are_____	amide fertilizers	ammonia fertilizers	nitrate fertilizers	ammonia nitrate fertilizer	amide fertilizers
34	Most effective pesticide is _____	carbamates	organophosphates	organochlorines	phosphates	carbamates
35	Which is true for DDT	not a pollutant	an antibiotic	an antiseptic agent	a non degradable pollutant	a non degradable pollutant
36	Which is major component of bordeaux mixture?	copper sulphate	sodium chloride	calcium chloride	magnesium sulphate	sodium chloride
37	Which one is correctly matched	carbamates-malathion	organophosphates-cabofuran	carbamates-malathion	organochloride-endosulphan	organochloride-endosulphan
38	IPM stands for	integrated plant manufacture	integrated plant management	integrated plant management	integrated pest management	integrated plant management
39	Which is major component of bordeaux mixture?	copper sulphate	sodium chloride	calcium chloride	magnesium sulphate	sodium chloride
40	Insecticides generally attack	respiratory system	muscular system	nervus system	circulatory system	muscular system
41	Organisms associated with sorghum and cotton which provide nutrition to them are	Azospirillum, Azotobacter	Azotobacter, Azospirillum	Anabena, Rhizobium	Rhizobium, Azotobacter	Azotobacter-Azospirillum
42	Azolla as biofertilizer, increase the yield of rice fields by _____	10%	20%	30%	50%	10%
43	Denitrification is _____	reduction of nitrate to nitrogen gas	reduction of nitrate to organic nitrogen compounds	both a and b	reduction of ammonia	Both a and b
44	Which of the following soil microorganism is involved	Thiobacillus	Desulfotomac	Rhodospirilli	Rhodomicro	Desulfotomaculum

	in the reduction of sulfates to hydrogen sulphide	thiooxidans	ulum	um	bium	
45	Which one of the following structure is formed in plant roots by mycorrhizae	Arbuscles	Hartig net	Haustoria	Rhizomorph	Hartig net
46	Except Rhizobium, which one of the following bacteria forms nitrogen fixing nodules in plants__	Actinorhiza	Burholderia	Micrococcus	Pseudomonas	Burholderia
47	Which one of the following genes is responsible for nod factor in bacteria	fix gene	gag gene	nif gene	nol gene	nol gene
48	In which one of the following relationship one partner benefits but the other is neither hurt nor helpless	Amensalism	Commensalism	Parasitization	Predation	Commensalism
49	The proteinaceous compounds ae converted to ammonia in the presence of which one of the following bacteria	Ammonifying bacteria	Denitrifying bacteria	Nitrifying bacteria	Putrefying bacteria	Ammonifying bacteria
50	In soil, which one of the following bacterial genera is responsible for degradation of cellulose	Escherichia	Pseudomonas	Salmonella	Staphylococcus	Pseudomonas
51	Which one of the following compound is known as the most resisant to microbial degradation during organic matter decomposition	cellulose	chitin	hemicellulose	lignin	lignin
52	Soil microorganisms influence above ground ecosystems by contributing to except which one of the following	plant nutrition and health	soil fertility	soil structure	soil texture	soil texture
53	Mycorrizha is a symbiotic association between a fungus and the roots of a vascular plant, was first observed by which one of the following scientist	Crick	Fisher	Frank	Funk	Frank
54	Denitrification is done only by microorganisms, usually by which one of the following	Facultative anaerobes	obligate aerobe	phototrophic aerobe	Microaerophilic	Facultative anaerobes
55	The plant disease control agents inclde to which one of the following microorganism, except?	Ampelomyces quisqualis	Bacillus subtilis	Trichoderme sp.	Bacillus anthrax	Trichoderma sp.
56	In plants, the strains of which one of the following bacterium initiates to the formation of galls?	Agrobacterium	Rhizobium	Pseudomonas	Ralstonia	Agrobacterium
57	In 1888, a dutch microbiologist Beijerinck succeeded in isolating which one of the following bacterial strain from root nodules	Bradyrhizobium japonicum	Rhizobium leguminosarum	Sinorhizobium meliloti	Azolla	Rhizobium leguminosarum
58	Ammonia produced in the bacteriod needs to be transported to the plants through which one of the following membrane	lipid membrane	periplasmic membrane	symbiosome membrane	plasma membrane	symbiosome membrane

59	Bacillus thuringiensis produce _____	Insecticidal protein	Nematocidal protein	Funigicidal Protein	Bactericidal protein	Insecticidal protein
60	Insecticides generally attack	respiratory system	muscular system	nervus system	circulatory system	muscular system