SOIL AND AGRICULTURAL MICROBIOLOGY

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Bio-fertilizers are living agents which improve the fertility of soil is called as bio-fertilizers. Nitrogen fixing microorganism, phosphate solubilizing microbes and organic matter decomposers are examples for bio-fertilizers.

Rhizobium

***Rhizobium* is** a Gram- negative rod , motile(bipolar,sub- polar and peritrichous) , Symbiotic Nitrogen fixing bacteria. The size is 1.2 to 3.0µ. Under phase contrast microscopy, the cells have a refractive granules of B. Polyhydroxybutyrate.

PHB – Polyester, bioplastics (biodegradable, ecofriendly) reserved food material as carbon and energy source, stained with Sudan.B black stain -- dark purple colour. Microbes synthesized PHB under stress condition, when it grow in excess carbon and limited nitrogen,sulfur, magnesium.

Medium- Yeast extract mannitol medium(YEMA) is useful for isolation of this bacterium. Most strains produce extracellular polysaccharides (gum).

Isolation. –plants are carefully uprooted and rootsystem is washed in running water to remove adhering soil particles. Pink nodules are selected and washed in water. They are immersed in solution containing 3 – 5 per cent H_2O_2 as sterilizing agent. The nodules are crushed with the help of glass rod in the presence of small amount sterile water. Dilution – spread on YEMA medium , incubate at 26^0C for 10 days. Watery or white , large gummy colonies emerge within 4-5 days. *Agrobacterium* may also grow on agar plates along with *Rhizobium.*

Peptone glucose agar, Hofers alkaline medium.

Microscopy - Size -1.2-3.0 µ in diameter, Gram –negative rod, motile bipolar or sub-polar or peritrichous flagella, irregular shape.

Differentiation of Rhizobium from Agrobacteria sp.

Congo red test - On YEMA , the rhizobium produces white colonies, where as Agro-produces reddish colonies.

Hofer's alkaline broth test – Agr can tolerate pH of 11, while Rhi –is unable to do this.

Lactose agar – Agr- can utilize lactose with the help of enzyme ketolactase, whrereas rhiz- cannot utilize the agar.

Measurement of Nitrogen fixation – 1,The Kjeldahl method is used for measurement of biologically fixed nitrogen.

2, Acetylene reduction method – is also used for measurement of biologically fixed nitrogen.

Because the nitrogenase can also rduce acetylene C_2H_2 to C_2H_4 ethylene.

Important species of Rhizobium.

R. leguminosarum	*Pisum*
R.phaseoli	*Phaseolus*
R.trifoli	*Trifolium*
R.lupini	*Lupinus*
R.japonicum	*Glycine*

Fast growing species - Rhizobium (G+C content is low) , Peritrichous.

R.phaseoli,R.trifoli, R.leguminosarum, R.meliloti are acid producers.

Slow growing species -Bradyrhizobium (G+C content is high), Sub-polarly.

R.japonicum, R.lupini are non-acid producers.

Strains identification - Serological methods useful for identification of strains. Two antigens are used somatic antigen and flagellar antigen. Antigenic character of bacteroids differ from cultured rhizobial cells of same species.

Maintenance of rhizobial cells.

Agar culture – Maintaining of cultures on YEMA after periodic sub-culturing at intervals (15-30 days)

Agar culture stored under paraffin oil – sterile paraffin oil is poured over the agar slopes.

Stored in (4^0C)

Porcelain(glass) bead method- Cells are stored in glass beads covered with silica gel. The beads are washed well and air –dried and both the gel and the bead are sterilized in a hot –air oven. As and when required, one bead is transferred to fresh YEMA for isolation.

Lyophilization. Bacterial cells are placed in sterile10% sucrose and 5% peptone are subjected for rapid freezing followed by drying in a vaccum container(ampoules) contains $P_2 0_5$ (Phosphorous pentaoxide – it remove all the moisture)

Sterilization

Inoculating rooms are equipped with U.V.rays with wavelengths around 2650 A^0., which have maximum bactericidal effect.(Thymine dimer in N.Acid)

Gamma rays are used to sterilize the peat soil, which are capable of penetrating microorganism and destroying them.

***Rhizobium* in soil** – tolerate upto 50 ^{0}C for more than a few hours. It is sensitive to pesticide,insecticide and other chemicals, also sensitive to bacteriophages,predators soil amoeba. Fluorescent antibodies used to identify the strains.

Rhizobiotoxin – toxin produced by *Rhizobium japonicum* that cause chlorosis in soybean.

Azorhizobium – *Rhizobium* that induces nodules on stem and roots of *Sesbania*, and *Aeschynomena.* 200 kg /ha in 50 days.

Rhi –in root nodules. Bacterial cells enter through the root hairs produce the infection thread, bacteroids are non-motile in nodules , which are pink colour due to leghaemoglobin, surrounded by three membranes.

Function of the nodules.

Nitrogenase is an enzyme which reduce N_2 to NH_3 , has two components , one with both iron(Fe) and molybdenum(Mo) having m.weight 200,000(dinitrogenase) and another with Fe without Mo (dinitrogenase reductase))having m.weight of about 65,000. Nitrogen fixation is an anerobic process. It requires ATP and reductant derived from photoproducts, 12 ATP molecules required for fixation of one molecule of N_2 is fixed.Ferredoxin or flavodoxin are electron supplier, the first stable product in nitrogen fixation is ammonia. The intermediate products are hydrazine,hydroxylamine,dimide, carbamyl phosphate. The ammonium ion is assimilated by glutamine synthatase-glutamate synthase for glutamic acid synthesis involved in protein synthesis.

Factors that influence nodulations

PH, soil nutrition(molybdenum,cobalt), light intensity,photoperiods, temperature etc.

Optimum temper- 20-30^{0}C, Lengthening day time decreases the nodule formation.

Mass production –

YEMA liquid medium containing sucrose or glucose.

Inoculam- obtained from lyophilized culture.

Strain selection- it should produce effective nitrogen fixing root nodule, should tolerate wide range of field condition.

Multi -strain inoculants (infect many host) is more effective than mono-strain inoculant

Preparation of broth - the selected strain is cultivated on YEMA for 3-9 days, is transferred to large flasks for 4-9 days is starter culture., later transferred seed tank fermentor and incubated for 4-9 days, later transferred to production fermentors , the pH to 6.5 to 7.0 with KOH or H_2SO_4, 30^0C, the sterilized medium is inoculated with 1% volume of inoculam, stainless sparger is used for oxygen supply.

Checking the broth – 1, pH test if above 8 or below 6, it indicate contamination.

2, agglutination test for strain identification.

3, Gram smear test to identify gram positive, spore former.

Counts the cell in the broth- 0.1ml of fermentation broth is inoculated on YEMA medium.if plate contains 75 to 150 colonies at the desired dilution is best result.

Storage of broth- it is not advisable to store broth for more than 24 Hours at 4^0 C after fermentation.

Carrier based inoculants.

Powdered beat is pass through 100 mesh sieve, heat treated followed by neutralization with Ca CO_3 to raise pH to 6.8. .The cell count is 300 million(3×10^8) per gm of peat. The broth is sprayed to powdered peat , covered with polythelene bags. If it is stored at 25 to 35^0C for upto 2-3 months. If it is 4^0 C for 12 months.

Other carrier – peat soil, farmyard manure, compost and charcoal.

In Australia, the expiry date is 6 months.

Informations – The following information should be marked on polythelene packet

A, name of the product, as Rhizobium inoculants.

B, leguminous crop for which inteneded

C,name and address of the manufacturer

D, type of carrier

E,batch number

F,date of manufacture

G,date of expiry

H,net quantity for hectare

I, storage information.

Methods of inoculation –

Carrier based cultures are mixed with water to form slurry, the seeds are added to the slurry. Later the seeds are dried in shade and sown immediately,

Transplanted crops roots are dipped in slurry for 30 minutes.

Seed inoculation – for black gram 20 kg of seeds + 3 packet of rhizobium /hectare.

Seed+ carbendazim(fungicide) for 24 hours, seed + rhizobium with rice gruel, shade dried for 15 minutes

Crop response

10 to 30% increases crop yield, adding of super phosphate along with rhizobial cells increases yield.

AZOTOBACTER

A free living nitrogen fixing bacteria , a non-symbiotic nitrogen fixing bacteria , this kind of nitrogen fixation is known as non-symbiotic nitrogen fixation. Azotobacteriaceae.

Aerobic Nitrogen fixer – *Azotobacter, Azomonas, Beijerinckia, Derxia, Mycobacterium, Azospirillum*(microaerophilic).

Nitrogen fixer facultative anaerobes – *Klebsiella sp, Rhodopseudomonas ,Rhodospirillum,*

Nitrogen fixer strict anaerobes – *Chlorobium, Chromatium, Desulfovibrio, Clostridium,*

Beijerinck was first person to isolate this bacterium *A. choococcum, A.agilis(*little gum producer *and* water-borne).

Important species – *A. vinelandii, A.beijerinckii(*both are gum producers*) A. insignis, A. macrocytogenes, A. paspali,* They are soil inhabitants.

Isolation - soil dilution plating method, nitrogen free medium(Ashby's medium, Jensen's medium, Burk's medium, Beijerinckia medium. incubated at28^0C,for three days , producec soft ,

milky and mucoid colonies. older culture produces Black colour due to oxidation of tyrosine by tyrosinase enzyme , contain copper atoms (*A. chroococcum* is a dominant species in aerobic soil, slime, capsulated)

Identification - polymorphic, 1.0 to 2.5µ . Gram negative, motile (peritrichous), cysts to withstand adverse condtion. The cyst accumulate poly hydroxylbutyric acid (PHP). Under favourable condition it produces vegetative cells. Polysaccharide gum producers.

Pigments - *A. vinelandii* – Green –yellow water soluble fluorescent pigments.

A. insignis, Yellow brown. *A.beijerinckii* yellow pigment.

Growth factors

Root exudates contain aminoacids, sugars,vitamins and organic acids that enhance the A.chro.

A.chroococcum in Indian soil is 10^4 to 10^5/g soil. *Cephalosporium* spp, addition of nitrogenous fertilizers are inhibits the growth of *A.chroococcum*. Addition of phosphatic fertilizers improves the growth of *A.ch*

A.ch. is also produces fungicidal agents that inhibit the growth of *Alternaria, Helminthosporium* and *Fusarium.*

A.chr. secrete thiamine, riboflavin, pyridoxine,cyanocobalamine, nicotinc, pantothenic acid,indole acetic acid, auxins , fixed nitrogen and gibberellins.

The overall reaction in the enzymatic reduction of atmospheric nitrogen to ammonia could boe stated as follows:

Six electrons and 12 ATP are needed to reduce one mole of N_2 to two ,moles of NH_3. . The compound pyruvate is a supplier of ATP and electrons. The N2 fixation is an anaerobic process.

Maintenance – maintained on nitrogen free media and periodically sub- cultured.

Cultivation - like *Rhzobium*.

Carrier -based inoculants – powdered lignite, peat soil, farmyard manure are neutralized with Ca CO3 and mixed with broth culture of Azo. Cured in trays for 2-5 days and then packed in polythene bags. The slurry is used for inoculation. For sugarcane and millets the inoculants are mixed in farmyard manure and broadcast near the root zone.

Crop –response – crop plants like Maize, sugarbeet,oats,barley, wheat and potato , their yileds increased from 8 to 12%., Onion -22%, rice23%, brinjal42%, cabbage 45%.

Azospirillum

In 1925, Beijerinck described a nitrogen fixing bacterium under the name ***Spirillum lipoferum.*** The nomenclature of this organism was revised and designated *as* ***Azospirillum*** **(Tarrend *et al.*, 1978). Bullow and Dobereiner (1975)** described the nitrogen fixation by Az- in rhizo –soil of Sorghum, wheat, maize and rye.

Media for isolation –1, Okon et al ., (1977) medium, 2, Nitrogen free bromothymol blue (NFB) medium.

Identification - polymorphic, Gram-negative, contains PHB granules, it produces thin pellicles and acetylene reducing activity in semi solid medium, five days old culture produces thick pellicle leading to pH to 3.0 or below, this bacterium uses ethyl alcohol for growth , oxidizing it to CO_2 and H_2O, On potato medium it forms dark brown colonies, On mineral medium with 10% sugar and bromothymol blue ,it forms orange colonies, nitrogen fixation activities carried by this bacterium at pH 5.6 and 7.2, maximum activities was observed at pH 6.7 to 7.0,electro.microscopic study- Azo. Show lateral flagella and one polar flagellum, cells are vibrioid and helical , it ferment succinate,malate lactate, pyruvate as their carbon source. Two important species are *S.lipoferum is group-I is able to utilize glucose, S.brasilense* grou-II is incapable of glucose. Both are denitrifier, can reduce nitrate to nitrite. This are found in the root system of grass, sugarcane, wheat is capable of nitrogen fixation. Soil below PH 5.7 and devoid of organic matter is not support the Azospi. *A. lipoferum* is common in root system of rice plant.

Maintenance - On agar containing ammonium chloride.

Mass cultivation – flasks contain ammonium chloride for three days at 35^0 C, Later,the flask contents are incorporated into a carrier.

Carrier based inoculants- Powdered sterilized farmyard manure+ soil, manure alone,munure+ charcoal, survived up to 31 weeks. farmyard manure+ soil is best carrier.

Crop response - it can fixed 40 kg N/ ha / year in the field of wheat,barley,sorghum,pearl millet or bajra and rice, 120kg/ha in fodder oats.

Seed inoculation with *A.braz* increased grain yield from 15 to 63% over uninoculated control.

Azo . produces IAA, cytokinin, gibberellins.

Phosphate solubilizing Microorganisms

Phosphorous is a vital, macro nutrient for microorganisms and plants. Based on chemical structure it exists in two forms 1, inorganic forms Calcium ,aluminium,iron phosphate, 2, Organic forms phytins, phospholipids, nucleic acids. Based on solubility, soluble forms and insoluble forms. soluble form (ortho phosphate) is nutrient for mic. plants.

Super phosphate is a chemical fertilizer applied to crop plants. Rock phosphate is a basic raw material for phosphatic fertilizer. Microbes which solubilize insoluble phosphate into soluble forms is phosphate solubilizing microorganisms is also known as Biofertilizer.(PSM). E.g- fungi, bacteria. (PSF), (PSB - Phosphobacteria). The main mechanisms for phosphate solubilization was acid production that result in decrease in pH. E.g – aluminium phosphate is solubilized by gluconic acid.

Mechanisms of phosphate solubilization.

The major mechanisms of phosphate solubilization is action of organic acid produced by PSM. The strains of *Bacillus liqueniformis* and *Bacillus amyloliquefaciens* are produce mixture of lactic, isovaleric, isobutyric, acetic,glycolic , oxalic, malonic, and succinic acid.

Mineralization – it refers to conversion of organic phosphorous into inorganic phosphorous by soil microbes.

Phosphatase – produced by bacteria belongs to enterobacteriaceae , e.g- alkaline phosphatase (gene phoA) of *E.coli*.

Insolulble phosphate – soluble phosphate by organic acid, phosphatase enzymes.

Isolation – Pikovskaya's medium Pikovskaya (1948) was developed for isolation of Phosphate solubilizing microorganisms. This medium contain tri-calcium phosphate as a insoluble phosphate compound. Rhizo- oil, serial dilution, plating on Pikovskaya's medium (agar) incubated at 28^0C for 4-5 days Phosphate solubilizing microorganisms are able to produce organic acid and phosphatase in this medium and solubilize the insoluble phosphorous into soluble phosphorous produce clear zone around the colonies.

Phosphate solubilizer – The most efficient Phosphate solubilizer are *Bacillus,* ***Pseudomonas* sp,** *Aspergillus* and *Penicillium sp.*

Plant growtth- promoting rhizobacteria - Phosphate solubilizing microorganisms e.g – *Pseudomonas sp* + N2 fixing microbes e.g- *Rhizobium sp, Azotobacter* sp are PGPR. Because they promote plants growth. It is also otherways called as **rhizobacteria.**

Pseudomonas fluorescens is used as **biofertilizer** as well as **biocontrol agents, it produces siderophores** (it promote Fe+3 (ferric iron) transport, iorn- sequestering agents) and **HCN**

(Secondary metabolite) and protease that showed antagonistic activity against ***Rhizoctonia solani, pythium and Fusarium sp.***

Phosphate solubilizers

Bacteria

1, *Bacillus sp. B. pulvifaciens, B.megaterium, B. subtilis, B.mycoides, B. fluorescence, B.circulans, B.mesentricus.*

2, Pseudomonas sp. P. putida, P. fluorescence, P. calcis, P. rathonia,

3, Escherichia. intermedia.

4, Xanthomonas sp

5, Flavobacterium sp.

6, Brevibacterium spp.

7,Serratia spp.

8, Alcalgenes.

9, Achromobacter spp

10, *Aerobacter aerogenes,*

11, Erwinia spp,

12,Nitrosomonas spp.

13,Thiobacillus thioxidans

Fungi.

1, Aspergillus sp , A.niger, A. flavus, A.fumigatus, A.terreus, A. awamori.

2, Penicillium sp, P. digitatum.

3, Fusarium sp, F. oxysporum.

4,Curvularia lunata.

5, Humicola,

6,Pythium,

7,Acrothecium sp,

8, Phoma,

9, Cladosporium sp.

10,Rhizoctonia sp,

11,Rhodotorula sp,

12,Candida sp,

Actinomycetes

1, Streptomyces sp.

Quantitative measurement of phosphate solubilization in culture medium.

Selected culture - cultivated in Pikovskaya's liquid medium for 6-17 days at 28^0 C. In the case of fungi, culture is filtered through Whatman No.42 filter paper to remove hyphae and pigments. The filterate is treated with charcoal to get clean filterate. For bacteria use Whatman No.1. The filterate is centrifuged at 10,000 r.p.m for 10-15 minutes to get clean solution. The solution is made into 50-100 ml. Take 10 ml of clean filterate, to this add 2.5 ml of Barton's reagent and made into 50 ml, after 10 minutes, the colour is read in a colorimeter using 430 µ wavelength.

Mass inoculam production .

Selected bacteria e.g- *Bacillus megaterium, Pseudomonas sp.* are grown in Pikovskaya's liquid medium for 6-17 days at 28^0 C. The broth is mixed in a sterilized carrier such as peat soil, lignite powder. The mixture is cured for a week at 28^0C in a large trays. The inoculants is packed in a plastic packet and stored at 15 – 20^0C until use.

Seed inoculation – similar to *Rhizobium* inoculants.

Phosphobacterin – is a commercial name for P.S.B. e.g – *Bacillus . megaterium* var *phosphaticum* is widely used in U.S.S.R(Union of Soviet Socialist Republic) and other European countries. Yield is increase from 5- 10% over the corresponding controls.

Benefited crop plants - Wheat, maize, paddy potato and sugarcane.

Azolla – an organic manure

Azolla is a floating fresh water fern and is ubiquitous in distribution. These are Bryophyte (non vascular plants ,lack of vascular bundles. Mosquito fern because it will destroys mos.larvae. These are rich in protein, amino acid, vitamins and minerals and is used as feed for hen and milching cow. Hens laid carotenoid rich egg and milk yield becomes high.

Bioremediation – Azolla can remove nickel, zinc, copper, chromium and lead from contaminated water.

Important species – *Azolla caroliniana, A.nilotica, A.filiculoides, A.mexicana, A.microphylla* and *A.pinnata.*

Important species in India – *A. pinnata.*

Green mat – it forms green mat over water, which often becomes reddish due to accumulation of anthocyanin pigments.

Structure of fern

The fern has floating branched stem, true deeply bi lobed leaves and true roots which penetrate the body of water. The leaves are arranged alternatively on the stem. Each leaf has a dorsal and ventral lobe. The dorsal fleshy lobes is exposed to air and contains chlorophyll. It has an algal symbiont (*Aanbaena azollae*) within central cavity. It has two types of reproduction found ,sexual,vegetative.In sexual repro. Two gametophytes are present, macro, micro. Macro. produce egg , microgame. Produce eight sperms, motile.

Importance of *Azolla*

The importance of *Azolla* as an organic input in rice cultivation was first demonstrated in North Vietnam in 1957, its potentiality has been recognized in U.S.A., Indonesia, Japan, Phillipines and China in 1977.

Uses of *Azolla* in China.

Azolla in China the optimum tem. 20-28^{0}C., pH is between 6 and 7. The field prepared for rice cultivation is flooded with water followed by seeding with *Azolla* and maintained for 5-10 days. The water in the field is drained off and the *Azolla* mat is ploughed into soil by using tractor. *Azolla* can also be grown simultaneously with rice seedling after transplantation.

In China 50% of nitrogen requirement of rice is fulfilled by *Azollae*.

Use of Azolla in India.

Azolla as a green manure in India,

Production – prepare a concrete tank , filled water of 4-5 cm. To this add 4-8 kg super phosphate p_20_5 / ha, apply carbofuran 1-2 kg/ha for controlling of insect parasites, pH8, temperature 20-30^0C for 20 days. The azolla is harvested and accumulated as heaps and allowed for decomposition for 7-10 days. The *Azolla* compost contains 94% water, 5% of nitrogen, 1% of potassium, phosphorous, calcium,, manganese and iron. Azolla is easily decomposed into ammonia which is available to rice plants.

Factors that destroys the *Azolla* – high or low temperature, insect parasites.

Supporting factors – application of phosphate, organic fertilizers.

Application - two methods of *Azolla* application have been recommended in India. 1, Direct incorporation of *Azolla* compost into the crop field. 2, Dual cropping method , Azolla is allowed for grow along with rice plant.

Crop response

It fixed 25-30 kg of nitrogen / ha. It is equivalent to 40 kg of ammonium sulphate.

Blue –green Algal inoculants (BGA)

These are cyanobacteria, contain two pigments chlorophylls (green) and phycocyanin(blue). Photoautotrophs, prokaryotes, found in wet soil,and aquatic environment, e.g –*Anabaena, Anabaenopsis,, Aulosira, Cylindrospermum, Nostoc, Calothrix,,Scytonema, Tolypothrix, Fischerella, Haplosiphon, Stigonema, Campylnema and microchetae,* these are nitrogen fixers, dominant species found in rice (*Oryza sativa*) fields. Besides nitrogen fixing, they release Vitamin B_{12}, auxins and ascorbic acid are utilized by rice plants. Biofertilizer.

Heterocytes- Nitrogen fixation found in specialized cells called Heterocysts, it contains nitrogenase, photosynthetic cells are concerned with photosynthesis. Heterocyst received electron and ATP from vegetative cells (photosynthetic cells). This cells, in turn, depend on heterocysts for nitrogen nutrition in the form of glutamine, glutamate or other a.a.

Isolation of BGA.

BGA are generally covered with mucilage contains bacteria, floated in rice field,Media for BGA-1, Pringsheim's medium,2, Chu's medium , 3,Crone's plant nutrient medium. Soil/ water sample is serially diluted , Aliquots of appropriate dilution is inoculated liquid media in flasks and incubated for several weeks in an illuminated growth room at 28^0C – 32^0C, The individual colonies are picked up and stored on agar slants for growth, identification and preservation.

Agro-climatic variation- In Assam, Tamil Nadu the dominant species are *Nostoc*, Whereas in Himachala Pradesh and Kashmir, *Anabaena* is the dominant form.

Japanese studies – *Tolypothrix tenuis* is dominant species in rice field of Japan. The algal inoculated rice field increased their yield from 2% to 19%.

Carrier materials - fine porous gravel and sponge rock gravel are fused with magnesium phosphate are suitable carrier for long distance transportation.

Algal inoculation significantly increased soil nitrogen and rice yield. Addition of ammonium sulphate was inhibit the growth, whereas organic matter addition were helpful for nitrogen fixation.

In U.S.S.R(Union of Soviet Socialist Republic) **and China –** increase crop yield from 13- 20%, *Anabaena azotica* is dominant species.

Indian studies – Blue green algal inoculation can supply 25 – 30 kg N/hectare.

BGA cultivation. – Open Air Shallow Culture **–** In this system , take a big iron metal tray lined with polythene, add a mixture of 10 kg soil and 200 gm of superphosphate and filled with 2-6 inches of water , pH is neutral, liming is recommended for acidic soil, when the soil settles , soil based starter culture is consists of Tolypothrix, *Nostoc*, *Anabaena* and *P lectonema* is sprinkled over the water in the tray for one week, a thick algal scum is formed, the water is drained off, the algal content should be dried, the algal flakes are collected and stored in polythene bags. The inoculated amount is 10 kg/ hectare.

BGA cultivation in rice fields- 5 kg of algal preparation for one cent is spreaded, the water level is 2.5 cm followed by broadcasting of superphosphate , 2 weeks in clay soil, 3-4 weeks in sandy soil **,** apply pesticide to destroy pests like daphnids, snails, mosquitoes. The average yield algae is range from 16-35 kg for each cent area of land.

Crop response – An average increase of 300kg/hectare of grain is possible by algal inoculation.

Actinorhiziae – *Frankia*

Root nodules caused by *Frankia* – Actinorhizal Sybiosis

A positive interaction between *Frankia* and root system of *Alnus* and *Casuarina* that result in root nodules in plants. Nodules size is 5-6 cm in diameter somewhat resembling tennis ball. Nodules contain endosymbiont *Frankia*. The endosybiont of *Alder* can fix 60 kg N/ha/year to157 kg N/ ha/year. In *Casuarina* 60kg/ha/year.

24 genera from 8 Angiosperms families possess actinorhizal root nodules

Host specific – Frankia is host specific, *Frankia* isolated from *Casuarina glauca* does not nodulate *Casuarina japonica*.

Isolation

The root nodules are washed repeatedly and disrupted inorder to release of toxic compound that may suppress *Frankia* growth. The nodules are surface sterilized in 3.0% osmium tetraoxide, cut the lobes into small pieces. The pieces transferred to a vial containing Q mod liquid medium containing 0.3 % agar and cyclohexamide 50 µg/ml to avoid fungal contamination, pour the 3 ml of the same medium over the layer containind nodule pieces, thereby providing microaerophilic condition, the activated charcoal is added to the medium, for elimination of phenolic compounds present in the nodular tissues, incubated at 25-28^{0}C upto 2 months. The colonies are spherical forms with 0.5-1.00 mm in diameter, the colonies may be diffuse on agar plate with loose of network of hyphae around the centre bearing many sporangia.

Cultural characteristics of Frankia.

Frankia has a long lag phase up to 14 days, slow exponential phase and autolysis of vegetative cells before reaching stationary phase, ph is 6-7, succinate as a good source of carbon . two types of nitrogen fixing root nodules, 1, they are spore(-) for nodules where spores are absent, and spore(+) for those containing many spores. Colonies are starfish like structure, sporangia are round, cylindrical,irregular, sporangia are sub-terminal or terminally, filled with spores, the hyphae are poorly branched, may be colourless, pigmented.

Entry of *Frankia* to the host plant

The actual entry of Frankia into root hairs has not been seen, but hyphae is simple or multiple threads or branched in inside the deformed hairs. The infected cells and the nucleus appear enlarged with prominent nucleolus.. The nodule consists of a central vascular bundle surrounded by endodermis and parenchyma cells with pockets of infected cells, red colour due to presence of anthocyanin.

Structure of Actinorhizal Nodule

1, *Alnus* type, it have lenticels for ventilation2, *Casuarina* type, it has no lenticels. Legume root nodule contain centralized infected bacteroid and leghamoglobin surrounded by cortex and vascular bundles. In actinorhizal nodule , there is vascular bundle surrounded by infected *Frankia*

Nitrogen fixation and assimilation

The nitrogenase enzyme of *Frankia* in *Alnus* is similar to *Rhizobium* in leguminous plants.

Mycorrhizae and Actinorhizal plants – e.g. Both Alnus and Casuarina have both VM and AM.

Proteoid roots

The formation of dense cluster rootlets on elongating lateral roots in *Casuarina equisetifolia*.These roots to absorbs phosphorous from soil.

Genetics of Frankia

The genome size of *Frankia* is twice that of *E.coli* and *Streptomyces,* 10,ooo kilo bases. The G+C content is 68-72 %. .It has plasmids ranging from 8kb to190kb The *nif* genes are located on plasmid 190kb.

Leaf nodules - is found in families of Rubiaceae and Myrsinaceae. This nodule contains *Mycobacterium, Mycoplana rubra, Flavobacterium, Klebsiella rubiacearum, Bacterium rubiacearum, Phyllobacterium rubiacearum*

Lichens

A symbiosis between alga and fungus –Microbe – microbe interaction.

Lichens is a thallus of dual organisms i.e. a fungus and an alga that form a self supporting combination.. The fungal component is mycobiont. The algal partner is phycobiont. Both are live in close proximity and appear as a single plant. The fungus forms the thallus of the lichens.

In lichen fungal mycelium derives nutrients, fixed nitrogen from the alga(biotrophic nutrition), the fungal provides protection,and shelter to algae. The algae in lichen include cyanobacteria and chlorophyta e.g- *(Trebouxia).*.The BGA includes Nostoc, Rivularia, Stigonema. The fungal partners include Basidiomycetes and Ascomycetes, no zygomycetes (Phycomycetes)involved.

Classification- Ascolichens – fungal component is ascomycete., basidilichens. On the basis of lichens habitat saxicolous growing on rocks or stones(crustose-e.g-*Graphis,Verrucaria,Haematomma,Leconara*), growing on leaves(foliose-e.g –*Gryophora,Parmelia,Sticta,Cetraria.)* and bark of the tree corticolous, growing on soil terricolous. The lichens produce strands or upright stalks, can about 10 cm stalk is fruiticose lichens e.g- *Cladonia, Usnea, Evernia and Ramlina*.

Fruticose lichens thalli are more complex, slender and freely branched.

Haustoria (haustorium)– fungal hyphae that penetrate into the algal cells for obtaining nutrients, sometimes more than one haustoria peneterate into a single algal cells. The association between algae and fungi are not specific.

Reproduction – vegetative processes, by fragmentation occurs when bits of thallus is composed of algal cells and fungal cells broken from the parent plant and fall on a suitable substrate. Lichens produce asexual reproductive bodies is called as **soredia**.

Economic importance of lichens

Bioindicators – lichens are bioindicators – sensitive to air pollutants like ozone, sulphur dioxide, toxic compounds. This pollutants are easily absorbed and destroy the chlorophyll a and b. This decreases photosynthesis.

Organic acids – are secreted by lichens that chemically weather the rocks – small rocks – soil particles.

N_2 fixation – Lichens with cyanobacteria can fix atmospheric nitrogen.

Usnic acid – lichens produce more than 100 lichen acid, e.g- usnic acid, obtained from *Usnea, Cladonia*. That inhibit the growth of some microorganisms, incorporated into antiseptic shampoos, used in European countries as a chaemotherapeutic drug for external application, **Lobaria** is used for curing pulmonary diseases. **Cetraria** produce mucilaginous substances used as laxative agents. **Cladonia** for curing intermittent fevers.

Essential oil – *Pseudovernia* sp , *Evernia* sp is used in perfumes. *Ramalina, Evernia* used in soap manufacture.

Pigments – *Parmelia omphalodes, P.saxatilis* produce pigments used to color many of the woolen fabrics in England.

Chemicals – chemicals obtained from *Lobaria sp, Cetraria sp* used in tanning of leather.

Alcohols – *Cladonia , Usnea, Ramlina* are used in prepatration of alcohol.

Food – *Cladonia* –is food for herbivorous animals, it is due to the presence of carbohydrate lichenin.

Cetraria, Lacanora, Dermatocarpon, Paremelia and *Umbilicaria* spp are edible lichens, harvested,dried, and cooked for consumption in India and Japan .

Mycotoxin – *Letharia vulpine* produce vulpinic acid which is poison for wolves and foxes.

Plants diseases

Plant Pathology

Pathology - means study of suffering plants.

Etiology/Aetiology – Study of living or non-living agents responsible for diseases

Pathogenesis- mechanism of disease development**.**

Pathogenecity – ability to cause diseases

Disease - A reaction between host and pathogen or abnormal/ harmful physiological processes

Pathogen – agent that cause diseases,

Systemic disease – pathogen spread throughout the plant.

Localised disease – pathogen spread limited area of the plant.

Epidemiology- It is concerned with the spread of pathogens.

Endemic disease – The disease is natural to one country/part of the earth.

Epidemic disease – a disease occurs periodically/ rapid disease development occurs only periodically.

Sporadic disease - a disease occurs irregular intervals.

Certain fungi, viruses and bacteria cause plant diseases that result in great economic losses and even severe food shortages.

To avoid this problems , planting of disease resistant varieties and high yielding varieties, to increases the cropping area.

Necrosis- Death of plant cells, may appear as spots in localized areas.

Canker – localized necrosis on stem, fruits.

Wilt – Droopiness due to loss of turgor.

Blight – loss of foliage.

Chlorosis – loss of chlorophyll pigments.

Hypoplasia- stunted growth.

Hyperplasia - Excessive growth.

Gall – tumourous growth.

Diseases in plants that result in severe economic lose(starvation in human population) that indces migration of human population from area to other or one state to other or one country to other. Hence it is necessary to control theses diseases.

Viral plant diseases

1, Tobacco mosaic disease

Worldwide in distribution. This disease affects the tobacco,potato,cucurbits , tomato, flowers and weeds. This virus damages to leaf,flower and fruits.

Causal Organism – *Tobacco Mosaic Virus*. TMV is rod shaped 300 nm long and 15 nm in diameter. NA is a single stranded RNA, contains 6400 nucleotides. NA is covered by protein coat, Protein coat is made by protein subunits 2130. The protein subunits are arranged in a helix. The molecular weight of each virus is 39 to 40 million. Each protein subunit distance is $20A^0$.

Transmission – It is not transmitted through the pest. Transmitted through the hands of worker handling infected and healthy plants. TMV is thermostable virus 120^0C for 30 minutes in dried leaf. The TMV infected leaf kept dry in the lab the remains infectious for more than 50 years. TMV is easily transmitted through mechanical sap, grafting and dodder.

Symptoms – chlorosis, curling, mottling, dwarfing, distortion and blistering of leaves. dwarfing of entire plant, distortion and discolouration of flowers. Dark green or light green areas on leaves.

Control – 1, TMV resistant varieties of tobacco must be grown. 2, The worker should wash their hands with 3% trisodium phosphate or soap.

2, Yellow Vein Mosaic of Bhindi

Four to five week old plants are very much affected by this diseases.

Causal Organism - *Yellow Vein Mosaic of Bhindi Virus*

Transmission – It is transmitted through bite of infected whitefly *Bremisia tabaci* , and also by bhindi leafhopper.

Symptoms –The infected leaves exhibit a very distinct yellow net work of veins and veinlets, fruits are distorted, malformed and yellow green in colour.

Control-1. Resistant varieties should be grown. 2. Four to six sprays of rogor.3. Spray with Follidol (0.3%) insecticide.

3. **Tungro of Rice**.

Tungro means degenerative growth.

Causal Organism – *Rice Tungro Bacilliform Virus* and *Rice Tungro Spherical Virus*

This viruses are bacilliform capsid, size is 130 to 30 nm , double stranded DNA, both are multiply independently, both are transmitted by leafhoppers, plant debris is a source of this disease.

Transmitting agent – Both viruses are transmitted by Green Leafhopper ***(Nephotettix virescens)***

Symptoms

Stunting of plants and discoloration of leaves. On older leaves small, rusty, necrotic spots. This virus affect both vegetative and reproductive biology of plants. It reduces yield, weight and starch content of grain.

Control – Application of Furadan 3.5 kg/ ha or Furatox 1.5 kg/ ha.

4, Potato Spindle Tuber

Destructive disease in potatoes

Causal Organism – *The potato Spindle Tuber Viroid* . it contains an infectious RNA of low mol.wt, single stranded RNA molecule, it spreads mainly by knives used to cut healthy and infected potato seed tubers and during handling and planting of crop.

Transmission- by pollen and seed and by several insects including aphids, grasshoppers and bugs.

Symptoms – infected plants become dwarfed, leaves are small and leaflets are darker green. The tubers are elongated with cylindrical middle and tapering ends. Yield is reduced to about 25%. Or more

Control- Planting PSTV free potato tubers in the field.

Root Knot of Vegetables

Causal organisms – Four species of the genus Meloidegyne(Nematodes)

M.incognita, M.javanica, M.arenaria and M.hapla, it attack vegetables commonly in various part of the world. More common species are M.javanica and M.incognita. The nematodes starts feeding on root cells and injecting gland secretion and biochemical and cytological changes occur in the vascular system. These cells are abnormally develop and enlarge to form giant cells. Which serve as food for nematode. Threre is continuous in cell multiplication of neighbouring cells. The cells become hyperplasic and divide repeatedly to give rise to swelling and knot formation.

Symptoms- root galls or knot. Heavilly infected roots become shorter with fewer branched roots and root hairs..This disease is common in cabbage and cauliflower.

Control- Seedling roots be dipped in the chemical 500 ppm thionazin for 15 minutes. Crop rotation, planting of resistant crops, solarisation of soil.

Fungal plant diseases

Tikka Disease of Groundnut

This is an important fungal disease of groundnuts. There is a serious leaf-spotting and defoliation of groundnut plants.

Causal organisms – by two fungi, *Cercospora personata* and *Cercospora arachidicola.* Both may occur on same leaf at the same time.The symptoms produced by two organism are differ.The leaf spots produced by *C.personata* are more circular 1-6-mm in diameter and necrotic lesion on both surface. The colour of the lesion is brown to black colour. The mycelia of fungi are intracellularly.

Leaf spots produced by *C. arachidicola* are larger and irregular, 4-10 mm in diameter.

High humidity is enhancing factor for this disease.

Control

1, Crop rotation.2,Eradiction of diseased plant from crop cultivated area.3, Seed treatment with agrosan and Cu So4.4, A variety of systemic fungicides are used Bordeaux mixture, Dthane and

Fycol. Five to six sprays are needed.

Red Rot of Sugarcane

It is a serious disease of sugarcane.

Causal organism – *Colletotrichum falcatum* – hyphae are slender, branched septate , colourles,Inter and intracellular. The hyphae grow in vascular bundles and the protoplasm changes in colour and gummy dark substance oozes out of cells filling the intercellular spaces. The conidiophores produces conidia and disseminated by wind, rain and water of irrigation and insects.

Symptoms

Split open stems, reddening of vascular bundle and pith. The juice is bad odour due to conversion of sucrose to glucose to alcohol. A dark reddish area, which elongates rapidly, forming blood -red lesion with dark margin.

Control –Use of healthy sets, crop rotation, use of resistant varieties, good sanitation.

Blast of Rice

Worldwide in distribution, High humidity and high rainfall are conducive factor for this disease.Symptoms – symptoms appear on leaves, leaf sheath. It appears small, bluish flecks(spots) on younger leaves..Similar spots appeared on leaf sheaths. At later stage, the spot become grey or straw coloured in the centre. On mature inflorescence brown to black spots are formed.

Causal organism- *Pyricularia grisea.* This fungus produces toxin pyricularin which are toxic to plants. It also produced pectinolyase enzyme which dissolve cell wall of plants. The fungus produces septate, branched and multinucleate mycelium. The mycelium produces conidiophores produces conidia, conidia are three-celled into multinucleate. Each conidium produces several germ tubes on germination.This fungus rarely survive in soil due to high temperature, it also survive on grass hosts. The conidia may be disseminated by wind. Temperature and moisture are important factors for incidence of this disease in rice plants, dark period is favourable for this diseases.

Control – 1, Foliar spray of Bordeaux mixture. 2, Seed treatment with copper sulphate.

Brown /Leaf Spot of Rice

The disease was a major factor for failure of rice crop in Bengal famine in 1942-43. The yield loss was 90%. In India the disease is prevalent in U.P, Assam, T.N, Kerala.

Symptoms. – Grey to brown spots on leaves and leaf sheath. In severe infection the whole grains surface become blackened and discoloured.

Causal organism - *Drechslera oryzae (Helminthosporium oryze).* Ascomycete, in host cells the mycelium are inter and intracellularly, conidiophores are produces conidia, conidia are gold brown , smooth. This fungus grow in wide range of temperature, produces phytotoxin ophiobolin A and ophiobolin B these are toxic to plants. Seed-borne, the fungus survive in seed for 2-3 years. The fungus does not survive for long in natural soil. Several weed grasses like *Arundo donax, Leersia hemndra* are host for this pathogen. The diseases increases with both deficiency and excess of nitrogen.

Control - Seed treatment with Campogram followed by Dithane and vitavax . Spraying fungicides Bordeaux mixture(5:5:20) Dithane 0. 2% , Blitox 0.2% and Benlate 0.2%. at regular intervals.

Powdery mildew of Pea

The disease is more severe at the time of pod formation.

Symptoms - There is white floury patches on both side of leaf, tendrils, stems and pods. In advance stage the aerial parts may be covered with floury patches. The powdery mass consist of mycelium and spores of pathogens.

Causal organism – *Erysiphe polygoni*. Mycelium are white and septate, conidiophores are produces conidia, disseminated by wind. The conidia are barrel –shaped or cylindrical, hyaline and 1- celled.

Cleistothecia (ascus) produces on intact leaves on the plant, they may develop in diseased plant debris in soil, the ascospores are released after disintegration of the ascus wall . These ascospores infect first the lower older leaves in next season.

Control- Foliar sprays with chemicals sulphur dust 25-30 kg/ha, Karathane 0.2%, Morocide 0.1%. Cosan 2kg/ha, elosal 5kg/ha.

Bacterial Plant diseases

Citrus Canker

The disease is widespread in all citrus growing areas of the world. Originated from China. It is particularly in India, China, Japan and Java.

Symptoms- . The disease attack on leaves, thorns, old branches and fruits.The spots are white or grayish and surrounded by a yellow halo. On large branched spots are irregular, rougher. On fruits cankers are similar to those on leaves except that yellow halo is absent.

Causal Organisms- *Xanthomonas campestris* var- *citri*. A Gram-negative rod, forming chains and capsule but no endospores . Size is 1.5 to 2 µm,aerobic, nitrate negative, amber yellow colour colonies on beef agar, tis bacterium enters through the stomata and wounds. Multiplies in intercellular spaces, mild temperature and wet weather favoured the disease, disseminated by men.

Control- 1,Destruction of diseased plants by burning them.2, Use disease free stock for planting.

3, Spraying the plant before planting with 1% Bordeaux mixture. 4, Spraying with Streptomycin and phytomycin.

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Bacterial Leaf Blight of Rice. (Burnt appearance – death of plant parts)

This disease is more severe in India. Previously it remained confined in Maharashtra, but later appeared in Bihar and northern part of the country.

Symptoms – Yellow or straw coloured stripes with wavy margin develop on both edges of leaf. The stripes usually develop from the tip towards the base. At later stage the infected tips become dry and twisted. In severe stage the leaf sheath and culms also become blighted, causing the death of affected parts of plants.

Causal Organism - *Xanthomonas campestris* -var- *oryzae*, Gram negative, 1to 2µm,, no spores, no chains, with sigle polar flagellum,,aerobic,,nitrate negative,, this bacterium survive in crop debris,, disseminated by infected seeds , insects and wind, bacteria enters through the wounds and stomata. High humidity, high nitrogen fertilizers, temperature 22 -26^{0}C, poorly drained are enhancing factors.

Control. 1, Five foliar sprays of Agrimycin plus copper oxiumchloride , 2, Resistant varieties 3, Seed treatment it involves soacking of seed in solution of Agrimycin(0.025%) for 12 hours.

Little Leaf of Brinjal

The disease is common in India. First reported in Coimbatore. The affected plants are failed for flowering.

Symptoms – The diseased plant develop small leaves and they appear as sticking to the stem. The leaves are small , narrow, soft, smooth and yellowish in colour. Flowering and fruiting are rare.

Causal Organisms – *Phytoplasma* spp., size is 40 to 300µm. infect phloem of stem, roots, petioles, leaves. It also infect some weed hosts. From these weeds, this is transmitted to brinjal by insect vector.

Control – 1, Eradication of weed hosts like *Datura* and *Vinca* and diseased plant from cropping area. Spraying of pesticides to reduce spreading of pests.