Soil Fertility and Fertilizers
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Lecture 46
Biofertilizers and Management of Fertilizers and Manures in Soil

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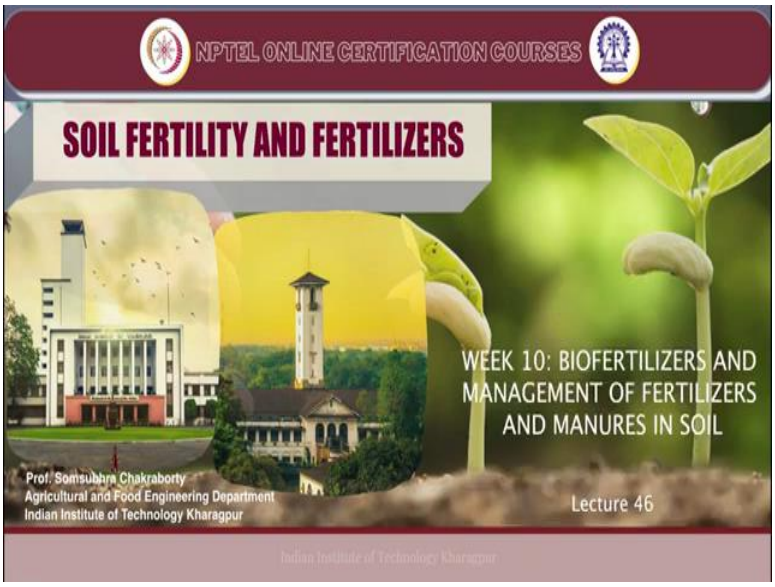

Welcome friends to this 10th week of lectures for this online NPTEL certification course of Soil Fertility and Fertilizers. This is a new week and in this week we are going to talk about Biofertilizers and Management of Fertilizers and Manures in Soil. Not only we are going to learn about biofertilizers, but also we are going to discuss about biochar and also other techniques like fertigation.

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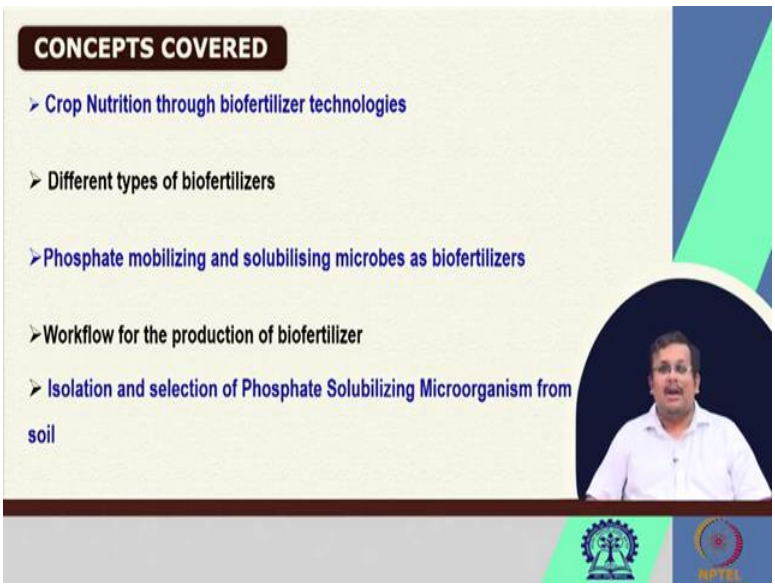

So, let us first start with bio fertilizer and in this lecture, lecture number 46, we are going to focus on this following concepts. So, first of all we are going to focus on the crop nutrition through biofertilizer technologies. Then, we are going to discuss about different types of biofertilizers, then we are going to discuss phosphate mobilizing and solubilizing microbes as biofertilizers, then we are going to see the workflow for the production of biofertilizers.

And finally we are going to see isolation and selection of phosphate solubilizing microorganisms from soil. So, in this lecture, we are going to have a comprehensive idea about biofertilizers and how we develop biofertilizers for commercial purpose.

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Now, these are the keywords for this lecture, first of all biofertilizers, then rhizobium, Azotobacter, Azospirillum, and phosphate mobilizing and solubilizing organisms.

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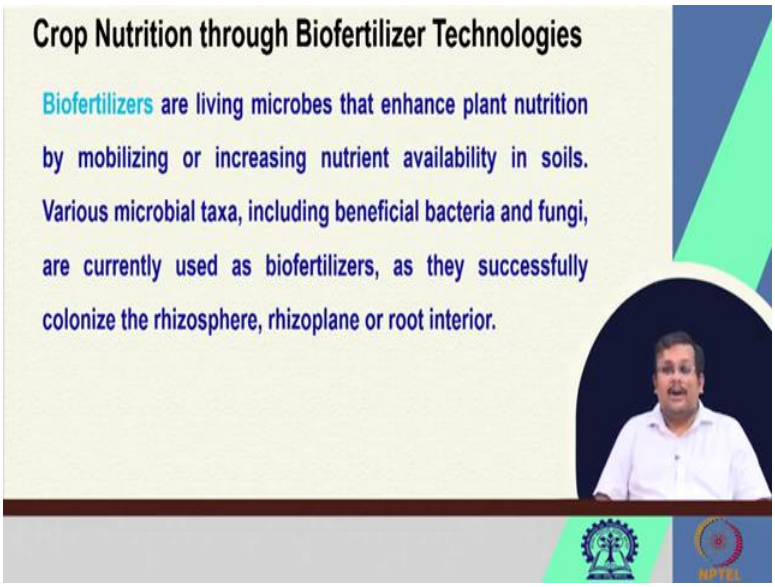

Now, let us start what is biofertilizer? Let us start with the discussion or definition of biofertilizer. Now, remember that biofertilizers are living microbes, these are living organisms that enhance plant nutrition by mobilizing or increasing nutrient availability in soils.

So, one of the most important feature of features of biofertilizers is, it consists of living organisms and these living organisms helps in plant nutrition by mobilizing or increasing nutrient availability in soils. Various microbial taxa, including beneficial bacteria and fungi are currently used as biofertilizer as they successfully colonize the rhizosphere, rhizoplane or root interior. And by colonizing in the rhizosphere or rhizoplane or in the root interior they help in the nutrition of the plant.

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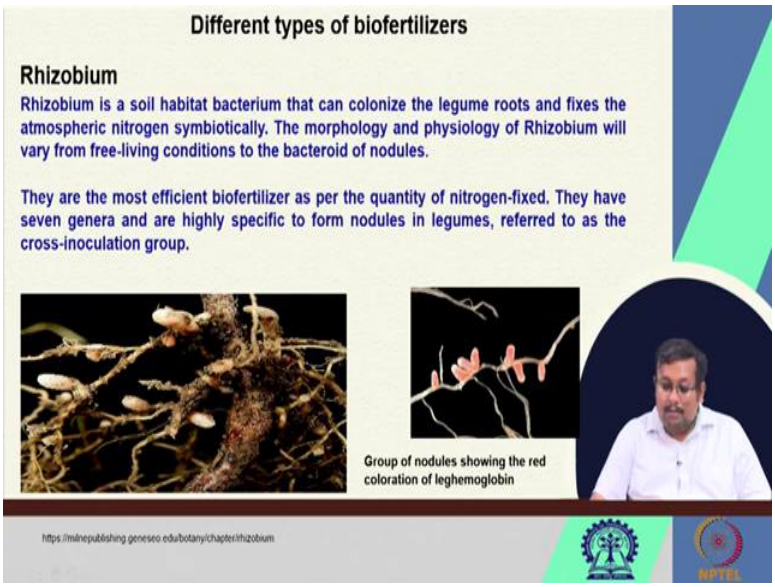

Now, one of the most famous I would say biofertilizers is rhizobium based biofertilizers, now rhizobium is a bacteria, so it is a soil habited bacterium that can colonize the legume roots and fixes the atmospheric nitrogen symbiotically. So, basically these rhizobium bacteria they live symbiotically in the legume plants root and they can synthesis atmospheric nitrogen. They can fix atmospheric nitrogen and synthesis organic nitrogenous compounds.

So, the morphology and physiology of rhizobium will vary from free living condition to the bacteroid of nodules. However, when fixing this nitrogen from the atmosphere of course rhizobium creates a symbiotic relationship within the plant root. Now, they are the most efficient biofertilizer as per the quantity of nitrogen fixed, they have seven genera and they are highly specific to form nodules in legumes, referred to as the cross inoculation group.

Now, in this picture you can see these nodules in the roots and these nodules are being created by these rhizobium bacteria. So, these are group of nodules showing the red coloration of leg haemoglobin, which are present due to and this leg hemoglobin is present in this nodules, where created by this rhizobium in the roots of the legume plants and they fix nitrogen from the atmosphere.

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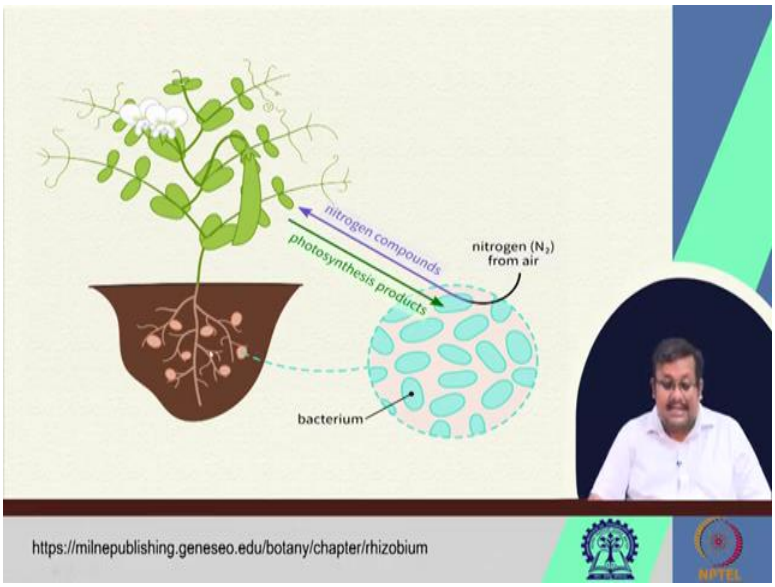

Now, in this picture things are a little bit clear, so you can see if there is a legume plant and these are their roots and these are the nodules. So, this bacterium leaves in these nodules and they fix the atmospheric nitrogen from air and they convert these atmospheric nitrogen into nitrogenous compound for the growth of the plant, in return they will get the photosynthesis products from the plant for their survival.

So, you can see in this root nodules this bacteria are colonizing and they are making a symbiotic relationship where they are converting the atmospheric nitrogen and giving this synthesized nitrogenous product to plant and in return in this symbiotic relationship they are getting the photosynthesis products for their survival.

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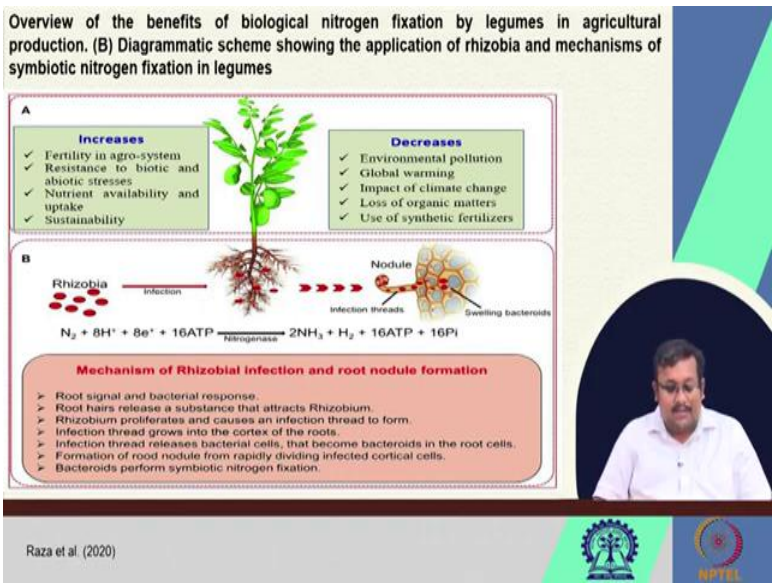

Now, if we see the overview of the benefits which we generally get from this legume rhizobia symbiotic relationship, this will this diagram will basically show that when there is a symbiotic relationship between rhizobia and the nodule and the legume plants, we can see there is an increase in fertility of the soil, because when the plant dies those nitrogen which have been synthesized which the nitrogenous compounds which are synthesized by these organisms will come into the soil.

So, that will increase the fertility of the system and also they will increase the resistance to biotic, and antibiotic stress and they can increase the nutrient availability and uptake and overall they help in the agriculture sustainability. At the same time they can decrease environmental pollution, global warming, they can also impact reduce the impact of climate change and also they can reduce the loss of organic matter and they can help in reducing the use of synthetic fertilizers, because these rhizobium when they synthesize atmospheric nitrogen that simultaneously reduces the need for synthetic fertilizers.

And also these by incorporating these legumes in the crop rotation can enhance the organic matter in the soil and of course that can reduce the impact of climate change and of course global warming. So, more fertility in the soil can enhance the group enhance the growth of the legumes and that can help in addition of organic matter in the soil.

So, you can see here, if these are rhizobium bacteria and they are creating the infection thread within the root and ultimately these nitrogen will get energy from this ATP in the presence of nitrogenous enzyme, which is there in this bacteria and ultimately they will convert into the ammonia and then hydrogen molecule. So, ultimately this rhizobia when they create the infection that initially creates this infection thread and then ultimately you can see swelling bacterioids and forming the nodules.

Now, mechanism of rhizobial infection and root nodule formation it consists of several step. First of all there is a root signal and bacterial response and secondly root hairs release a substance that attracts the rhizobium and thirdly the rhizobium proliferates and cause and causes and infection threat to form and infection thread grows into the cortex of the roots as you can see this infection thread goes into the cortex of the roots and infection threads release the bacterial cell.

So, you can see the bacterial cells are ultimately released by this infection thread that become bacteriod cells in the bacteria in the root cells and ultimately that help in formation of root nodule from rapidly dividing infected cortical cells.

So, using these infection thread these bacterial, the bacterial basically goes to the root cell and basically goes to the cortex and ultimately release into the cortical cell and then they divide vigorously and ultimately creating those nodules and then bacteriods perform symbiotic nitrogen fixation. So, this is how rhizobium bacteria affects atmospheric nitrogen by symbiotic relationship with the legume.

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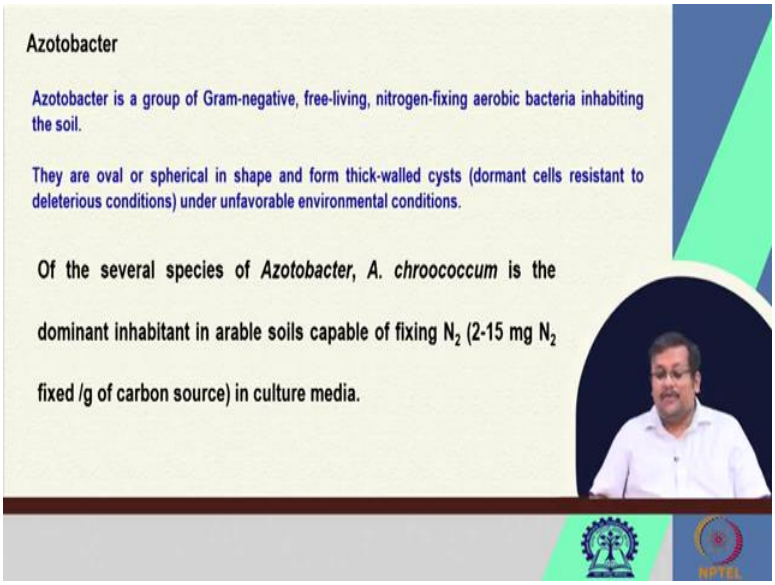

Another important bacteria which helps in nitrogen fixation is called the Azotobacter. Now, azotobacter is a group of gram-negative free living nitrogen fixing aerobic bacteria inhabiting in the inhabiting in the soil. Then they are generally oval or spherical in shape and form thick-walled cyst, what are the cysts? Cysts are basically dormant cells which are resistant to deleterious conditions, so the basically they can leave for extended period of time in harsh environment and under unfavourable environmental condition.

Now, there are several species of course of azotobacter, however azotobacter chroococcum is the dominant inhabitant in arable soils capable of fixing atmospheric nitrogen, that is 2 to 15 milligram nitrogen fixed per gram of carbon source in a cultural media.

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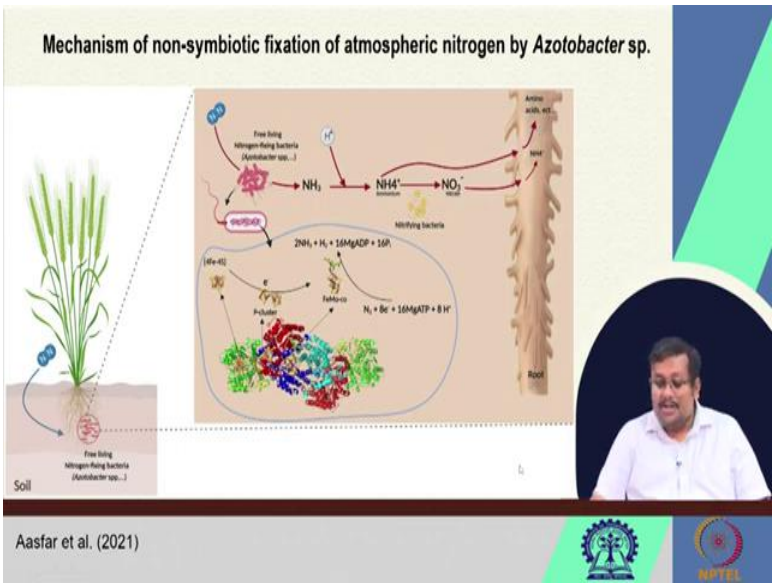

Now, what is the mechanism of non-symbiotic fixation of atmospheric nitrogen by azotobacter? So, azotobacter as I have told you these are the free living nitrogen fixing bacteria and this free living nitrogen fixing bacteria basically converts these atmospheric nitrogen into ammonia.

And then they create this ammonium ion these ammonium ions are taken up by the plants, roots to convert into amino acids and also this ammonium ion can convert into nitrate by nitrifying bacteria and these nitrate is also again taken up by the plant to convert into ammonium and other nitrogenous compounds. So, this is how these microorganisms fix non-symbiotically atmospheric nitrogen.

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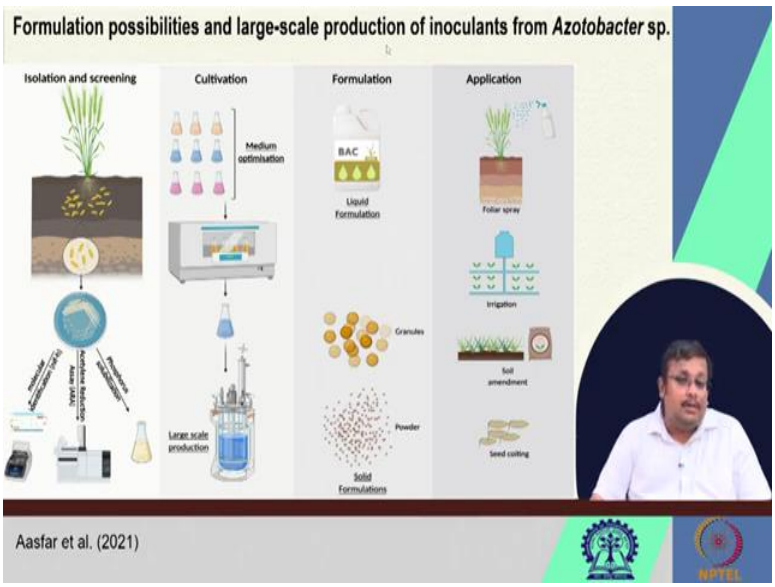

Now, the next question comes to our mind how we can develop the formulation in large scale for large scale production of inoculants from azotobacter species? So, first of all there are 4 different stages for large scale production, first of all isolation and screening, second one is cultivation, third one is formulation and fourth one is application.

So, in the first stage we isolate the bacteria from the soil by plating in the media and then once we isolate these colonies of azotobacter, we identify them by molecular techniques and also we use different types of other technique, like acetylene reduction S i and also phosphorus solubilisation.

So, basically we first isolate from the soil and then we do the molecular identification one and also once we do the molecular identification then we go to the cultivation steps, so first we inoculate them into the medium and then put them into the in the BOD chamber for their multiplication or we can go for the large scale production in a fermenter. And then the formulation we can create this liquid formulation or granules or powder form powders or granules are the solid formulations.

Now, once we develop this liquid or powder formulation or the solid formulation, we can either apply through foliate spray or through irrigation or through soil amendment or through seed coating. So, these are different ways to which we can go ahead and apply and first develop first and then apply the azotobacter by fertilizer formulations in the crop, to the crop.

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Now, another important biofertilizer is azospirillum. Now, azospirillum is a non-symbiotic associative nitrogen fixer, aerobic bacteria which can associate with the growing root system

of a variety of crop plants and increase their growth by different mechanism called induced systemic tolerance.

What is induced systemic tolerance? Induced systemic tolerance may include production of different metabolites, plant hormones, antioxidants, osmotic adjustments and defense strategies such as the expression of pathogenesis-related genes. So, using these strategies they can help in the growth of the plant.

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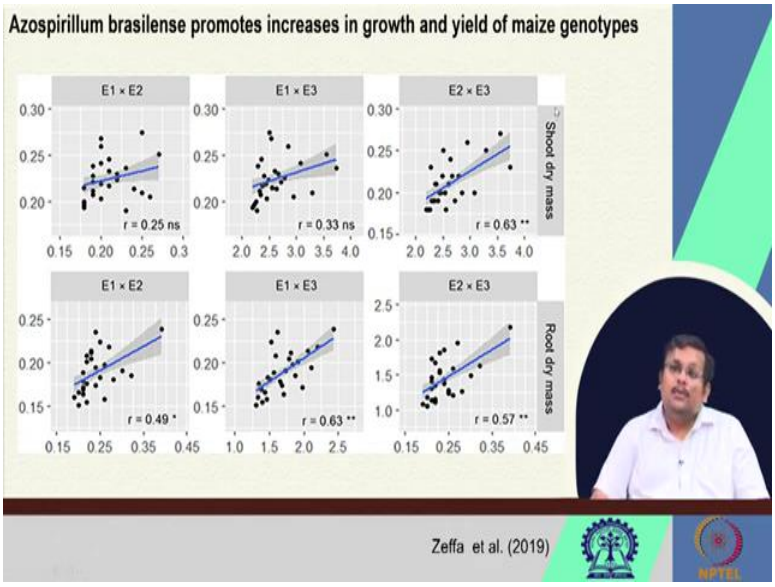

Now, we have seen that in a research the azospirillum brasilense can promote the increase in growth and yield of maize genotypes. So, we can see there is an increase of shoot dry mass and also root dry mass with the inoculation of with the application of azotobacter brasilense.

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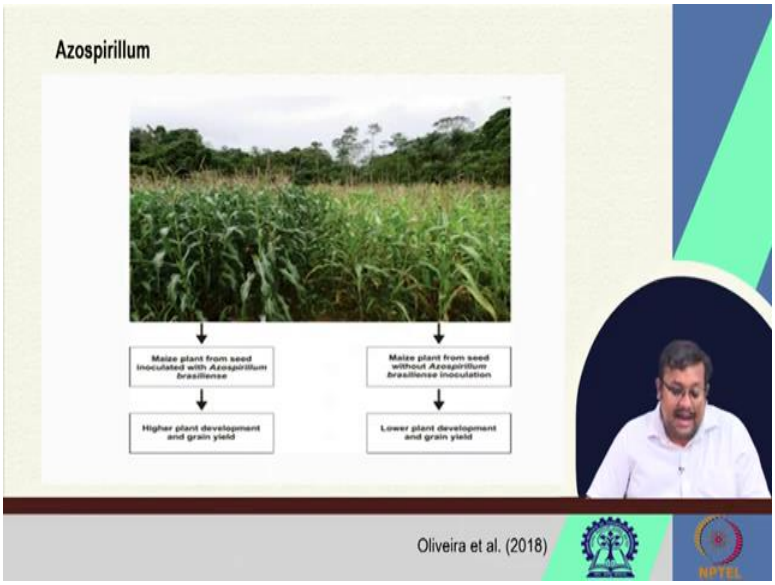

Also azospirillum you can see here, here this shows the maize plant from the seed inoculated with azospirillum brasilense and you can see higher plant developed and grain yield, so you can see increased plant development and granil, however in this half you can see maize plant from the seed without azospirillum brasilense inoculation and it shows the lower plant yield. So, you can clearly see the difference for azospirillum inoculation and how these biofertilizer can enhance the plant growth you can see by comparing these this maize crop growths.

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Now, cyanobacteria or blue green algae is are the group of photosynthetic organisms which can easily survive on bare minimum requirement of light, carbon dioxide and water. They are phototrophic and naturally occur and they naturally occur in several agro-ecosystems, like paddy fields and from Antarctica to Arctic poles.

Now, in the paddy fields you can see they can colonize, they can create a symbiotic relationship with a water fund called Azula and these is a specific blue green algae or cyanobacteria called anabina azuli they can create the symbiotic relationship with Azula and with those symbiotic relationship they can also fix atmospheric nitrogen. So, they fulfil their own nitrogen requirement by nitrogen fixation just like rhizobium and azotobacter and produce some bioactive compounds which promote the crop growth or protect them from pathogens and improve the soil nutrient status.

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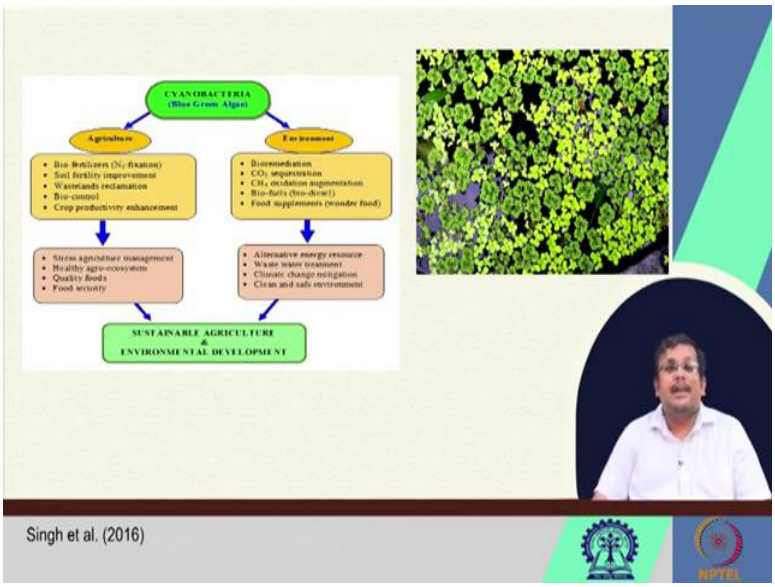

Now, this is the azola which you can frequently see in the paddy field and these anabina azuli which is an important blue green algae or cyanobacteria they can colonize at the bottom of this azola and they can fix atmospheric nitrogen. Now, cyanobacteria are also known as blue green algae, so in agriculture they can be used as biofertilizers, they can help in soil fertility improvement, they can also help in the wasteland reclamation, then also can help in bio control of pathogens and crop productivity enhancement.

At the same time from the environment point of view they can help in bioremediation, carbon dioxide sequestration, because they are adding the organic matter into the soil and then methane oxidation, augmentation and biofuels producing biofuels and food supplements also can be generated from this blue green algae.

So, while they are helping in the crop productivity enhancement ultimately they help in they can help in agricultural management, they also can maintain healthy agro ecosystem, they can ensure food security and also they can maintain the quality of the food.

At the same time from the environment point of view also they acts as an alternative energy source, they help in the wastewater treatment and they can help in the climate change mitigation and finally they can help in cleaning and saving the environment from different types of environmental hazards. So, in overall these biofertilizers can the cyanobacteria can help in sustainable agriculture and environmental development.

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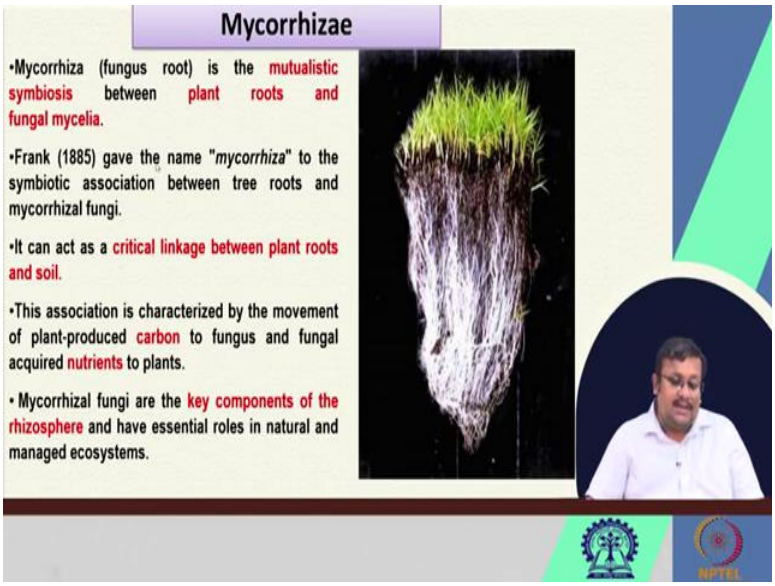

Now, let us talk about phosphate mobilizing and solubilizing microbes as biofertilizer. In the first let us talk about the mycorrhizae. Now, mycorrhizae, basically comes from 2 terms, one is myco, another is rhizae. The myco means fungus and rhizae, basically comes from the root.

So, the meaning of mycorrhizae is basically fungus root, so it is basically a mutualistic symbiotic relationship between plant roots and fungal mycelia. Frank in 1885 gave the name mycorrhizae to the symbiotic association between the roots, tree roots and mycorrhizal fungi. It can acts as a critical linkage between plant roots and soil.

So, this association between fungus and the plant root is characterized by the movement of plant-produced carbon to fungus and fungal acquired nutrients to plants. Now, mycorrhizal

fungi are the key components of the rhizosphere and have essential roles in natural and managed ecosystems.

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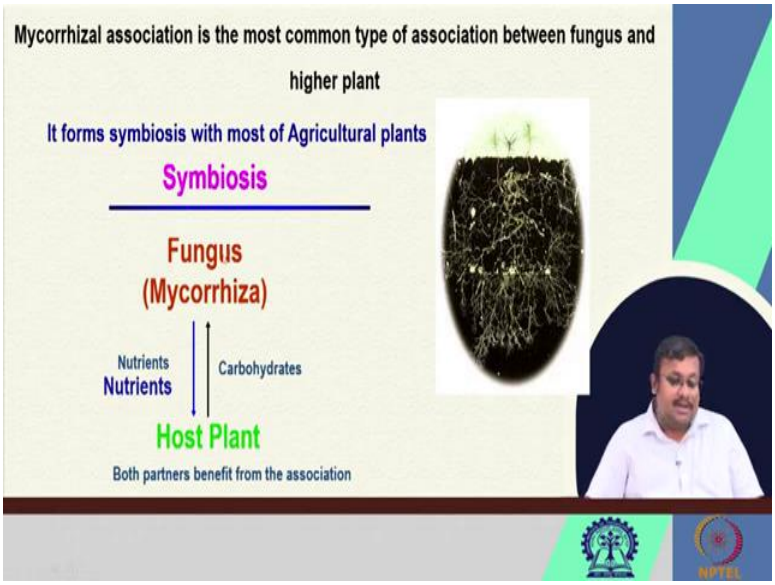

Now, remember that mycorrhizal association is the most common type of association between fungus and higher plant and we can see a symbiotic relationship between fungus and higher plant. So, in this symbiotic relationship mycorrhizae or the fungus basically gets carbohydrate from the plants in return they give the nutrients which are required by the plant for their growth. So, both partners benefit from their association. So, this is a symbiotic relationship between the host plant and fungus.

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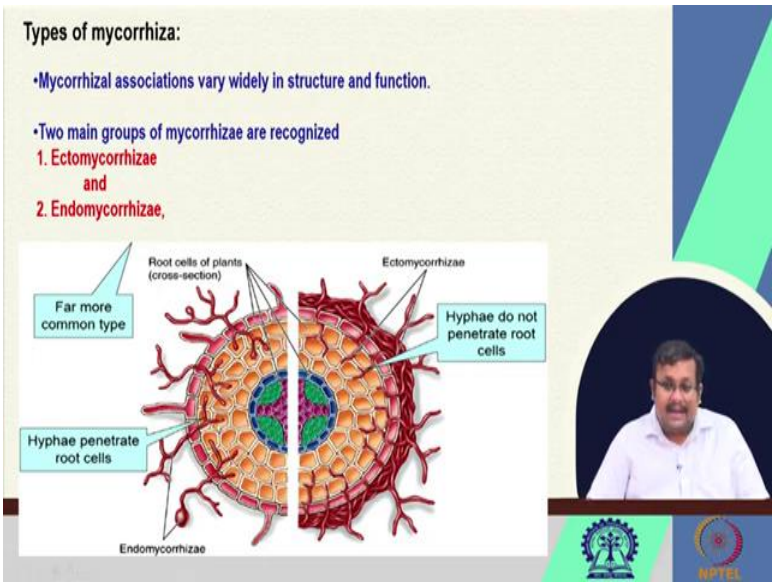

Now, what are the different types of mycorrhizae? Mycorrhizal association vary in structure and function and there are 2 major groups of mycorrhizae, first of all ectomycorrhizae and endomycorrhizae.

Now, let us see ectomycorrhizae, ectomycorrhizae are those mycorrhizal association where we can see the fungus is creating a mantle or sheath outside the plant root and hyphae do not penetrate the root cell. So, here you can see the hyphae, hyphae do not penetrate the root cell, so these are ectomycorrhizae.

On contrary Endomycorrhizae, you can see the root cells of plants, so you can see these are the root cells of the plants in cross section, so in case of endomycorrhizae as the name suggests the hyphae penetrate the root cell. So, you can see the hyphae is directly penetrating the root cell. So, these are 2 different types of mycorrhizal association.

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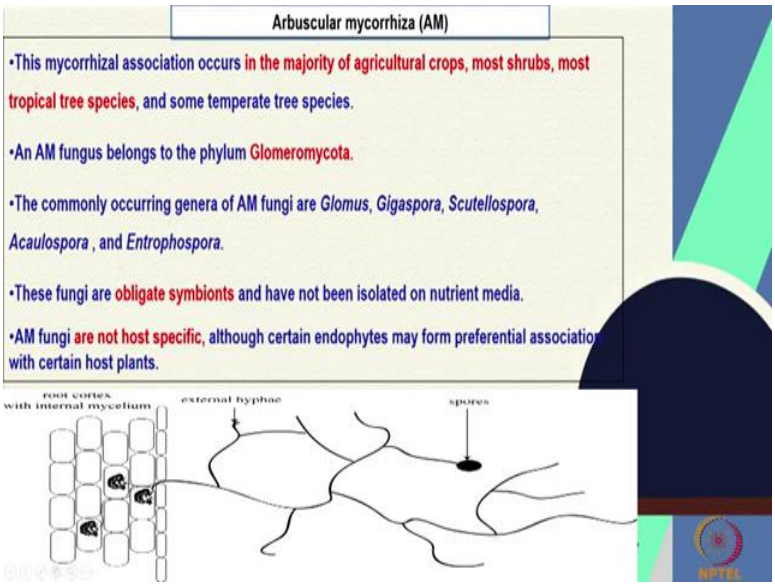

Now, arbuscular mycorrhizae is a specialized endomycorrhizae which can help in plant nutrition. Now, these arbuscular mycorrhizae or AM association occurs in the majority of agricultural crops most shrubs and most tropical tree species and some temperate tree species.

Now, an arbuscular mycorrhizae fungus belongs to the phylum Glomeromycota and the commonly occurring genera of these AM fungi are Glomus, Gigaspora, Scutllospora, Acaulospora, and Entrophospora. And these fungi are obligate symbionts and have not been isolated or nutrient media.

So, they have to be in symbiotic relationship to survive for their survival, so that is why they are called obligate symbionts. Also these AM fungi are not host specific although certain

endophytes may be from preferential association with certain host plants. So, you can see that if this is a root cortex with internal mycelium, so you can see this hyphae are growing inside and these are examples of arbuscular mycorrhizae.

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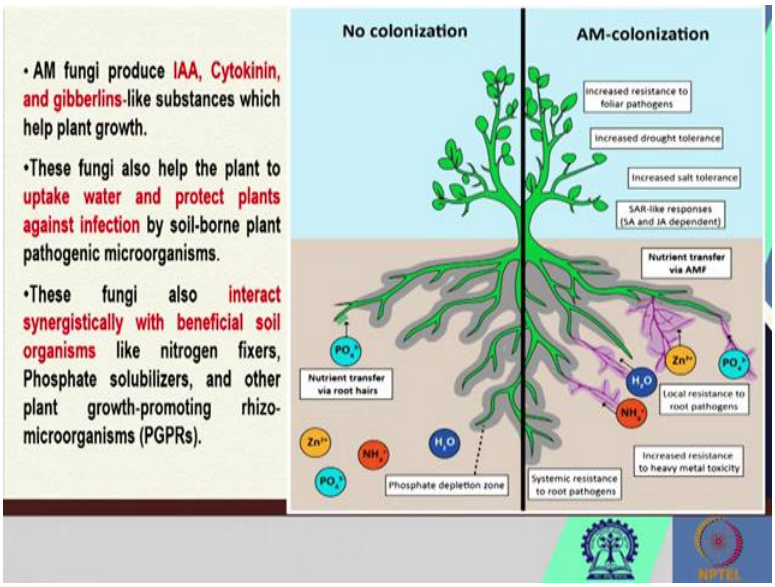

Now, but the how does AM fungi helps in plant nutrition? Now, these AM fungi produce indole acetic acid, cytokinin and gibberelins-like substances, this IAA, cytokinin and gibberellin, these are plant hormones, plant growth hormones. So, these AM fungi produces these hormones, like substances which help plant growth, then these fungi also help the plant to uptake water and protect plants again infection by soil bone plant pathogenic microorganisms.

And also they interact synergistically with beneficial soil organisms, like nitrogen fixer, phosphate solubilizers and other plant growth-promoting rhizo-microorganisms or rhizo plant growth promoting rhizobacteria or rhizo microorganisms, we call it PGPRs.

So, you can see where the 2 conditions side by side when there is no colonization of course nutrient transferred to the plant root, so whatever phosphate is nearby to the roots they can be uptaken, however the zinc, phosphate, ammonium, which are far apart from the root cells, root zone cannot be uptake by the cannot be uptaken by the plant.

However, when there are some mycorrhizal association, these mycorrhizal hyphae can go to these non-accessible spaces, because they are very fine and thin, so they can go to those non accessible space and they can access these zinc, phosphate and other nutrients from these un-accessible points and plant can uptake.

So, you can see here they can be these microorganism association can help in uptaking of these different types of nutrients. So, ultimately they also helps in increasing the resistance to foliar pathogens, they also help in increasing drought tolerance, increasing salt tolerance and also they can also help in the nutrient transfer. So, this is how these arbuscular micorrhizal helps in plant nutrition.

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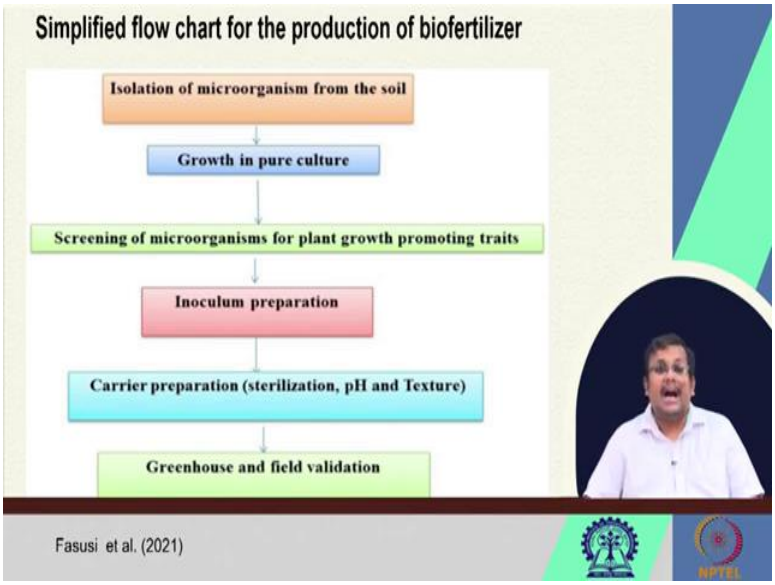

Now, if we see the simplified flowchart for the production of the biofertilizer, of course it starts with isolation of microorganisms from the soil and then we isolate it from the soil and then we grow in pure culture and once we develop them in the pure culture then we can screen the microorganisms for plant growth promoting traits and then we can pre-create the inoculum for subsequent application.

And then once we prepare the inoculum, we add the carriers, carriers are those materials which are required for development of solid and liquid formulation and then we sterilize that and then we adjust the pH and the texture and finally once this biofertilizer material is produced then we apply in the greenhouse and we test them in the greenhouse and field. So, we call it greenhouse and field validation. So, these are the 6 major steps of the production of biofertilizer.

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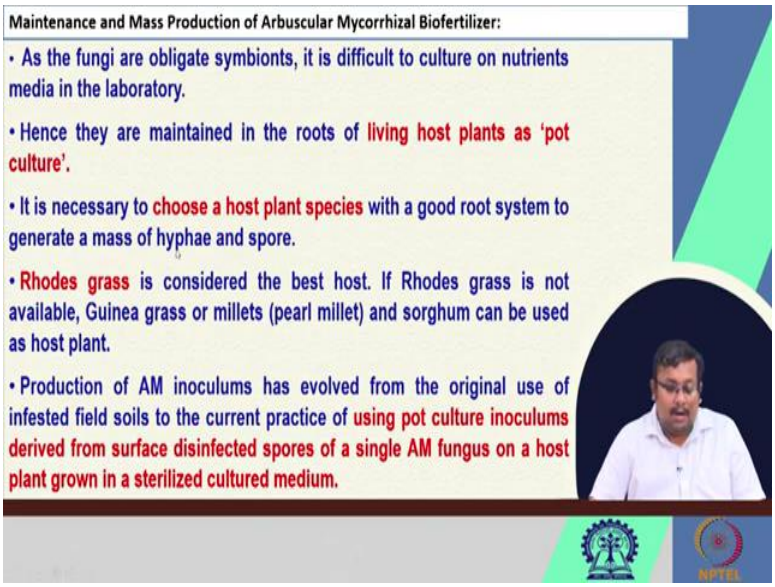

Now, if we consider the maintenance and mass production of arbuscular mycorrhizae biofertilizer as the fungi are obligate symbionts, and it is difficult to culture on nutrient media in the laboratory. So, they are maintained in the roots of the living host plants as pot culture, so it is necessary to choose a host plant species with a good root system to generate a mass of hyphae and spore. And it has been found that the rhodes grass is the best host, so if rhodes grass is not available then Guinea grass or millet or sorghum can be used as host plant.

Now, production of this arbuscular mycorrhizae inoculum has evolved from the original use of infested field soils to the current practices of using pot culture inoculums derived from sulphate disinfected spores, a surface disinfected spores of a single arbuscular micorrhizal fungus on a host plant grown in a sterilized culture medium. So, this is how these arbuscular mycorrhizal fungal inoculums are prepared.

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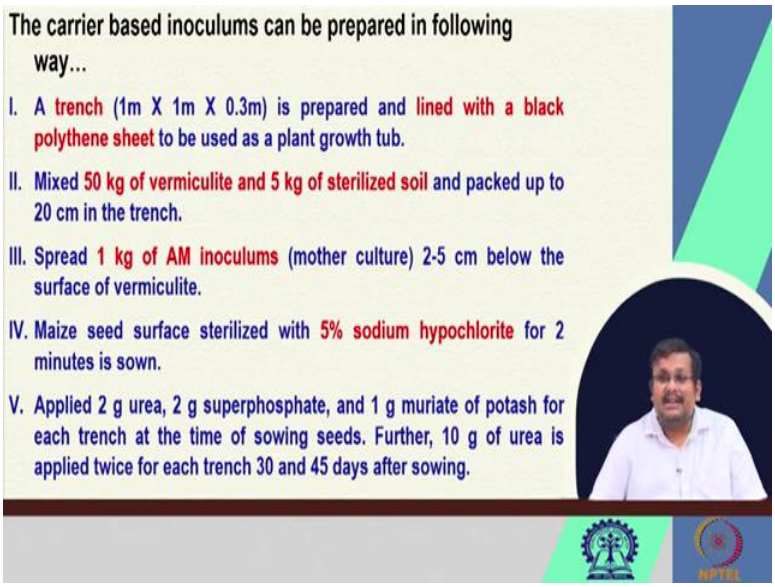

Now, the carrier based inoculums can be prepared in the following way, first of all with a trench of 1 meter by 1 meter by 0.3 meter and lined with a black polythene sheet, which should be used as a plant growth tub and then mix 50 kg of vermiculite and 5 kg of sterilized soil and packed up to 20 centimeter of the trench. Then we sprayed 1 kg of this AM inoculum from the mother culture, 2 to 5 centimeter below the surface of the vermiculites.

And then maize seed surface sterilized with 5 percent sodium hypochlorite for 2 minutes is sown and then we just apply 2 gram of urea, 2 gram of superphosphate and 1 gram of muriate of potash for each trench at the time of sowing seeds. Further 10 gram of urea is applied twice for each trench 30 and 40 days after the sowing.

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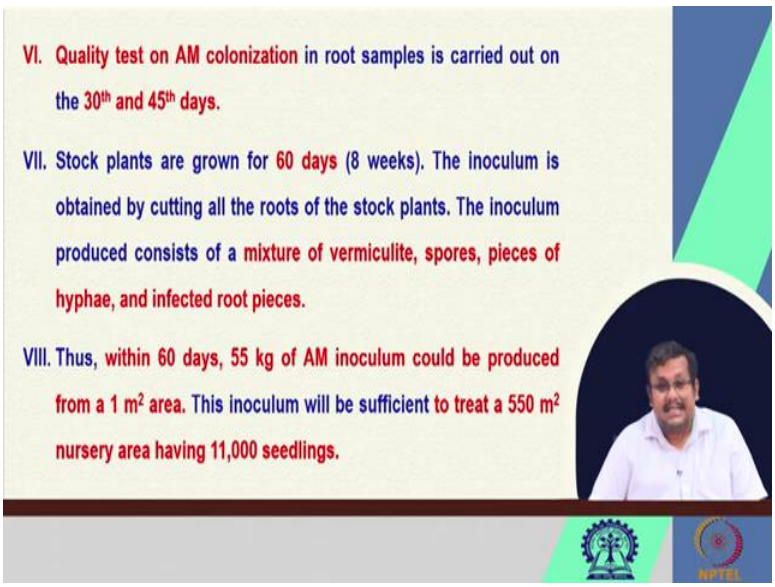

Then quality test on AM colonization in root sample is carried out on 30th and 45th days and stock plants have grown for 60 days or 8 weeks and the inoculum is obtained by cutting all the roots of the stock plants and the inoculum produce consists of a mixture of vermiculite, spores, pieces of hyphae, and infected root species.

Thus within 60 days 55 kg of AM inoculum could be produced from 1 square meter area and this inoculum will be sufficient to treat 550 square meter nursery area having 11,000 seedlings.

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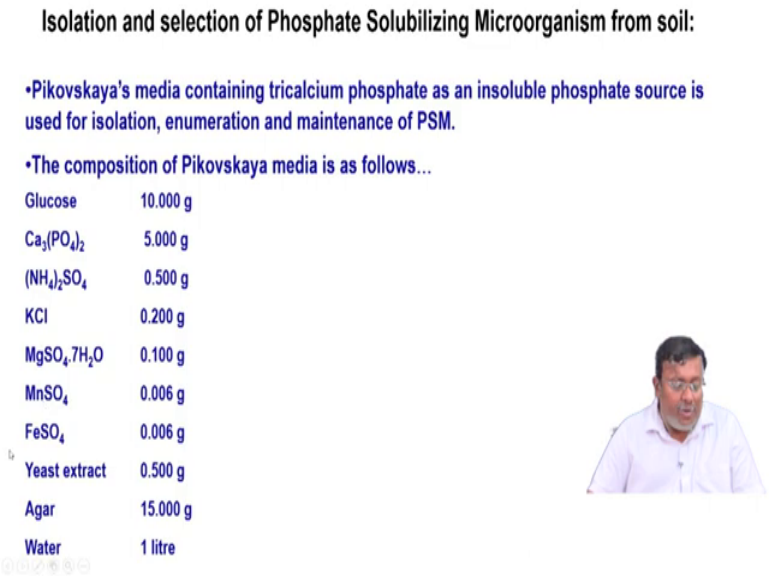

Now, if we want to isolate and select the phosphate solubilizing microorganisms for soil, we have to use the Pikovskaya medium. So, this Pikovskaya medium containing tricalcium phosphate as an insoluble phosphate source and it is used for isolation and enumeration and maintenance of PSM or phosphate solubilizing microorganism. So, this is the composition of Pikovskaya media you can see Glucose, tri-calcium phosphate, ammonium sulphate, KCl, manganese sulphate, heptahydrate and all these things yeast extract, Agar, water all these are there in this Pikovskaya media.

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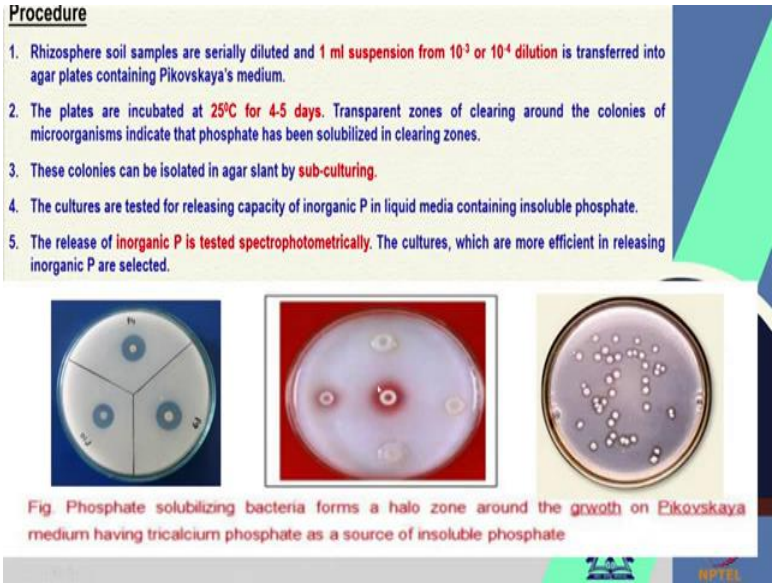

And the procedure says the rhizosphere soil samples are serially diluted and 1 ml of suspension from 10 to the power minus 3 to 10 to the power minus 4 dilution is transferred into the agar plate containing this Pikovskaya medium. And the plant and the plates are incubated for 25 degree centigrade for 4 to 5 days.

And then transparent root zones of clearing around the colonies, so you can see this clearing if you see the clearing around the colonies of the microorganisms indicate that phosphates have been solubilized by this in this cleared zone, in this clearing zones.

So, these colonies can be isolated in agar slant by sub-culturing and the cultures are tested for releasing the capacity of inorganic phosphate in liquid media containing insoluble phosphate and the release of inorganic phosphate is tested spectrophotometrically. And the culture which are more efficient in releasing inorganic phosphate are selected for subsequent use.

So, you can see here phosphate solubilizing bacteria from a halo zone or clearing zone around the growth in this Pikovskaya medium having tri-calcium phosphate as a source of insoluble phosphate. So, basically they solubilize this phosphate which is present in tri-calcium phosphate within the Pikovskaya medium and they create this the halo zones.

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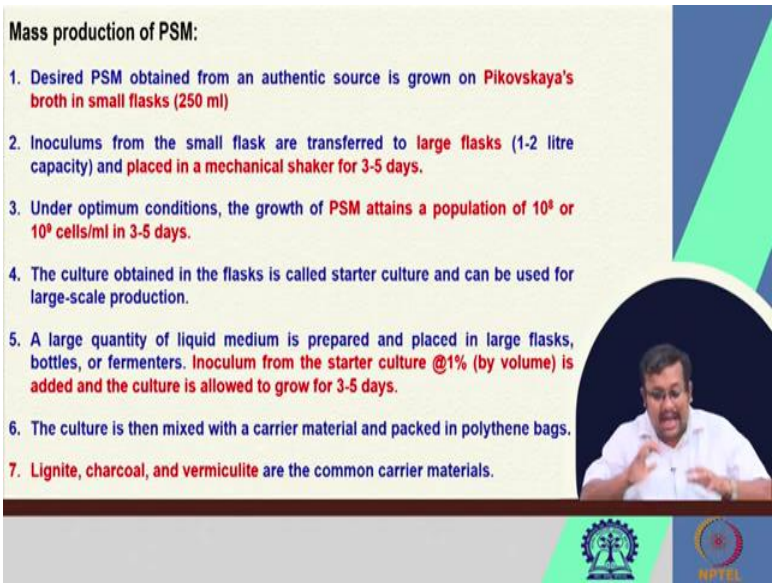

So, how to mass culture these phosphate solubilizing microorganism, so desired PSM obtained from an authentic source is grown in Pikovskaya medium, Pikovskaya medium broth in small flask of 250 ml and then inoculums from the small flask are transferred to large flasks of 1 to 2 litre capacity and placed in a mechanical Shaker for 3 to 5 days under optimum condition the growth of the PSM attains a population of 10 to the power 8 to 10 to the 9 cell per ml entry 3 to 5 days.

The culture obtained in the flask is called starter culture and can be used for large scale production. So, a large quantity of liquid medium is prepared and placed in large flask, bottles and fermenters and inoculum from the starter culture at 1 person by volume is added and the culture is allowed to grow for 3 to 5 days. And the culture is then mixed with a carrier material and packed in polythene bags and then lignite, charcoal or vermiculite are used as common carrier materials to finally develop this biofertilizers.

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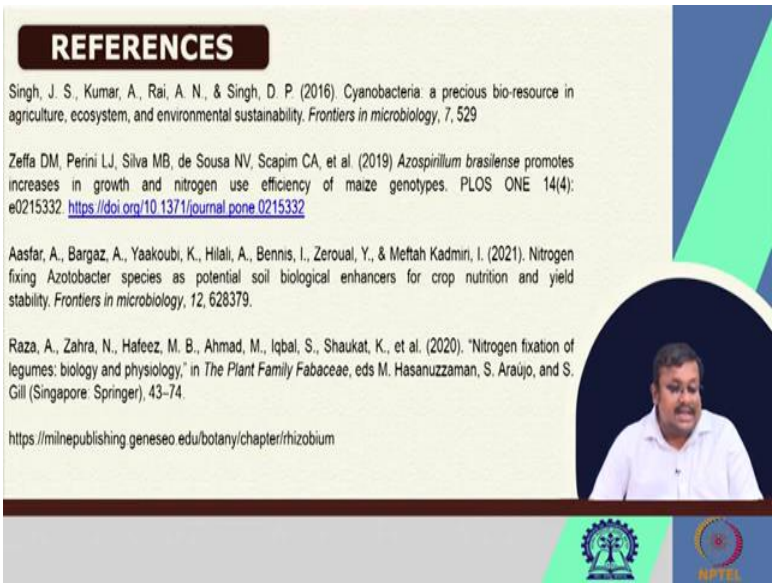

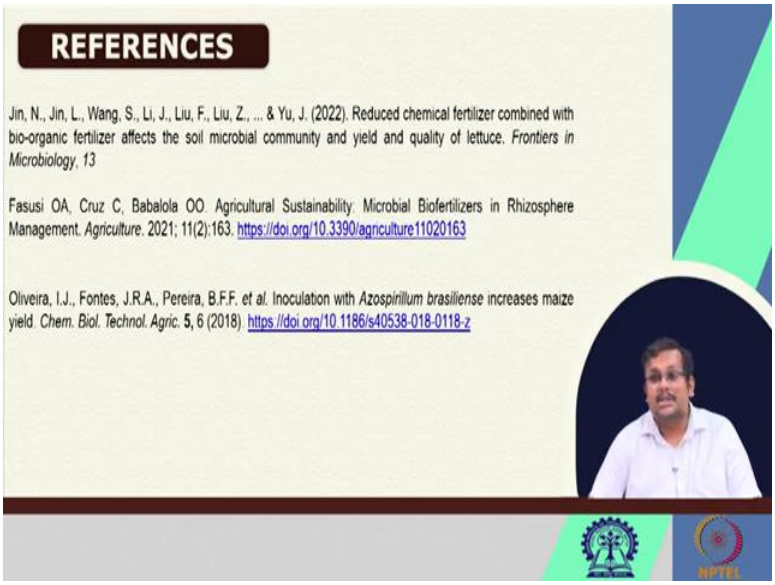

So, guys I hope that you have gathered some knowledge about biofertilizers and know what are the important biofertilizers and how we can prepare them for mass production, how we can isolate them, we have discussed them in brief. So, these are the references and please go through these references for more comprehensive knowledge on biofertilizer. Thank you very much, let us meet in our next lecture. Thank you.