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BIO-FERTILIZERS APPLICATION IN AGRICULTURAL BIOTECHNOLOGY

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Abstract*—Nutrient supplementation of soil is important as the soil is the medium for the plants for growth as the soil supplies the nutrients needed by the plants. It includes phosphorus, nitrogen and potassium. In the meantime, the farmers use chemical fertilizers in order to increase the fertility of the soil. However, the uses of chemical fertilizer have given many effects to the environment and also society as it increases the nitrogen content which will cause a decrease in the colonization of plant roots of mycorrhizae and prevent the rhizobacteria from fixing the nitrogen. As the cause of that, it continuously will reduce the soil fertility which also reduces the productivity crop yield for the farmers. New technology in the agricultural sector that uses renewable and eco-friendly bio-fertilizers have become a new approach to the researchers and to the farmers as other alternatives in increasing the nutrient content in the soil. The formation of bio-fertilizers can be obtained from several sources of microorganisms such as azospirillum, azolla, arbuscular mycorrhizal fungi, rhizobium, azotobacter, silicate solubilizing bacteria cyanobacteria, phosphate solubilizing microorganisms, and plants growth promoting rhizobacteria. The bio-fertilizer also can be classified into several types according to its function and purposes which is phosphate solubilizing bio-fertilizers, nitrogen fixing bio-fertilizers, micro-nutrients phosphate mobilizing bio-fertilizers, and plants growth promoting rhizobacteria. Bio-fertilizers can be performed using different methods such as seed treatments, seedling root dip, soil treatment, VAM bio-fertilizers, blue green algae, azolla, pH, electrical conductivity, organic carbon, nitrogen and phosphorus. The use of bio-fertilizer has become a preferable bio-fertilizer in replacing the use of chemical fertilizers. It gives many benefits and advantages in sustainability and reasonable prices, as it improves the soil fertility towards the plant productivity. As it has positive effects on the plants and environment, at the same time, it also gives effects to society as it has the ability to increase the market production of products which at the same time will reduce the negative effects on the people and society. As the bio-fertilizers increase crop productivity, it will give a higher potential to provide larger and more sustainable yields and healthier soils for the society, especially for the small-scale farmers. In fact, bio-fertilizers have become a new way to develop crop yield productivity. There are great renewable products that can be used to have a better life in the future to achieve sustainability. This chapter will be focused on the application of bio-fertilizer in agricultural biotechnology.

***Keywords*—** *Bio-fertilizers, Agriculture, Biotechnology, Soil fertility, Sustainable, Crop productivity, Natural.*

5.1 Introduction

Agricultural biotechnology is where the technology that applies to the biological or chemical process on the farm. The use of agricultural biotechnology includes the breeding techniques, modifying products, improved plants and developing the microorganisms for specific agricultural uses. Agricultural biotechnology can be categorized into several types, which are genetics, breeding, animal health, microbiome research and also synthetic chemistry. However, this chapter focuses on crop productivity production to improve and enhance food production at a global level. As agricultural biotechnology is used, it will increase the production of the product to a high level that is cheaper and easy to manage. The advance of agricultural biotechnology would provide the farmers with high nutritional and longer lasting food products which at the same time reduce the level of use of chemical and toxic material that present in the food products. As biotechnology research is performed, the other purpose of the research is to conserve the natural sources sustainability so that it will not have negative effects on the future environment. An example of biotechnology research in the agricultural sector is the researcher investigating another way to reduce chemical fertilizer uses by replacing it with bio-fertilizer that is more sustainable to the environment and earth.

Bio-fertilizers are micro-organisms that enhance plant nutrient availability, contribute to plant nutrition by facilitating nutrient uptake and increasing the rhizosphere's primary nutrient supply [1]. Bio-fertilizer is a type of fertilizer that consists of living microorganisms and enriches the soil's nutrient quality. These fertilizers are aimed to supply and to increase the nutrients contents in the soil. Chemical fertilizer consists of high quantities of nitrogen, potassium and phosphorus which can cause a negative effect such as water and air pollution. Therefore, bio-fertilizer becomes more significant to be used in agriculture. The price of chemical fertilizer production is also more expensive compared to bio-fertilizer. The excessive use of chemical fertilizer may lead to damage in soil’s texture. and increase other environmental problems such as mineral depletion and raise the acidity of the soil. **Figure 5.1** shows an example of bio-fertilizers, which is Azotobacter bio-fertilizers.

Figure 5.1: Azotobacter bio-fertilizers [1]

Bio-fertilizer can be classified into several microorganism which is:

I Rhizobium

Rhizobium is a type of bacteria that is part of legume roots, which fix atmospheric nitrogen and live together symbiotically. The morphology that studies the forms of rhizobium and physiology of Rhizobium states that rhizobium varies as the bacteroid of nodules is from a free-living condition. Rhizobium has become one of the most efficient uses of bio-fertilizer as the quantity of nitrogen fixed is more focused. The bio-fertilizers have seven classifications of groups and are more particular to form nodules in legumes, which can be preferred as of cross immunization. The rhizobium that presents in the nodules root of the leguminous plants can convert the nitrogen atmospherically in a form that can be used by the plants to grow well. Usually, rhizobium is present on roots of leguminous plants such as bean plants, chickpea plants or cowpea plants. Rhizobium can be classified on the basis of the types of the plant such as *Rhizobium leguminosarum, Rhizobium trifoli, Rhizobium alamii, Rhizobium japonicum, Rhizobium phaseolii, Rhizobium smilacinae* and also *Rhizobium lantis*. **Figure 5.2** shows the root nodules of the leguminous plants.

Figure 5.2: Roots nodules of leguminous plants [2]

II Azotobacter

As for the azotobacter, azotobacter are known as free-living nitrogen-fixing bacteria mostly used in cultivation of most crops. It is used to raise hormones in soil, solubilize phosphates, aerobically fix the nitrogen, and also defeat the phytopathogens as it reduces its harmful and damaging effects. Azotobacter is classified into several types of species such as *Azotobacter Chroococcum, Azotobacter agilis, Azotobacter paspali, Azotobacter salinestris and Azotobacter vinelandii*. Usually, *Azotobacter Chroococcum* is commonly found in the soils. Azotobacter is used to bind the atmospheric nitrogens that are unreachable to the plants and produce in forms of ammonium soils into the soil [2]. These show that the azotobacter has played its significant part in the cycle of nitrogen in nature. Azotobacter has properties as it can give effects to the development of plants by controlling the producing substances. Therefore, azotobacter sp. is usually considered as a group of Plant Growth Promoting Rhozobacteria (PGPR). Next, there are a few characteristics of azotobacter where it has the ability in producing amino acids as it has grown in the culture medium. The culture media is modified with dissimilar nitrogen and carbon sources. The amino acids produced by these azotobacter are required in vary processes that enhance the plants growth.

III Azospirillum

Azospirillum which is also categorized as bio-fertilizer sources and also known as nitrogen fixing same to the azotobacter and enhances the growth promoting substance production as it has the ability to produce the phytohormone [3]. Azospirillum can be classified into several species, which are *Azospirillum canadense, Azospirillum oryzae, Azospirillum brasilense, Azospirillum halopraeferens, Azospirillum lipoferum and Azospirillum doebereinera*e. Azospirillum acts to transform atmospheric nitrogen into ammonium under micro-aerobic state at low levels of nitrogen through the nitrogenase complex. Azospirillum also can promote plant growth by the influence of the mechanisms of abiotic stresses, which affects the mediated by antioxidants, systemic tolerance, osmotic adjustments and also acts as an interdiction strategy.

IV Cyanobacteria

Another microorganisms that are classified as bio-fertilizer are cyanobacteria. Cyanobacteria are symbiotic cyanobacteria which can be recognized as Blue-Green Algae (BGA). Cyanobacteria are generally unicellular and usually grown in large colonies of the group. It is composed of a wide range of bacteria with different sizes and shapes. Cyanobacteria are classified into several types which are Chroococcales, Nostocales, Oscilltoriales and Stigonemetales. Cyanobacteria is an autotrophic type of bacteria and usually can be found in multiple environments, especially in freshwater and marine. It is also used to fix nitrogen, which is used as a significant and vital nutrient for supporting plant growth and improving the fertility of the plant and environment quality. Cyanobacteria act to trap the sun's energy throughout the photosynthesis to fix the nitrogen content in the air and transform it into a form that can be used by the plant [4]. Cyanobacteria is usually used as an eco-friendly and sustainable agricultural application for the production of biomass of a very high value and also acts to decrease the levels of carbon dioxide. **Figure 5.3** shows cyanobacteria or BGA.

Figure 5.3: Cyanobacteria or BGA [4]

V Azolla

Azolla are another source of bio-fertilizer, a type of fern that floats in the water, which moves freely and improves the nutritional content of the soil. Azolla also can be classified into several species which are *Azolla nilotica, Azolla filiculoides, Azolla caroliniana, Azolla Mexicana, Azolla primaeval, Azolla rubra, Azolla microphylla, and Azolla pinnata* which usually found in wetland rice paddy fields, ponds and wetlands of warm-temperate and tropical regions. It is also used to fix the nitrogen atmospherically in alliance with the nitrogen fixing from the blue green algae by carrying out photosynthesis and uptake the nutrients from its surrounding environment through its roots system [5]. These sources of bio-fertilizers can be used in two ways, which are as green manure that assimilated before transplanting and as an intercropping that assimilated after transplanting. **Figure 5.4** shows azolla type bio-fertilizers.

Figure 5.4: Azolla type bio-fertilizers [5]

VI Phosphate Solubilizing Microorganisms (PSM)

For PSM, these microorganisms in bio-fertilizer are capable in hydrolysing organic and inorganic insoluble phosphorus compounds to soluble the phosphorus form to be assimilated by the plants easily. The mineral phosphate solubilization occurs as the pH value of the soil is lowered by producing microbial from the organic acids and conversion of organic phosphorus by using acid phosphate [6]. PSM is used to increase the solubilization of phosphorus compounds that are insoluble using the ransom of organic acids, phosphatase and the enzymes phytase which is present in a broad variety of soil microorganisms. A powerful phosphate solubilizer consists of two strains, which are bacterial strains and fungal strains. Examples of bacterial strains are such as rhizobium, pseudomonas, enterobacter, and bacillus while fungal strains are such as penicillium and aspergillus.

VII Arbuscular Mycorrhizal (AM) fungi

AM fungi is a source of bio-fertilizer that was used as an agricultural biotechnology application. AM fungi is a kind of mycorrhiza microorganisms which is a symbiont fungus that perforates the cortical cells which are from the roots of vascular plants such as ferns and forming arbuscules. AM fungi can be classified into several types which are, Monotropoid mycorrhiza, Orchidaceous mycorrhiza, Ectendomycorrhiza, Ericoid mycorrhiza, Arbutoid mycorrhiza, and Ectomycorrhiza. AM fungi are used to assist plants express nutrients such as phosphorus, nitrogen, sulphur and micronutrients from the soil and promote the growth hormones. It is also used to increase the defiant in plants in opposition to the surface area of the root and pathogens for achieving a significant absorption of nutrients in the soil [7]. Usually, AM fungi can be found in stressed and polluted soils. AM fungi are one of the most systematic applications to enhance plant toleration to the environmental stresses. Recent studies state that the contribution of AM fungi in improving the stress plant toleration to water deficiency by strengthening the nutrient content and water uses as beneficial of vary mycorrhizal [8].

VIII Silicate Solubilizing Bacteria (SSB)

After that, SSB is another source of bio-fertilizer bacteria as it plays its characters not only solubilizing potassium in insoluble forms, but also silicates and phosphates at the same time increasing the fertility of soil and magnify the plant productivity [9]. SSB is used to supply the hydrogen ions to the medium and enhance the hydrolysis process and the organic acids such as oxalic acid, citric, keto acids and hydroxy carbolic acids which is a complexes form with cations as it enhanced the withdrawal and hold the medium in a dissolved condition. There was a study to determine the use of SSB to improve photosynthetic function of brassica juncea. The utilization of SSB are proven as it significantly increased the layer in mesophilic layer, the amount of mesophyll cells, the volume of the plastid type and the content in the photosynthetic pigment.

IX Plant Growth Promoting Rhizobacteria (PGPR)

Another of bacteria used in bio-fertilizer is PGPR which also recognized as Nodule Promoting Rhizobacteria (NPR). It can be classified into two types of groups which is symbiotic bacteria and

rhizobacteria that can move freely [10]. It is usually used as plant and soil substance to prevent less adverse effects on the environment by promoting the yields and handling in the diseased plant [11]. The crop yields can be increased by facilitating the plant growth throughout the direct or indirect mechanism. The mechanisms of PGPR are hormone controlling and to achieve equilibrium content of nutrients, producing repellent in opposition to plant pathogens and solubilizing the nutrients [12].

Bio-fertilizer acts by fixing the nitrogen atmospherically from the action of adding the nutrients required by the natural process, emulsifying of the phosphorus, and restoring plants growth by integrating of the growth-promoting substance. In fact, the biofertilizers can be classified into several types, where one of them is nitrogen biofertilizers. These types of bio-fertilizers have a function by fixing the nitrogen symbiotically and enhancing the nitrogen levels in the soil. The other type of bio-fertilizer is phosphorus bio-fertilizers. Phosphorus bio-fertilizers are used to develop the soil nutrients into the optimum levels of phosphorus to enhance plant growth same as to the nitrogen bio-fertilizers. However, the use of phosphorus needs to be used with other types such as rhizobium, azotobacter, azospirillum and acetobacter due to the independent properties of it.

Next, compost bio-fertilizers are also classified as a type of bio-fertilizers used to enhance the bacterial process to break down to compost waste. Usually, compost bio-fertilizers uses are such as cellulolytic fungal cultures, phosphotika and azotobacter cultures. Moreover, bio-compost also one of the eco-friendly organic fertilizer that consist of nitrogen, phosphate-solubilizing bacteria and various types of fungi such as trichoderma viridae. Trichoderma viridae can be used to raising the fertility of the soil which leading to a great product quality for the farmers in agricultural sectors. Another types which is nitrogen fixing bacteria that move freely where these types of bacteria used as nitrogen fixation. An example of the nitrogen fixing bacteria are rhodopseudomonas, rhizobium, chromatium, and rhodospirillum. After that, free-living nitrogen fixing cyanobacteria such as anabaena, nostoc, aulosira, totypothrix, cylindrospermum and stigonema which functioning to add the organic content as well as the high level of nitrogen to the soil. Usually, aulosira fertilissima used in rice fields while cylindrospermum licheniforme grows in maize and sugarcane fields. Next, associative symbiotic bacteria which live within the root and the part outside has a relation between the host and the bacteria where azospirillum were important used for grass, wheat, maize and rice. **Table 5.1** shows the type of bio-fertilizer according to its function and type of microorganisms.

Table 5.1: Type of bio-fertilizers according to its functions [13], [14]

Function	Nature of Microorganisms	Example
N_2 fixing bio-fertilizers	Free living	*Azotobacter, Beijerinkia, Clostridium, Klebsiella, Nostoc, Anabaena*
	Symbiotic	*Rhizobium, Anabaena azollae, Frankia*
	Associative Symbiotic	*Azospirillum*
P Solubilizing Biofertilizers	Bacteria	*Bacillus megaterium var. phosphaticum, Bacillus circulans, Bacillus subtilis, Pseudomonas striata*
	Fungi	*Aspergillus awamori, Penicillium sp.*
P Mobilizing Biofertilizers	Arbuscular Mycorrhiza	*Glomussp., Gigaspora sp., Acaulospora sp., Scutellospora sp. & Sclerocystis sp.*
	Ectomycorrhiza	*Amanita sp, Laccaria sp. Boletus sp., Pisolithus sp*
	Ericoid mycorrhizae	*Pezizellaericae*
	Orchid mycorrhiza	*Rhizoctonia solani*
Biofertilizers for Micro-nutrients	Silicate and Zinc solubilizers	*Bacillus sp.*
Plant Growth Promoting Rhizobacteria (PGPR)	Pseudomonas	*Pseudomonas fluorescens*

As modern technology has developed nowadays, bio-fertilizer are now preferred instead of chemical fertilizers because it is harmful as it contain organic materials while chemical fertilizers are made up of chemicals that are harmful to consumers. Another reason is that bio-fertilizers do not decrease the fertility of the soil, while the continuous use of chemical fertilizers would be decreasing the soil fertility and also results in the increase of the soil acidity. The use of chemical fertilizers can also cause the waterways as the chemical run off the excess fertilizer which will

reduce the amount of oxygen. It will give continuous effects as the oxygen depletion which will give effects such as the fish will die. All of those negative effects have caused by the use of chemical fertilizers. In facts of that, the farmer in agricultural sectors are preferable to use Bio-fertilizers instead of chemical fertilizers.

5.2 Biotechnology Application on Bio-Fertilizers

From nature, few functional soil microorganisms can allow performance of plants to absorb nutrients. Their utility can be modified with human intervention by choosing effective organisms, growing them and adding them directly into the soil or through the seeds. The microorganisms that have been cultured are packed in selected carrier material for easier application in the field are called as bio-fertilizers. Bio-fertilizer treatment will affect relatively low pH, which is suitable for the solubilization of bound elements. Soil lead to alkaline after has been treated with chemical fertilizers [13]. The treatment soil with containing bio-fertilizer had increase in EC which is good for perfect plant growth. Therefore, the highest amount of organic carbon in served followed by chemical fertilizer that have been treated soil and reduce value of bio-fertilizer treated soils. The concentration reduce production of nitrogen was found in treated soil with containing bio-fertilizer. Application of bio-fertilizers will reduce the nitrogen number in soil. Chemical and also bio-fertilizer that have been treated affect in optimum value of potassium which is low in control condition. It was simplified that the bio-fertilizers soil that has been treated will show good concentration of phosphorus. Exceed availability of phosphorus in soils is commonly limited by fixation reactions, which produce the ion into few types insoluble forms and better availability of Phosphorus compounds that use as energy currency which is by the plants and include in wide range of plant processes started from permitting cell division then producing good root system [14].

A Seed Treatment

Seed treatment is one of the ways application of fungicide, insecticide, or mix of both, to seeds so that it will turn to disinfect and it also disinfect them from seed-borne or soil-borne pathogenic organisms and storage insects. It is also used to subject seeds toward solar energy exposure, immersion in conditioned water, etc.

One of the seed treatment applications is to prevent the spreading of plant diseases, avoid seed from seed rot and seedling blights, increase germination, produce protection from storage insects and also prevent soil insects [17]. First, mix each packet (200g) of inoculant with 200 ml of rice gruel or jaggery solution. Then, amount of seeds needed for one hectare are combine into the slurry so that it will resulting uniform coating of the inoculants over the seeds. Next, let the shade dried in duration of 30 minutes. The seeds that have been treated should be used within 24 hours. One packet of inoculant is suitable and can use to treat for 10 kg seeds. Rhizobium, Azospirillum, Azotobacter and Phosphobacteria are an example of microorganisms that listed out as seed treatment.

B Seedling Root Dip

Few parts of procedure to carry out the treatment of roots of seedlings which the roots are containing a water-rich coating, then providing a surface optimum dry. Other than that, moisture that suits to permit the seedlings to let them discrete and to provide the integrity of the coating from the ordinary handling and planting operation. Besides, effective moisture-sensitive results after the planting carry out, the coating disintegrates and provides the pesticide. Thus releasing the plant protection toward pests over an extended duration of time. This procedure is applicable with insecticides, nematicides and fungicides, either pesticides are systemic or non-systemic.

This procedure is carried out for transplanted crops. First, prepare five packets (1.0 kg) of the inoculants for one ha and let it mix with 40 liters of water. Then, dip the root portion of the seedlings into the mixture in the duration of 5 to 10 minutes and then transplant. Azospirillum is one of the organisms used for seedling root dip, especially for rice [2].

C Soil Treatment

Soil treatment is carried out for turning contaminated soil to use for agriculture and other purposes. If soil stores chemicals or wastes such as oil, alkali or few other non-degradable materials, this can be handled by treatment using microbes. Soil contamination can be caused by many activities, such as the inappropriate disposal of industrial waste, sewage, agricultural chemicals, etc. Amount moisture holding, aeration and nutrient are reduced caused by the polluted soil. First, mix 4 kg each of the recommended bio-fertilizers into 200 kg of compost and let for overnight. This mixture is communicated and reacts in the soil at the time of sowing or planting [2].

D Use of VAM Biofertilizer

Vesicular-arbuscular mycorrhiza (VAM) is produced from the symbiotic association, normally certain phycomycetous fungi and angiosperm roots. The fungus colonizes will encourage the root cortex forming a mycelial network and characteristic vesicles (bladder-like structures) and also arbuscules (branched finger-like hyphae) [16].

First, apply 2-3 cm of the inoculum under the soil in the range duration of sowing. Cut or sow the seeds, then planted above the VAM inoculums therefore the roots will let in contact with the inoculums and can affect the infection. While for one-meter square area sufficient amount of bulk inoculums is 100gm. Polythene bag filled with 5-10kg bulk inoculums is used to raise the seedling. During the planting process, at rate 20g /seedling of VAM inoculums is supplied for each bag but for trees 200g of inoculums are needed.

E Use of Blue Green Algae (BGA)

Blue-green algae is one of the common types of bacteria known as Cyanobacteria. They often appear in green and sometimes it could turn bluish when scums are dead. Taste and odour also one of the problems normally came with high concentrations of blue-green algae and some of those types have possibility for producing toxins.

First step taken is 10 kg/ha of algal act as dried flakes culture over the standing water in the field rice. It will be consumed two days after having been planted in loamy soil and after that it will continue for six days after planted in clayey soils. After the algal application, the field will let the waterlogged for some days. Lastly, bio-fertilizer will be applied for 3-4 consecutive seasons at the same field. **Figure 5.5** shows the algal culture.

Figure 5.5: Algal culture [4]

F Use of Azolla

Azolla is one of the unique species because it is only consumed in the shortest time for growing plants on the planet. It also does not require any soil to grow differently with almost all other plants. Azolla has a tendency to get its own nitrogen fertilizer directly from the atmosphere.

So, it can be concluded that it is able to produce bio-fertilizer, livestock feed, food and biofuel with the amount that they are needed and in the same time, drag down large amounts of CO2 from the atmosphere, thus helping to decrease the tendency of climate change.

First, applied 0.6-1.0 kg/m2 (6.25-10.0 t/ha) of green manure also called Azolla incorporated before transplanting of rice. Azolla is applied 100 g/m2 (1.25t/ha) and it is considered a dual crop after one to three days after transplanting of rice and furthering to multiply for 25-30 days. Azolla fronds can be directly reacted into the soil at the time of first wedding.

G pH (Pouvoir Hydrogen)

pH is one of the parameters commonly used to analyse in soil and water testing. It is a parameter used to indicate the sample acidity. pH is actually allowed to measure the activity of hydrogen ions in the sample. pH measurement is recorded on a scale 0 to14, and it will assume 7.0 as neutral value.

Took 20 gram of soil and placed it into a 100ml beaker. Then, filled with 50ml distilled water. Continue by stirring at regular intervals and let for 30 minutes. By using a pH meter, record the pH value.

H Electrical Conductivity

Soil electrical conductivity is an equipment which is not direct one that correlates effectively with several soil physical and also chemical properties. Electrical conductivity is an ability of an equipment to conduct an electrical current and it is normally shown in units of milliSiemens per meter (mS/m).

First step was to weigh 20 gram of the soil and directly add 40ml of distilled water in 150ml of conical flask and let it rest for 1 hour. Then, record the pH and using the same clear solution measure electrical conductivity by using a conductivity meter.

I Organic Carbon

Components of organic soil are usually measured with their soil organic carbon. Organic matter rises up in the range of 2–10% which is divided by the soil's mass. Organic matter plays an important role in the physical, chemical and biological function of agricultural soils. Organic matter prioritized nutrient retention and turnover, soil structure, moisture retention and availability, degradation of pollutants, carbon sequestration and also soil resilience.

Dry soil weighs in the range of 0.1 gram - 2.0 gram. 10ml of 0.167M $K_2Cr_2O_7$ is added and followed by adding 20 ml of conc. H_2SO_2. Gently mix the mixture well. Let it rest for 30 minutes. Try to avoid rapid heat loss by placing it on an insulation during the process. Next, dilute the mixture with 200ml distilled water. Further the test added 10ml of 85% H_3PO_4 and 0.2g of NaF. Lastly, 10 drops of ferroin indicator was added and let the wine turn red colour end point.

J Nitrogen

Nitrogen is playing an important building block which is proteins, nucleic acids and other cellular constituents which are suitable for all forms of life. Nitrogen is always an important key nutrient element especially for plants that its overall cover careful management, and if it is not handled in an appropriate way it can lead to some environmental problems.

Place 1-gram sample and 0.5 grams soil that is rich in organic matter into the digestion tube. Further the test by adding 2 gram of catalyst mixture and also 7ml concentrated $H_2SO_4$and and slowly stir and mix it. Let the mixture rest for 3 hours and either clear or pale yellow will form. Then add 50ml of distilled water and let it cool again. 10ml of boric acid is added followed by 2 drops of the indicator solution then placed into the steam distillation unit. Next, switch on the distillation process which is to add 3 strokes of 40% NaOH and let it for 0.5 minutes and 2.5 minutes steam distillation. After completing the process, continue by adding a stirrer bar. Then titrate the solution and ensure the solution from green over colourless to a pink is formed with 0.1N H_2SO_4. So, the amount of H_2SO_4 used is recorded.

K Phosphorus

Phosphorus is one of the sources of macro-element and it is needed for plant nutrition. It includes metabolic processes such as photosynthesis, energy transfer and also synthesis and breakdown of the carbohydrates. Phosphorus is usually found in the soil which is in organic compounds and also in minerals. Furthermore, the amount of phosphorus that already exists is very low compared with the total amount of phosphorus already in the soil. So that, in some cases phosphorus fertilizers will be used in order to fulfil crop requirements.

In volumetric flask, 35ml of aliquot is added and continued by adding 10ml of vanadate. Dilute the molybdate reagent with distilled water till it reaches the mark. Distilled water is also used to prepare blank solutions. After 10 minutes, set 400nm of wavelength to test the absorbance of the sample. Lastly, prepare the standard curve for measuring the value of the results.

5.3 Advantages of Bio-Fertilizers

A bio-fertilizer is not only about organic fertilizer or manure. It is a carrier medium with a lot of live microorganisms. When it is applied to the seed, soil or living plants, it will promote soil nutrients and make them present biologically. Bio-fertilizers have many different types of fungi, root bacteria and also some microorganisms. They are forming a mutually beneficial or symbiotic relationship with host plants growing in the soil.

Bio-fertilizer also promotes the ultimate goal by increasing productivity and economically viable support to small and marginal farmers. Bio-fertilizer are low cost, effective and a renewable source of plant nutrients rather than supplementing chemical fertilizers. Microorganisms such as bacteria, fungi and blue green algae can act as bio-fertilizer. These organisms are added to the rhizosphere of the plant to promote their performance in the soil. The use of good soil health will enhance sustainable crop production. Soil health ensures the optimum combination of either organic or inorganic components of the soil. Frequently use of chemical fertilizers will destroy soil biota.

In nature, there are few numbers of good soil microorganisms that can improve plants to absorb nutrients. Their utility can be improved by human intervention and choosing efficient organisms, culturing them and directly adding them to soils or through the seeds. The cultured microorganisms packed in the carrier material for easier application in the field are called bio-fertilizers. Bio-fertilizers are living microorganisms such as bacterial, fungal and algae origin. Each of them has their own mode of action and can be used alone or in combination. From research, effective strains are classified to suit given soil and climatic conditions. Some strains need to be measured, multiplied in the laboratory and distributed to the farmers. They are in the form of carrier materials such as peat, lignite powder is a better way that will enhance sufficient shelf life.

A Sustainability

Bio-fertilizers can improve the nitrogen and phosphorus presence in the soils more naturally than chemical fertilizer. The presence of varieties type available will be promoting growers to tailor for the microorganisms used to the needs of particular plants. Bio-fertilizers are easier to use, even for small novice growers. Bio-fertilizers will not affect or pollute the soil and the environment, while chemical fertilizers commonly affect because of too much

phosphate and nitrogen in the soil. The excess then flows into the lakes and streams through runoff. Waters reduce in quality and face an overgrowth of algae and the death of aquatic animals. One example is the microbiome which is a potential significance of beneficial microbes for achieving sustainability in agriculture.

The rhizosphere is one of the narrow zones of soil growing at the plant roots, and can grow up to 1011 microbial cells per gram of root and more than 30,000 prokaryotic species that often increase plant productivity. The amount genome of rhizosphere microbial community enveloping plant roots is larger rather than of plants and is referred as microbiome, whose communicate for determine crop health in natural agro-ecosystem by supplying numerous services to crop plants viz., organic matter decomposition, nutrient acquisition, water absorption, nutrient recycling, weed control and bio-control.

Azotobacter is playing an important role in the nitrogen cycle in nature as it contained various type of metabolic functions such as thiamine and riboflavin, and plant hormones viz., indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). A. chroococcum enhances the plant growth by improving seed germination and increasing performance of the root architecture by limiting pathogenic microorganisms around the root systems of crop plants. Rhizobium has always been used as an efficient nitrogen fixer since the previous decade. It played an important role in improving yield by converting atmospheric nitrogen into usable forms. It is also resistant to different numbers of temperature ranges Rhizobium often enters the root hairs, multiplies there and forms nodules. Not only playing a role in nitrogen fixation, Azotobacter also has the tendency to produce vitamins. **Figure 5.6** shows the microorganisms used in sustainable agriculture.

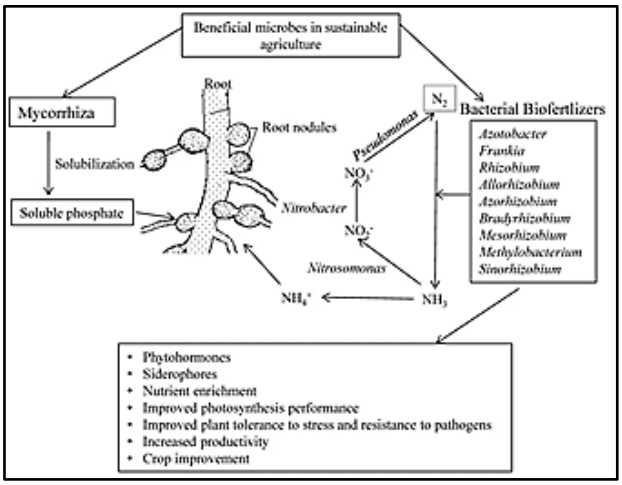

Figure 5.6: Microorganisms used in sustainable agriculture which are bacterial biofertilizers and mycorrhiza that give benefits in agriculture such as enrich nutrients and improve crop [15].

The beneficial soil micro-organisms sustain crop production either as biofertilizers or symbiont. They perform nutrient solubilization which facilitate nutrient availability and thereby uptake. It improves the plant growth by advancing the root architecture. Their activity allows several significant traits to plants such as increased root hairs, nodules and nitrate reductase activity. An effective of strains of Azotobacter, Azospirillum, Phosphobacter and also Rhizobacter can supplying significant amount of available nitrogen through nitrogen cycling [15]. The bio-fertilizers produced plant hormones such as indole acetic acid (IAA), gibberellins (GA) and cytokinins (CK). Biofertilizers encourage photosynthesis performance for prefer plant tolerance to stress and also improve resistance, so that resulting in crop improvement.

B Affordability

Bio-fertilizers will decrease dependence rather than expensive petroleum sources of chemical fertilizers. Refer to the "Journal of Phytology," use of chemical fertilizers will exceeding the supply which is more than 7 million tons by 2020. The shortage of fossil fuels for producing the chemical fertilizers may rise up the prices above the reach of small users. Bio-fertilizers is better causing its price is cheaper, easy-to-use alternative to produce petrochemical products.

C Improved Soil

Soils may contain natural production of plant nutrients, but these reserves are highly different in forms which are encourage for plant growth. Then, Plant nutrients are limited to plants, because of exceed use of the chemical fertilizers. Chemicals

produce the nutrients, which leaved in soil, in active and therefore it will be not use by plants and also make the soil reduce it quality.

Bio-fertilizers is containing normal fertility for the soil and allow it biologically alive. They are producing the amount of organic matter and also increasing soil texture and structure. The sample of soil holds water better than before. Bio-fertilizers added useful nutrients for the soil, such as nitrogen, proteins and vitamins. They consume nitrogen from the atmosphere while phosphates from the soil, then converting them into forms that easier plants to use. Other species also producing natural pesticides.

Cyanobacteria is one of commonly natural donor of fixed N as they are good quality soil fertility by compact the atmospheric Dinitrogen (N_2) and it will be affecting in improve of growth and yield. Cyanobacteria also encourage the soil fertility by making the soil pore size and water storage capacity due to exceed mucilage production and it also can communicate for higher dose of pesticides rather than the recommended levels applied in the field.

D Improved Plant

Important microbial combination that are improving plant nutrition and health, often more popular. For the mixture with some of organic materials will lead to produce organic fertilizers. Bio-fertilizers improving production by up to 30 percent because of the nitrogen and phosphorus that added to the soil. The increases quality in soil texture and quality encourage plants grow much better during time of drought. Bio-fertilizers allow the plants growth stronger root systems and rise better. Bio-fertilizers also decrease the effects of dangerous organisms that found in the soil, such as fungi and nematodes. Plants will be facing stress better and live longer.

In general, bio-fertilizers especially living microorganisms which is supplying nutrients and encourage growth and also the development of plants by the natural processes. For the example nitrogen fixation, phosphate solubilization, production of hormones, and other plant growth-promoting substances of rice. Other than N2 fixation, they also make other types of growth hormones, vitamins, bioactive compounds, organic acids, antagonistic compounds, and act as an important function in nutrient cycling.

5.4 Effects of Bio-Fertilizers on Today Society

The application of agricultural biotechnology can have many positive effects to today's society. One of the effects is that it would increase the market production in the agriculture sector. As the use of bio-fertilizer are nowadays becoming the main role to a grown healthy plant, promoting the sustainability and the growth health of the soil, the demand of the bio-fertilizer product becoming more popular and increases as it functions to increase the crop yield productivity.At the same time, it also reduces the water and air pollution as the fertilizers used are organic, safely to be used and also eco-friendly [17].

Chemical fertilizers are inorganic materials that undergo chemical treatments. If the chemical treatments are used continuously towards the soil, the depletion of the soil of essential nutrients will occur. After that, the productivity of the food product will decrease due to the lack of vitamins and mineral contents in the soil. Then, the problem from the use of chemical fertilizer can be faced by using bio-fertilizers as another of alternatives to promote the plant's productivity and at the same time to reduce the use of chemical fertilizer that gives effects to the nutrient in the soil. This way could give opportunities for the farmer in increasing crop yield productivity and fulfil the customer demands without any problems faced.

The use of bio-fertilizer has given great news as its benefits and advantages towards the environment and ecosystem at the first beginning. It has given an approached for the researchers as it has become a new way to achieve sustainable agriculture by continuing stability of the economics of the farms, raise the productivity for human food and fibre needs and helping the farmer to improve the techniques and the quality in producing products but at the same time has minimal effects on the environment. By doing so, the researchers would investigate, experimenting, and also researching to ensure the lands and crops stay healthy, viable and enough with nutrient supplies. There was a study on bio-fertilizer that focused on investigating the continuously accessible and eco-friendly nutrients, type and its potential for crop production based on the applicable practices and research work that have been gone through by the numerous researchers [19]. Based on the study, it shows that the existence of the bio-fertilizer has caused the other researchers to urge, investigate and research in depth analysis of the components that need to be used and the effect of using the sources in other aspects.

The other effects of the use of bio-fertilizers are it reduces the amount of money used for the farmer in agriculture application to invest money in bio-fertilizer as it functions to increase the crop productivity through these types of nutrients supplementation. As the use of Bio-fertilizers only uses microorganism, bacteria, algae and fungi, the

cost of Bio-fertilizers is cheap as it is renewable such as it is biological fertilizers and easy to obtain the plant nutrients. This is known as cost effective resources technology as it reduces the amount of chemical fertilizers to be used for crop production. The need for decreasing the costs of fertilizing crops with reconvert able energy forms has revitalized the importance of bio-fertilizer all around the world while at the same time improving the fertility of the soil without leaving any damaging or dangerous effects in the soil and sustainability can be achieved [18].

Furthermore, the use of bio-fertilizer also reduces poisoning effects and chemical burns to people and society. The poisoning effects and chemical burns mentioned are such as skin and eye irritation, burning, redness, numbness and pain in the affected area. The worst effects for humans can be seen in the use of chemical fertilizers such as nitrate in chemical fertilizer. These conditions would be very dangerous when present at high levels in human bodies, the nitrate can lower the ability of Red Blood Cells (RBC) to carry and deliver the oxygen. These effects can be attributed to methemoglobinemia diseases in which the amount of methaemoglobin produced is abnormal. All of these types of dangerous and poisoning effects are caused by the utilization of chemical fertilizers. By using bio-fertilizers which are eco-friendly, all of those effects can be prevented as the composition of Bio-fertilizers consist of microorganisms instead of dangerous chemicals that would give effects to human society but also to the environment.

Another effect of bio-fertilizer is it creates job opportunities as the demand of the bio-fertilizer increases. As the industrial biotechnology in the agricultural sector has become favoured by today's society, it has been creating a new great job of opportunities for society such as in the research and development sector or in factories in the biotechnology industry on producing bio-fertilizer and improving the plant development. This sector includes choosing the active organisms and handling the microbes according to its optimum condition for the organisms. In this situation, all of those activities require manpower to ensure the bio-fertilizer production can be performed and fulfill customer requirements and at the same time to develop and maintain the sustainability in a long term of soil fertility.

CONCLUSION

It can be concluded that the bio-fertilizer is very significant as it is used towards the agricultural sector in application of biotechnology. Bio-fertilizer plays a significant character in supplying the organic nutrients to the plants and replacing the potency of the soil and improving soil yields that lead to sustainable farming. As the population of the world increases, the demand for the food is increasing rapidly. This happens especially in those developed countries where land resources are not a contributing part in crop production as the population on a daily basis requires enough food. As the farmer is still using chemical fertilizers, not only does the fertility of the soil decrease, but the nutrient of the soil also decreases. By using bio-fertilizer as a new way to achieve sustainability in the future, it can promote plant growth and the quality of the crop yield can be improved through better nutrient supplies.

In conclusion, new improvised fertilizers, which are bio-fertilizers, have been commercially popular in the market due to its benefits not only to the environment, but to human society. Due to this condition, the use of bio-fertilizers has replaced the use of chemical fertilizers and becoming more preferable for the farmers in managing crops yields plants. Bio-fertilizers help to increase the accessibility of plant nutrients which can help to keep maintaining the fertility of the soil for over a long period so that it will not give negative effects in the future.

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