**Subject:** Biotechnology

### Production of Courseware - Content for Post Graduate Courses

- **Paper No.:** 05 Environmental Biotechnology
- **Module:** 19 Biopesticides; biofertilizers

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1) Learning objectives:
This module is intended to apprise students about the developments in the area of applications of biopesticides & biofertilizers in agriculture sector for sustainable and environment friendly agriculture practices. Since, the application of synthetic pesticides and fertilisers in agricultural field has seen a sharp increase in recent years and in some cases it has been reached at alarming levels with grave consequences on human health and on the ecosystem. It is therefore increasingly importnat that eco-friendly methods of improving soil health and nutrients must be used. In this module will discuss about the potential and limitations in the use of biopesticides and biofertilisers in general and in the perspective of Indian agriculture. We will further explores the factors responsible for the limited use of these agents, based on the detailed analysis with a large number of publications, reports from various organizations engaged in the promotion and applications of biopesticides and biofertilisers.

2.) Introduction:
2.1) Agriculture and food security: Agricultural activities play a very important role in the economic growth of any countries apart from providing the food security to the growing population. However, worldwide millions of people lack access to adequate nutritious food worldwide. It is assumed that by 2050 the agricultural productivity is expected to be higher so as to provide the needs of ~9.7 billion people globally. Currently, millions of people are chronically hungry in developing world. Hence, agricultural sector is facing increasing challenges such as water availability, climate changes and raising the risk of production shortfalls. Since agriculture sectors have the potential for food security, environmental sustainability and economic opportunity and growth worldwide. So the future vision is to adopt new technologies to increase agricultural productivity sustainably. India is a lower middle income country and agriculture is a leading economic sector in India. It is a primary source of food and raw materials that provide livelihood to about 80% of the population. Agriculture sector in India accounts for about 17.9 % of GDP growth. In 2014 agricultural land in India was reported at 60.41% (World Bank collection of development indicators). It has record production of food grains during fiscal year (FY) 2016; the consolidated food grain productivity was recorded as 253.16 million tonnes (MT), which increased to 273.83 MT in FY 2017. Moreover, India is among the largest producer of rice, wheat, fruit, and vegetables and among the largest producer of pulses, spices, jute and tea.

2.2) India agriculture facts overview: The increased production in agricultural sector in the past few decades has been demonstrated a higher increase in the use of chemical pesticides and fertilizers. Also
the green revolution leads to higher gains in crop production but with lower concern for environmental sustainability. Further, dependence on chemical fertilizers for our agricultural growth would mean further loss in soil health and chances of water contamination. In fiscal year 2017, higher food grain production was due to great demand and higher population which are the key driver of demand for agricultural activity. India has competitive advantages such as high proposition of agricultural land (157 million hectares). By 2020-21 the agricultural grain production is expected to be ~280.6 MTs. Hence, there is an attractive potential opportunities and increasing demand for agricultural inputs such as fertilizers and hybrid seeds.

3. Challenges in agriculture crop cultivation: The major challenges in agriculture sector are to minimize the causes of declining crop productivity. Youssef et al, 2014 have reported that agricultural crops are largely damaged by 10,000 species of insects species, 30,000 species of weeds, 100,000 diseases caused by fungi, viruses, bacteria and other microbes and 1000 species of nematodes. Therefore, in common practices the indiscriminate and imbalanced use of the chemical fertilizers, especially urea along with chemical pesticides leads to increasing cost of chemical fertilizers. Also this indiscriminate use of synthetic fertilizers has imposed potential threats to sustainable agriculture due to pollution and contamination of the soil, polluted water basins and killing of friendly non micro-organisms and insects. Therefore, there is an urgent need for environmental friendly methods of improving plant resistance to diseases, improving soil fertility; plant nutrients as well as pests control alternative methods. Biopesticides and Biofertilizers are potential environment friendly methods that are used to supplement the soil nutrients for proper plant growth in long term and these are economical, eco-friendly, more efficient and reported to be highly productive in agriculture.

Moreover the usages of synthetic pesticides and chemical fertilizers in agricultural sector has lead to detrimental consequences on environment by affecting the soil health, water quality, development of insect resistance; accumulation of toxic residue through food chain. Further, the current progresses in food habits are more focused towards organic agricultural produces. Hence, it has become indispensable to adopt the best of scientific practices and a technological solutions that are crucial to satisfy today’s complex challenges. Therefore, biofertilizers and biopesticides are emerging as important areas to fulfil the human challenges in a sustainable way.

Figure 1: Plant pathogens (Source: https://4.imimg.com/data4/RL/TJ/MY-535538/nano-shield-250x250.jpg)

4) What are Bio-Fertilizers and Bio-Pesticides?
Biofertilizers are biologically active organic products containing specific living micro-organism (bacteria, algae or fungi) in cultured form which are isolated either from roots of certain plant species or from root zone soil of some specific plants. These biofertilizers improve the nutrient level and quality of soils that facilitate the plants to absorb the essential nutrients. Hence, the soil becomes more healthy and helps the budding seeds and roots to grow to their full potential. Biofertilizers work by activating the soil microorganisms, thus restore the soils' natural nutrients and protect it against diseases and other harsh conditions, and promotes the growth of plants. When Biofertilizers are applied to plant, it colonizes the rhizosphere in root and help promotes growth of plant by augmenting
the supply of nutrients to the plant. Hence, biofertilizers enhances nutrients uptake through the natural processes of N₂ fixation, phosphorus solubilizing, and stimulating the plant growth. Additionally, biopesticides, or also called as biological pesticides, are natural pest controlling agents that are isolated from natural sources. Using biopesticides has advantages over using conventional pesticides, since biopesticides are less harmful to the environment (Raja N, 2013).

**Figure 2:** Examples of different types of biofertilizers (Source: http://agritech.tnau.ac.in/org_farm/orgfarm_biofertilizers.html)

Biopesticides are natural pest control agents obtained from natural sources e.g. bacteria, animals, plants etc. Biopesticide kills pathogens through specific biological phenomenon rather than as a synthetic pesticide (poison) for example baking soda and canola oil have pesticidal properties and considered to be as a good biopesticides. Till 2016, there are about 299 registered biopesticides and its active ingredients and about 1400 active biopesticides product registered. Application of biopesticides has advantages over using conventional pesticides, as biopesticides are less harmful and they play very important role in the protection of agricultural foods as well as protection against pathogenic organisms.

**How does Biofertilizers work?**

Biofertilisers work by fixing atmospheric N₂ in root nodules of legume crop or in the soil and make it available to the plants and it solubilize the insoluble forms of phosphates into readily available forms. It also produce hormones and antimetabolites which enhances root development, decompose the organic matter and that help in soil mineralization. They supply the plants with high amount of nitrogen and phosphorus in the readily available forms. In the contrary the chemical fertilizers deteriorate the environment and leads to harmful impacts on living beings. The microorganisms that are present in these bio-fertilizers also reduce the stress in plants by competing with the plant pathogens and suppressing them (Araujo., 2008). However, the quality and productivity is a major factor in their limited acceptance by the local farmers. This limitations are primarily linked to strains selection and efficient biofertilisers development and production technology.
Figure 3: Effects of biofertilizers on physiological and biochemical properties of soil. (Source: https://www.researchgate.net/profile/A_Chatterjee/publication/316700544/figure/fig1/AS:490981482078209@1494070515590/FIGURE-101-Effects-of-biofertilizers-on-physiological-and-biochemical-properties-of.ppm).

Objectives of producing biofertilizers in India
Since, biofertilizers are one of the essential inputs in organic farming they not only stimulate plant growth and product yield but also enhances the soil fertility and soil health. The promotion of biofertilisers applications in India is mainly monitored by the National Biofertilisers Development Centre established in 1987. The major objectives of this center is to develop, produce and market the different types of biofertilisers of standard quality. The center is also involve in isolation and maintainance of biofertilisers strains suitable to various agricultural climatic regions. They also provide training to farmers and promote biofertilisers applications through demonstrations on site; preparation of quality parameters, monitor and test the samples of biofertilisers produced by others. It also provide important technical and other assistance to biofertilisers producing unit and to the farmers involved. Currently, the demand for good quality biofertilizers is on the rise since the last few years owing to its eco-friendly characteristics and a global trend to reduce the reliance on chemically derived pesticides and fertilizers.

Advantage of Biofertilizers:
The major advantages is it has longer shelf life and use to improve the soil profile and fertility, and to promote plant growth without any adverse effects on ecosystem. It increases the crop yields by 20-25% and improves the soil structure by enhancing the aggregation of the soil particles, help in better water retention. It also improve the leaf water and turgor potential that maintain the stomatal functioning as well as transpiration, increasing root length and development. Biofertilisers are formulated mainly from commonly available organic materials such as agricultural residues e.g. rice husks hence, it has low the input expenses and lower production costs in compare to the chemical fertilizers or pesticides. It helps in improvement and sustainability of agriculture by secretting growth factors, e.g. aquatic cyanobacteria provide natural growth hormone, protein, vitamins and minerals to the soil which help in growth of crop and yield. They have excellent buffering capabilities because of their organic matters contents that help in balancing of the pH of the soil. The organic materials and the acids that are found in the bio-fertilizers include humic and fulvic acids, fungi, and organic fertilizer nutrients. Humic acids are plant biostimulants that can increase soil fertility, enhance microbial activity and reduce water evaporation. Ultimately, bio-fertilizers are environmentally friendly that not only help protect against the drought and diseases, but also prevent from damaging...
the natural sources, as well as cleansing the plant from precipitated chemical fertilizers. Often effective in very low concentration and decompose in shorter time in nature.

**Disadvantages of Bio-fertilizers**
On the other hand, sometimes bio-fertilizers aren’t readily acceptable by the farmers primarily because of the delay in response and wide spectrum activity. However, this depends on the quality of the biofertilizers used. Some bio-fertilizer batch may have insufficient or poor population of microorganisms or may have some levels of contaminants or have insufficient amounts of phosphorus and nitrogen, which can affects the plant growth. It get destroyed by ultraviolet light, so basic action is slow, effective dose can be higher. Since seasonal availability of plant residues leads to need for storage, hence, it is not readily available everywhere on demand.

**What type of Biofertilizers and Biopesticides are available?**
There are several types of biofertilizers are available with varying effects on the soil, depending on the plant selected. Generally they fall into two categories: i) N$_2$ fixing biofertilizers and II) Phosphate solubilizing. N$_2$ fixers are as the name suggests they fixes the atmospheric nitrogen into readily available forms for the plants. They help balance the nitrogen levels of the soil and help the growth of plants. The N$_2$ fixers include rhizobium, azatobacter, blue green algae (BGA), azospirillum, and azolla. However, Rhizobium always requires a symbiotic association with the root nodules of many legume plants to fix nitrogen, while others can independently fix nitrogen. Similarly, phosphorus solubilizing biofertilizers work almost like nitrogen biofertilizers as phosphorus is also an important factor for plant growth. However, unlike nitrogen biofertilizers, phosphorus biofertilizers could be applied on almost all types of crops and soils. Phosphate solubilizing microorganisms secrete organic acids which enhance the uptake of phosphorus by plants. Hence, all biofertilizers provide nutrients to crops through either nitrogen fixation and phosphorous solubilization processes. A more details about different types of biofertilizers are given below:

1. **Bacterial biofertilizers:** These biofertilisers contain living bacteria that contain a specific gene called “Nif-Gene” which is responsible for fixing of atmospheric nitrogen. These types of bacteria live under two conditions, either as symbiotically and as free living. Symbiotic species of bacteria are Rhizobium and Azospirillum. Where Rhizobium can fix 50-300 kg/ha and survive in the root nodules of legumes plants where root supply essential minerals for survival of the bacteria. Azospirillum are mainly present in root cells of cereal plants forming symbiotic relation and increasing N$_2$ fixing potential of the plant. Interestingly, significant quantity of nitrogen fertilizer upto 20-30 % can be saved by the using *Azospirillum*. These biofertilizers have been commercially exploited for the use as nitrogen supplying agents. Non symbiotic bacteria are those that does not make any nodules but have association in the rhizosphere in soil. Commonly found non symbiotic bacteria are *Azotobacter*, *Klebsiella*. Where *Azotobacter* is a heterotrophic free living N$_2$fixing bacteria. These bacteria can also synthesize growth promoting hormones auxins and gibberellins that enhances germination of seeds and plant growth. Phosphate solubilising bacteria are *Pseudomonas*, *Bacillus megaterium*.

**What is liquid Biofertilizers?** Currently, biofertilizers are being supplied to the farmers in the form of carrier based inoculants. alternately, a liquid formulation has been developed by Department of Agricultural, TNAU, Coimbatore. This liquid formulation has more advantages than the carrier beased inoculants.

**Benefits of Liquid Biofertilizers:** The benefits of liquid biofertilizer over conventional are: It has longer shelf and minimum contamination. It has greater potentials to fight against the native population. Dosages are less compared to the carrier based bio-fertilizers. High export potential and high commercial revenues and have very high enzymatic activity and no contamination.

**What is Vesicular Arbucuscular Mycorrhiza (VAM)?**
VAM is an association formed by the symbiotic relationship between a specific phycomycetous fungi and the angiospermic roots. In VAM specific types of fungus grow and colonizes the plant root
cortex and forms a mycelial network comprised of characteristic vesicles and arbuscules structural branched hyphae. VAM help in plant growth and productivity. It help the plant in tolerance to various abiotic and biotic stresses e.g. alkalinity heavy metals and acts as biofertilizers. It substitute the fertilizer requirements of plants in areas of low fertility and minimizes the needs of chemical fertilizer. This symbiosis of mycorrhiza plays a very crucial role in changing the ecology of a surrounding and promotes mineral recycling an important component of natural ecosystems.

Risk of chemical pesticides and Potential for the Biopesticides.
Currently, around ~80,000 tons of pesticides are used in agriculture in India annually. Indiscriminate use of chemical pesticides leads to three sad R’s; Resistance, Resurgence and Residues and elimination of natural enemies of pests, disturbance of the ecological balance. These pesticides enter the food chain and causes bioaccumulation and biomagnification. Further, due to misuse and overuse of these pesticides, drop in crop productivity has been consistently reported. The problem is very serious because of the development of resistance to pesticides in the target pests and presence of the pesticide residue in food products. It is, therefore, important that alternative, eco-friendly methods for pest management techniques for plant protection should be adopted, including usages of biopesticides.

Why bio-pesticides?
Biopesticides are derivatives obtained from natural materials such as plants, animals, and bacteria (Gupta et al., 2010). These are widely used for controlling insects and disease causing pathogens. The biopesticides are categorized under microbial pesticides, plant-incorporated-protectants and biochemical pesticides, and are produced through various naturally occurring substances that control pests by non-toxic mechanisms. Biopesticides are generally inherently less toxic; affect only the target pest, it has highly effective in very small quantitie and are easily biodegradable, thereby resulting in lower exposures and pollution problems. When used as a component of pest management strategies, it can greatly control major pest problems while keeping the crop yields high. Increasing demand for organic farming are expected to be the driving force for high global demand of biopesticides in future pest controlling agents.

Advantages:
Biopesticides have high human and environmental safety. In contrast to conventional pesticides: 14% of all known occupational injuries and 10% of all fatal injuries are solely caused by chemical pesticides. Biopesticides are amenable for small-scale local production. Biopesticides are a prime component of any pest management strategies. Through application of biopesticides natural enemies protected. Growing awareness among consumers for environmental and food safety and organic product & chemical free crops. Bio-pesticides fits where few chemicals exist e.g. Nematode control. Many bio-pesticides reduces the pre-harvest intervals, which allow more flexibility in harvesting and shipping schedules. Conventional technique or methods can be used for application. Tightening regulations on pesticide residues, especially in developed world

Types of Biopesticides: There are different types of biopesticidaea are available nowadays and each type can be used to utilize or target a specific group of pest. Such as specific types of fungi can kill certain types of unwanted weeds, while various group of bacteria can kill different types of insect larvae, and mosquitoes. The different types of biopesticides are discussed below:

I) Biochemical pesticides: These are naturally occurring compounds that check the pests populations by non-toxic mechanisms. Since, it interfere with mating procedures or insect sex pheromones, as well as various plant extracts that attract insect pests to traps such as plant extracts, pheromones, and fatty acids, natural plant growth regulators (PGR), e.g. Avermectin, Pyrethrins, Spinocid from natural products.

II) Microbial pesticides: It consist of specific microorganism e.g., bacteria, fungi, viruses as an active ingredient. Microbial pesticide are able to control many different kinds of pests, although each
ingredient is specific for its target pests. e.g., specific fungi can control certain weeds and a different
fungi kill specific insects. Bacillus thuringiensis strains or Bt. strains produces a mix of proteins and
that specifically kills certain species of insect larvae. Certain strains of Bacillus e.g. Bacillus subtilis,
B. pumilus, Pseudomonas spp. and Streptomyces spp. increase crop yield and prevent plant diseases
by competing with other plant pathogens. Certain viruses and fungi used as biopesticides are:
Cydia pomonella granulosis virus (CpGV), cytoplasmic polyhedrosis virus and fungi include-
Aschersonia aleyrodis, Metarhizium anisophliae

III) Plant-Incorporated-Protectants (PIPs): These are the pesticidal molecules produced by plant
from its genetic material that has been deliberately added to the plant. e.g. plant producing Bt toxin. In
this case the Bt toxin or pesticidal protein coding gene can be introduce into the plant's genome. Then
the plant, starts manufacturing the substance that destroys the pest.

Advantages of using Biopesticides as biocontrol Agents.
Biopesticides are more target specific than chemical pesticides as it affect only the target pests and
their close species. In contrary, chemical pesticides destroy friendly insects, and other non target
organisms e.g. nnmammals and birds. Biopesticides are effective in small concentrations and degraded
quickly and do not leave any harmful residues.

Microorganisms involved and the Metabolic Processes.
First of all in biofertilizers, the four types of microorganisms that are involved are rhizobium,
azola, azotobacter and the blue-green algae. Rhizobium are soil bacteria that can fix nitrogen found in
the air. Inside the root, they invade the cortex cells, and then differentiate into "bacteroides" as
nitrogen forming structure. The nitrogen fixation within the root nodules results from a complex
metabolic exchange processes between the Rhizobium and the host plant. With this, nutrients are
supplied to the bacterial cells or bacteroides and in exchange, bacteria transfer the fixed nitrogen to
plant. Azolla is a type of fern that contains an endosymbiont the nitrogen-fixing cyanobacterium
Anabaena azollae that dissolves the nitrogen rapidly and made available to the plants in shorter time
period.

Synthetic pesticides Vs. Biopesticides: Generally, bio-pesticides are considered to be less harmful
that the synthetic pesticides because, as mentioned earlier, they are obtained from natural sources.
These are effective in very small concentrations and often get decompose very quickly in compare to
synthetic pesticides so avoid the associated pollution problems.

Disadvantages of Bio-Pesticides: The major disadvantage of biopesticides are if they are used in
large quantities then they can start harming non-targeted organisms, including humans. Therfore, to
target a particular pest different types of pesticides must be used. This is due to very high specificity
of biopesticides that cannot be used on any plant without a test. However, the constant use of these
pesticides would be harmful for plants. Also, since their reactions take place slowly, bio-pesticides
would be very unsuitable if there is a pest-breakout. This is because if they don’t work effectively at
that time, there is a possibility of being an immediate threat to the crops. Since bio-pesticides are also
living organisms, their efficiency is influenced by other biotic and abiotic factors. And if bio-
pesticides are constantly used, the targeted organisms would also become resistance to their control.
The only way to remove this possibility is if the targeted population is completely exterminated before
they reproduce or they become incapable of reproducing.

How are Bio-Fertilizers and Bio-Pesticides beneficial to the society and the environment?
Bio-pesticides and Bio-fertilizers are used widely by the agricultural society. This is because they are
beneficial to the environment and to the farmers. Since they are the opposite of conventional
fertilizers and pesticides, which include chemicals, they are greatly accepted. In the environment,
naturally occurring pesticides and bio-fertilizers are known to cause less pollution than other chemical
ones (Bale et al., 2008). They are also known to reduce the amount of synthetic fertilizers and
pesticides that most farmers use. And in most cases, they are not harmful to the society or to the
organisms around the plant (Aktar, et al., 2009). These bio-fertilizers and bio-pesticides also have the ability to produce greener and healthier leaves. Although, they may show slow results but the benefits are worth it.

How does EPA (Environment Protection Agency) encourage the development and use of Biopesticides?

Biopesticides and Pollution Prevention Division was established by EPA in 1994 to regulate the usages and registration of biopesticides developed (Dutta S., 2015). It monitor the application of environmentally safe pesticides, including biopesticides as part of the integrated pest management programs. Since biopesticides have lower risks than the chemical pesticides, EPA generally requires less information to register a biopesticide than to register a chemical pesticide. In fact, new formulated biopesticides get registered in less than a year, in compared with an average time of more than 3 years for a chemical pesticides. Further, EPA always conducts analysis and reviews to ensure that registered pesticides is not harmful to public and to the environment. For further safety, EPA make sure that the relevant agency submit the results of a variety of studies and other information about the formulations, its toxicity, degradation, and other properties of the submitted pesticide.

Summary

Biofertilizers and biopesticides are one of the essential inputs in organic farming they not only stimulate plant growth and product yield but also enhances the soil fertility and soil health. The biological pest management is an important areas that require intensive research to enhance its effectiveness mainly to achieve food security of increasing population and restore soil health. The potential uses of biopesticides and biofertilisers are in sustainable agriculture. A number of government agencies are already involved in production and application of these biopesticides. However still, their uses are limited in developing countries. These areas need to be prioritized in the agricultural research by various research organizations in technology development for the farmers.