Biological control and PGPR – Scope and importance – Role and mechanisms of biological control and PGPR with examples. Plant growth promoting rhizobacteria

Biological control is defined as the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant stage by one or more organisms accomplished naturally or through manipulation of the environment, host or by introduction of one or more antagonists or by mass introduction of one or more antagonists.

Biological control is but control of plant diseases using living microorganisms. Root rot disease *(Macrophomina phaseolina)* is a major disease in pulses, oilseeds, cofton, etc., and the most common method of control is using fungicides. But the chemical methods are uneconomical and law effective, as seed treatment with chemical may give protection only in the early stages of crop growth 2 weeks.

In addition, it is harmful to the beneficial microorganisms in soil and creates residual problems. So, the biological control can be very efficasy used for the root rot disease management as the biological agent multiply in soil and offer protection throughout the crop growth. The four main mechanisms involved in the biocontrol are (i) the biological agent (antagonist), may parasite the other organism, (ii) antagonist may secrete metabolites (antibiotics) harmful to the pathogens (Antibiosis) (iii) antagonist may compete with the pathogens for nutrients or space (Competition) and (iv) may cause death of the parasite by producing enzymes (Lysis).

Parasitism and Lysis

The biocontrol against parasitizes the pathogen by coiling around the hyphae, e.g., *Trichoderma* viride; various bacteria and fungi secrete hydrolytic about the degradation of cell wall of pathogens.

e.g. (i) *Bacillus* sp. causes hyphal lysis of *Gaeumanornyces* graminis

(ii) The chitnolytic enzymes of *Serratia marcescens* caused cell wall lysis of *Scierotium rolfsii.* (iii) *Trichoderma* sp. produces chitinases and β-1,3 glucanases which lyses the cell wall of *Rhizoctonia solani.*

Antibiosis

The antibiotic compounds secreted by the biocontrol agent suppress the growth of the pathogen. e.g. Phenazine-l-carboxylic acid produced by *P fluorescens* plays an important role in suppressing the take all disease of wheat.

Competition

The biocontrol bacteria and fungi compete for food and essential elements with the pathogen thereby displacing and suppressing the growth of pathogen.

e.g. (i) the competition for nutrients between *Pythium aphanidermatum, P ultimum* and bacteria suppress the damping off disease in cucumbers.

(ii) Fluorescent siderophores (iron chelaters) such as pseudobactinis & *pyoverdins* produced by P *fluorescons* chelates iron available in the soil, thereby depriving the pathogen of Its Fe requirements.

A. TRICHODERMA VIRIDE

The fungus, *Tfichoderma vitide* is one such biocontrol agent, mainly used for the control of root rot diseases of pulses and oil seeds in Tamil Nadu. A mass production technology for *T.viride* has been developed by Tamil Nadu Agricultural University, Coimbatore.

Systematic Position

Asexual **(conidial) Sexual (sscospore)**

Sub division : Deuteromycotina Ascomycotina

Class : Hypomycetes Pyremomycetes

Order : Moniliales Sphaeriales

Family : Moniliaceae Hypocreaceae

Genus : Trichoderma Hypocrea

Isolation of *Trichoderms* from soil

Trichoderma is isolated from the soil by using *Trichoderma* selective medium developed by Elad and Chet (I 983). Collect soil samples from the field, mix well and make it into fine particles. Soil samples should be collected in root zone at 5-1 5 cm depth and from rhizosphere wherever possible. Ten gram of soil sample is taken, and suspended in 100 ml of sterile distilled water and stirred well to get I : IO dilution. Transfer one ml from this to 9 ml of sterile water in a test tube to get 1: 100 dilution. Make serial dilutions by transferring one ml of suspension to subsequent tubes to get dilution of 1: 1 0,000. Transfer one ml of the desired soil suspension to

sterile petriplates. Pour 15 ml of melted and cooled *Trichoderma* selective medium in the same petriplates. Rotate the plate gently and allow to solidify, incubate at room temperature for 5-7 days and observe for the development of fungal colonies. Trichoderma colonies will be white initially and turn to green. Count the number of colonies developing in individual plates. Transfer the individual colonies to potato de)drose agar slants.

Testing Method

Dual Culture Technique

It consists of growing the test organism and the pathogenic organism on the same plate. This ran be done by the following procedure. Transfer 15-20 ml of melted and cooled PDA to sterilised petridishes. Allow it to solidify. Transfer 8 mm disc of test organism to one end of the petriplate. In the opposite end, 8 mm disc of the pathogenic culture is transferred in the same petriplate (if the antagonistic micro-organism is slow growing it should be plated in the previous day itself). Incubate the plate at room temperature. Observe the development of inhibition zone. Observe under microscope where both the test organism and the pathogen come in contact.

Mass Production

Molasses yeast medium (Molasses 30g + yeast 5g + water 1000ml) is prepared in conical flasks and sterilized at 1.1 kg/CM 2 for 20 minutes. *T.viride* culture is inoculated by taking a fungal disc from 10 day old culture and incubated for 10 days. This serves as mother culture. Molasses yeast medium is prepared in a fermenter and sterilized. Then, the mother culture is added to the fermenter @ 1.5 litre/50 litres of medium and incubated at room temperature for 10 days. The fungal biomass and broth are mixed with talc powder at 1:2 ratio. The mixture is air dried and mixed with carboxy methyl cellulose (CMC) @ 5g / kg of the product. It is packed in Polythene covers and used within 4 months.

Quality Control Specifications

1. Fresh product should contain not less than 28 X 10 6 CfU / g
2. After 120 days of storage at room temperature, the population should be 10 x IO 6 cfu / g.
3. Maximum storage period using talc as carrier is 120 days.
4. Size of the carrier (talc) should be 500 microns.
5. Product should be packed in white Polythene bags.
6. Moisture content of the final product should not be more than 20%.

B. *Bacillus subtilis*

This bacterium is widely used for the control of soil-bome plant pathogens like *Macrophomina phaseolina, Rhizoctonia solani, Fusarium* spp. etc. This treatment also considerably improves the plant growth and yield. *Bacillus subtilis* is a rod shaped, thermophilic gram positive, aerobic bacterium. Roots may be formed in chains. It is 5-6 mm in length and 2-3 mm in width. It forms endospores during adverse conditions.

Isolation

One gram of soil sample is mixed with 9 ml sterilized nutrient broth in a test tube. This has to be kept on a boiling waterbath at 800C for 10 minutes. Then it is kept for incubation at root temperature for 24-48 hrs. From this serial dilution is prepared upto 10-6 dilution. Dilution 10-5 and 10 -6 are plated In Nutrient Agar and incubated for 24- 48 hrs. *B. subtilis* colonies will be rough, opaque with irregular margins.

Staining for Identification

Bacterial smear is prepared with 24 hours old culture, air dried and heat fixed. The slide is flooeded with crystal violet for 60 seconds and then washed with tap water. Then, the slide is flooded with Grams iodine mordant for 60 seconds and washed with tap water. It is then the smear is counterstrained with safranin for seconds, washed with tap water, blot dried and observed under microscope. *Bacillus subtilis* appeared violet since it,is gram positive.

Biochemical tests for Identification

The following biochemical tests are carried out for identification.

1. Starch hydrolysis
2. Catalase test
3. Nitrate reduction test
4. Acid and gas production test

Bacillus subtilis is amylase positive catalase positive, nitrate positive, acid positive and gas negative.

Mass multiplication

Nutrient broth (Peptone 5g, beef extract 3g, sodium chloride 3g in 1 litre of distilled water, pH7) is prepared and sterilized at 1.1 kg/ CM 2 pressure for 20 minutes. One loopful of *B. subtilis* is inoculated and incubated for 24 hours. This serves as mother culture. One litre of mother culture is transferred to 100 litres of sterilized nutrient broth in a fermenter and the

bacterial growth is harvested after 72 hrs. Then it is mixed with 250 kg of sterilized peat soil amended with 37 kg Calcium carbonate, dried in shade and packed in Polythene bags. This product can be stored upto 6 months.

C. *Pseudomonas fluorescens*

This is another bacterium effectively used in controlling sheath blight and blast of paddy, wilt diseases of redgram, and banana. *Pseudomonas fluorescens* is a gram negative, rod shaped nonspore forming bacteria which may be mono or lopotrichous or non motile. It produces greenish, fluorescent and water soluble pigment, pyoverdin. The direct influence of pseudomonas on plant growth is mediated either by release of auxin-like substances or through improved uptake of nutrients in the environment. The indirect promotion of plant growth is achieved when fluorescent *Pseudomonas* decreases or prevents the deleterious influence of phytopathogens.

lsolation

One gram of rhizosphere soil sample is mixed in 100 ml of sterilewaterto give 1:100dilution. From this serial dilutions upto 10 -7 level are made by repeatedly transferring 1 ml of 1:I00 dilution to 9 ml sterile water stants 10 -5 , 10 -6 and 10 -7 dilutions are plated in kings B Agar medium and incubated for 24-48 hours. P *ftuorescens* appears as smooth, slimy, circular translucent colonies.

Mass production

P. fluorescens is multiplied in sterilized Kings 'B' broth for 48 hours. The pH of the substrate (Peat soil or talc powder) is adjusted to 7 by adding calcium carbonate @150 g / kg. The substrate is then sterilized at 1.1 kg/cm 2 pressure for 30 minutes for two successive days. Four hundred ml of *P. fluorescens* suspension is added to 1 kg of substrate containing 5 g of carboxy methyl cellulose and mixed well.The formulation is packed in Polythene covers and can be stored for one month.

Quality Control

1. Fresh product should contain 2.5 x I 0" cfu / g
2. After 3 months of storage at room temperature, the population should be 8-9 x 10 7 CfU / g.
3. Storage period is 3-4 months
4. Minimum population load for use is 1.0 x I0 8 cfu / g.
5. Product should be packed in white Polythene bags.

6. Moisture content of the product should not be more than 20% in the final product.

7. Population per ml of the broth is 9 ± 2 x 1 0 8 cfu / g.

Methods of Application

Crop: Paddy -blast, sheath blight

1. Seed Treatment

Mix paddy seeds with the formulation at the rate of 10 g per kg of seeds and soak the seeds in water for ovemight. Decant the excess water and allow to sprout the seeds for 24 hrs and then sow.

2. Seedling root dipping

Apply 2.5 kg of the formulation to the water stagnated in an area of 25 sq.m. The seedlings, after pulling out from the nursery can be left in the stagnating water containing the bacteria. A minimum period of 30 minutes is necessary for soaking the roots and prolonged soaking will enhance the efficacy.

3. Soil application

Apply the product @ 2.5 kg / ha after 30 days of transplanting (This product should be mixed with 50 kg of well decomposed FYM / sand and then applied).

4. Foilar application

Spray the product at 0.2% concentration (1 kg/ha) commencing from 45 days after transplanting at 10 days interval for 3 times depending on disease intensity. If there is no disease incidence, a single spray is sufficient. Crop: Groundnut, Gingelly, Sunflower, Redgram, Greengram, Blackgram - root rot and wilt

Seed treatment : 10 g /kg of seeds

Soil application : Apply 2.5 kg/ha. mixed with 50 kg of well decomposed FYM / sand at 30 days after sowing.

Crop : Banana - Fusarium wilt

Sucker treatment: 10 g/sucker

Capsule application: 50 mg / capsule / sucker.

Apply once in 3 months from 3 months after planting

Soil application: 2.5 kg / ha + 50 kg FYM / sand

Apply once at the time of planting and repeat it once In 3 months.

Plant Products and Antiviral principles in plant disease management

Plant products play an important role in evolving an ecologically sound and environmentally acceptable disease management system. Plant products have been found to have fungicidal, bactericidal and antiviral properties. It is well established that about 346 plant products have fungicidal properties, 92 have bactericidal and 90 have antiviral properties. This clearly indicates that the plant kingdom is a vast storehouse of chemicals that can check several plant pathogens. As many of them have more than one type of activity there is a less chance for development of resistance and moreover, the plant products are saferto non-target organisms.

Neem Products

Among the plant products, the neem derivatives are reported to be effective in controlling several diseases. The neem tree *(Azadirachta indica),* popularly called as china berry, crackjack, Nim, Indian lilac, margosa and paradise tree, contains several active principles in various parts. The important active principles are Azadirachtin, Nimbin, Nimbidin, Nimbinene, Nimbridic acid and Azadirone which have anitifungal and insecticidal properties.

(i) Neem Seed Kernel Extract (NSKE)

It is prepared by soaking 5 kg of powdered neem seed kernel (in a gunny bag) in 100 litres of water for 8 hours. The gunny bag is then removed afterthorough shaking. Then, 100 ml of teepol is mixed thoroughly, before spraying. The quantity of extract required for a liectare is 500 litres,

(ii) Neem oil solution

One hundred nil of teepol is mixed first with 100 litres of waterThen, 3 litres of neem oil is slowly added to this solution with constant shaking. The milky solution formed is ready for spray. The spray volume is 500 litres/ha.

(iii) Neem cake extract

Ten kg of powdered neem cake in a gunny bag is soaked in 100 litres of water for 8 hours. The gunny bagis removed after thorough shaking. Then, 100 ml of sticker is added and mixed well. The quantity of spray fluid required is 500 litres / ha.

(iv) Neem cake

Powdered neem cake is directly applied to the field at the time of last ploughing. The quantity applied is 150 kg/ha.

Diseases controlled by neem products

(a) Paddy: Tungro (virus) (Vector: *Nophotettix virescens)*

Neem cake is applied at 150 kg/ha as basal dose. In addition, 3% neem oil or 5% NSKE @) 500 l/ ha can be sprayed. If one jassid is noticed in a plant. Three sprays have to be given at 15 days interval.

(b) Paddy : Sheath rot *(Acrocyfindrium oryzae)*

Five per cent NSKE or 3% neem oil can be sprayed @ 500 lit/ ha at the time of grain emergence.

(c) Paddy: Blast *(Pyricularia oryzae)* Spraying 5% neem oil is effective

(d) Paddy: Sheath blight *(Rhizoctonia solani)*

Application of 150 Kg of neem cake/ha

(e) Groundnut : Rust *(Puccinia arachidis)*

Application of 3% neem oil @ 500 lit/ha. The first spray should be given immediately on noticing the symptom and second 15 days later.

(f) Groundnut : Foot rot *(Sclerotium rolfsii)* Application of 1 % neem oil is effective.

(g) Coconut: Wilt *(Ganoderma lucidum)*

Application of 5 kg of neem cake/ tree/ year during the rainy season.

(h) Black gram: Powdery mildew *(Erysiphe polygoni)*

Two sprays with 3% neem oil or 5% NSKE, starting first spray at the initiation of the disease and second 15 days later are effective.

(i) Black gram: Root rot *(Macrophomina phaseolina)* Application of neem cake @ 150 kg/ha

(j) Black gram: Yeliow mosaic (Virus) Application of 3% neem oil is effective.

(k) **Soybean:** Root rot *(M. phaseolina)* Application of neem cake @ 150 kg/ha.

Other Plant Products

In addition to the neem products, products from several other plant species are also found to be effective in disease management. The leaf extract of tuisi *(Ocimum sanctum)* is found effective against *Helminthosporium oryzae* (paddy brown spot). The leaf and pollen extracts of vilvam *(Aegle marmolos)* effectively reduced early blight of tomato *(Altenaria solani)* and blight of onion *(A. porri). A. solani* is also effectively checked by flower extract of periwinkle *(Catheranthus roseus)* and bulb extract of garlic *(Allium sativum).*

Rice discolouration caused by *Drechslera oryzae* is effectively reduced by leaf extract of mint *(Mentha piperita).* The bulb extract of garlic is also effective in reducing leaf blight of finger millet *(H. nodulosum)* and blast of paddy *(Pyricularia oryzae).* The root exudates of kolinji and rhizome extract of banana are effectively used against *Ganoderma lucidum, the* pathogen of Thanjavur wilt of coconut. The seed oil of pinnai *(Calophyllum inophyllum)* is effective against *Puccinia arachidis* causing groundnut rust. Leaf extract of nochi *(Vitex negundo)* effectively reduced, Rice Tungro viruses by checking the vector, Nephotettix *virescens.*

Anti Viral Principle (AVP)

Plants are also known to contain some compounds which are inhibitory to virus. They are called Anti-Viral Principles (AVP) or AntiViral Factors (AVF). The leaf extracts of sorghum, coconut, bougainvillea, *Prosopis juliflora and Cyanodon dactylon* are known to contain virus inhibiting principles.

Preparation of AVP extract

Dried coconut or sorghum leaves are cut and powdered. Twenty kg of leaf powder is mixed with 50 litres of water and heated at 60 0 C for one hour. It is filtered and volume is made upto 200 litres. This gives 10 per cent extract. Five hundred litres of extract is required to cover one hectare. The 10 per cent AVP extract is very effective in controlling groundnut ring mosaic virus (bud necrosis).

Two sprays are to be given at ten and twenty days after sowing. Similarly of percent leaf extracts of *P. juliflora* and C. *dactylon* effectively reduced the tomato spotted wilt virus in tomato. The leaf extracts are known to contain some proteinaceous substances which induce virus inhibition in the plants.

PGPR

Plant growth promoting rhizobacteria are bacteria that colonize plant roots, and in doing so, they promote plant growth and/or reduce disease or insect damage. There has been much research interest in PGPR and there is now an increasing number of PGPR being commercialized for crops. Organic growers may have been promoting these bacteria without knowing it. The addition of compost and compost teas promote existing PGPR and may introduce additional helpful bacteria to the field. The absence of pesticides and the more complex organic rotations likely promote existing populations of these beneficial bacteria. However, it is also possible to

inoculate seeds with bacteria that increase the availability of nutrients, including solubilizing phosphate, potassium , oxidizing sulphur, fixing nitrogen, chelating iron and copper. Phosphorus (P) frequently limits crop growth in organic production. Nitrogen fixing bacteria are miniature of urea factories, turning N2 gas from the atmosphere into plant available amines and ammonium via a specific and unique enzyme they possess called nitrogenase. Although there are many bacteria in the soil that ‘cycle' nitrogen from organic material, it is only this small group of specialized nitrogen fixing bacteria that can ‘fix' atmospheric nitrogen in the soil. Arbuscular mycorrhizal fungi (AMF) are root symbiotic fungi improving plant stress resistance to abiotic factors such as phosphorus deficiency or deshydratation.

The fourth major plant nutrient after N, P and K is sulphur (S). Although elemental sulphur, gypsum and other sulphur bearing mined minerals are approved for organic production, the sulphur must be transformed (or oxidized) by bacteria into sulphate before it is available for plants. Special groups of microorganisms can make sulphur more available, and do occur naturally in most soils.

One of the most common ways that PGPR improve nutrient uptake for plants is by altering plant hormone levels. This changes root growth and shape by increasing root branching, root mass, root length, and/or the amount of root hairs. This leads to greater root surface area, which in turn, helps it to absorb more nutrients.

Disease control

PGPR have attracted much attention in their role in reducing plant diseases. Although the full potential has not been reached yet, the work to date is very promising and may offer organic growers some of their first effective control of serious plant diseases. Some PGPR, especially if they are inoculated on the seed before planting, are able to establish themselves on the crop roots. They use scarce resources, and thereby prevent or limit the growth of pathogenic microorganisms. Even if nutrients are not limiting, the establishment of benign or beneficial organisms on the roots limits the chance that a pathogenic organism that arrives later will find space to become established. Numerous rhizosphere organisms are capable of producing compounds that are toxic to pathogens like HCN

Challenges with PGPR

One of the challenges of using PGPR is natural variation. It is difficult to predict how an organism may respond when placed in the field (compared to the controlled environment of a

laboratory. Another challenge is that PGPR are living organisms. They must be able to be propagated artificially and produced in a manner to optimize their viability and biological activity until field application. Like Rhizobia, PGPR bacteria will not live forever in a soil, and over time growers will need to re-inoculate seeds to bring back populations.

PGPR in Research

Over the years the PGPR (plant growth promoting rhizobacteria) have gained worldwide importance and acceptance for agricultural benefits. These microorganisms are the potential tools for sustainable agriculture and the trend for the future. Scientific researchers involve multidisciplinary approaches to understand adaptation of PGPR to the rhizosphere, mechanisms of root colonization, effects of plant physiology and growth, biofertilization, induced systemic resistance, biocontrol of plant pathogens, production of determinants etc. Biodiversity of PGPR and mechanisms of action for the different groups: diazotrophs, bacilli, pseudomonads, Trichoderma, AMF, rhizobia, Phosphate solubilising bacteria and fungi, Lignin degrading , chitin degrading , cellulose degrading bacteria and fungi are shown. Effects of physical, chemical and biological factors on root colonization and the proteomics perspective on biocontrol and plant defense have also shown positive results. Visualization of interactions of pathogens and biocontrol agents on plant roots using autofluorescent protein makers has provided more understanding of biocontrol processes with overall positive consequences.

Ways that PGPR promote plant growth

- Increasing nitrogen fixation in legumes
- Promoting free-living nitrogen-fixing bacteria
- Increasing supply of other nutrients, such as phosphorus, sulphur, iron and copper
- Producing plant hormones
- Enhancing other beneficial bacteria or fungi
- Controlling fungal diseases
- Controlling bacterial diseases
- Controlling insect pests