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# Harmonizing Bio-Agents for Effective Control of Soil-Borne and Foliar Diseases

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Biological control is the control of disease by the application of biological agents to a host animal or plant that prevents the development of disease by a pathogen. With regard to plant diseases the biocontrol agents are usually bacterial or fungal strains isolated from the endosphere or rhizosphere. The organism that suppresses the growth of pathogen is referred to as the biological control agent (BCA). Biological control is the suppression of damaging activities of one organism by one or more other organisms, often referred to as natural enemies. One of the basic principles of plant pathology is that interactions occur between hosts, pathogens and the environment and that disease severity is determined by the outcome of these interactions. The environment is a particularly important component of this 'disease triangle' and the role of environmental factors such as moisture and temperature in disease development is widely recognized. The abundance of any one organism is controlled by its food supply, the environment and by other organisms. These same principles also apply to plant pathogens. If the quantity and activity of a pathogen is kept in check by the microbial community with which it is associated, biological control has been achieved. Biological control occurs naturally on plant surfaces (e.g. in the rhizosphere, phylloplane and fruit surfaces) by the activity of epiphytic microflora.

## Modes of action of biological control agents

Microbial antagonism of plant pathogens occurs in several ways, the most common mechanisms being parasitism and predation, competition for nutrients or space, production of antimicrobial substances and induced resistance.

## Competition for nutrients and space

- Competition occurs when two or more organisms require the same resource for growth and survival. The use of this resource by one organism reduces the amount available to the other.
- The rhizosphere is a region of intense microbial activity where there may also be competition for oxygen. On the leaf surface, where nutrients are in short supply, competition for nutrients is thought to play a significant role in disease suppression.
- Competition for the same carbon source between *Pythium ultimum*, a common cause of seedling damping-off and rhizosphere bacteria has resulted in effective biological control of *P. ultimum* in several crops.
- Treating seeds with a strain of *Pseudomonas putida*, which utilizes ethanol as its sole carbon source in culture, reduces the concentration of volatiles released, lessens hyphal growth or sporangial germination of the pathogen.
- One of the best documented examples of nutrient competition in biological control involves competition for iron between fluorescent pseudomonads and soilborne fungal pathogens such as *Fusarium oxysporum*. Strains of bacteria including *Pseudomonas*

*fluorescens* and *P. putida* produce siderophores, metabolic products of micro-organisms that bind iron and facilitate its transport from the environment into the microbial cell.

- The siderophores pyoverdine and pseudobactin have a high affinity for the soluble ferric iron and inhibit the growth of pathogens by limiting the availability of iron. *Pseudomonas* species inhibited the germination of conidia of *Botrytis cinerea* by competing for amino acids.

## Production of antimicrobial substances

- When grown in culture, most micro-organisms produce secondary metabolites. They often have unusual structures and are toxic to other micro-organisms (e.g. antibiotics and mycotoxins). They may be volatile or non-volatile. Among the known volatile substances, hydrogen cyanide and ammonia have been studied.
- A phenazine antibiotic produced by a strain of *Pseudomonas fluorescens* in the suppression of the take-all pathogen of wheat, *Gauemannomyces graminis* var. *tritici*.

## Parasitism and predation

- The parasitism of one fungus by another is well documented and is manifested as morphological disturbance, direct penetration of hyphae and hyphal lysis. The genus *Trichoderma* contains some of the most studied mycoparasites. Formulations of some species are available commercially and are used to control fungal pathogens in the soil and on aerial plant surfaces. This mycoparasite penetrates resting structures such as sclerotia or may parasitize growing hyphae by coiling round them. *Trichoderma harzianum* degrades fungal cell walls by the lytic action of glucanases and chitinases, while other species also produce cellulase. The attributes of the partially successful mycoparasite *Ampelomyces quisqualis*, which can control powdery mildew in glasshouse grown cucumber.
- Other widely studied mycoparasites include *Coniothyrium minitans* and *Sporidesmium sclerotiorum*, which are antagonists of sclerotial fungi, and *Gliocladium* spp., which parasitise a range of soil-borne pathogens. Mycoparasitic *Pythium* spp. appear to have some affinity for plant-parasitic members of the same genus. On the phylloplane, several fungi, including *Verticillium lecanii*, *Sphaerellopsis filum* and *Cladosporium* sp., are known to attack rust fungi. Isolates of *Bacillus* obtained from the surface of cereal rust urediniospores can lyse germ tubes arising from urediniospores on agar.

## Examples of biological control of plant pathogens

Crown gall which is caused by the soil-inhabiting bacteria *Agrobacterium tumefaciens*, *A. rhizogenes* and *A. uitis*, has been reported from over 90 families of plants. Commercially, the biological control agent is applied by dipping plants or cuttings in a suspension of *A. radiobacter* strain K 84 during propagation and transplanting. This technique is so simple and effective that it has been widely adopted.

## Diseases of leaves and flowers

- There are several examples of potentially useful biological control systems that are currently being studied for pathogens of leaves and flowers. Cucumber powdery mildew (*sphaerotheca fuliginea*) has been successfully controlled in the greenhouse by a hyperparasitic fungus *Ampelomyces quisqualis*. Antagonism of the fireblight pathogen *Erwinia amylovora* by a closely related bacterium *Erwinia herbicola*.

## Diseases of fruits and vegetables-

- One of the most common pathogens targeted is *Botrytis cinerea*, the cause of grey mould in many fruits. The fungus is spread rapidly by airborne spores. Experimentally, control has been achieved by applying spores of *Trichoderma harzianum*.
- Apple scab, caused by the ascomycete *Venturia inaequalis*, also leads to both pre and postharvest loss of fruit due to reduction in fruit quality. *Chaetomium globosum* is one possible candidate for biological control of apple scab. The basidiomycete antagonist

*Athelia bombacina* has also been tested against apple scab and reduced the production of ascospores. Application of antagonists prior to harvest to control both pre and postharvest disease development.

## Biological control of foliar pathogens by means of *Trichoderma harzianum*

- Bio-control of foliar diseases is an alternative means of management of foliar pathogens. One of the most studied commercial biocontrol agents is isolate T39 of *Trichoderma harzianum* which can be regarded as a model to demonstrate biocontrol under commercial conditions. This biocontrol agent (BCA) controls the foliar pathogens, *Botrytis cinerea, Pseuperonospora cubensis, Sclerotinia sclerotiorum and Sphaerotheca fusca* in cucumber.
- Cells of the BCA applied to the roots and dead cells applied to the leaves of cucumber plants induced control of powdery mildew. The BCA suppressed enzymes of *B. cinerea, such as* pectinases, cutinase, glucanase and chitinase, through the action of protease secreted on plant surfaces. A combination of several modes of action is responsible for biocontrol. They have the potential to degrade cell-wall polymers, such as chitin and secretion of antibiotics.
- The bacterial and fungal genera that are commercially employed as biological control agents include *Gliocladium, Bacillus, Coniothyrium, Paecilomyces, Phlebiopsis, Pseudomonas, Rhizobium, Serratia, Streptomyces* and *Trichoderma*. The application of biocontrol agents in soil such as *T. viride, T. harzianum*, fluorescent Pseudomonas and *B. subtilis* have been found to be effective against root rot caused by soilborne plant pathogens in a number of crops.
- *Trichoderma* species are known to produce large quantities of fungi-toxic metabolites. They are the active mycoparasites which have been used as effective biocontrol agents against foliar and soilborne disease.
- Use of mycoparasite, *C. minitans*, to effectively manage *S. sclerotiorum* is widely used. *C. minitans* produce cell-wall degrading enzymes such as chitinases and glucanases that can enhance the colonization and degradation of sclerotia produced by *S. sclerotiorum.*
- The antifungal substance obtained from the bacterium *B. amyloliquefaciens* was found to be effective to inhibit the mycelial growth of some pathogenic fungi such as *Alternaria panax, Botrytis cinera, C. orbiculare, Penicillium digitatum, P. grisea* and *S. sclerotiorum.*

| Biocontrol Agents | Target Pathogen | Mode of Action |
|---|---|---|
| *Bacillus* spp. | *Pythium* spp., *Fusarium* spp., *Rhizoctonia solani*, *Aspergillus flavus* | Competition, direct antibiosis, induced resistance |
| *Pseudomonas* spp. | *Pythium* spp. and *R. solani* | Production of antibiotics, siderophores, volatiles |
| *Streptomyces* spp. | Species of *Fusarium, Rhizoctonia, Phytophthora, Pythium, Phytomatotricum, Aphanomyces, Monosprascus, Armillaria, Sclerotinia, Verticillium, Geotrichum* | Mycoparasitism |
| *Trichoderma* spp. | Species of *Rhizoctonia, Fusarium, Alternaria* and *Colletotrichum* as well as oomycetes, such as *Pythium* and *Phytophthora* | Competition, resistance and hyperparasitism |

## Commercialization of biological control

- Mass production systems for biological control agents involve growth of the organisms on a solid substrate such as cereal grains or in liquid culture. At present, nearly all biological control agents are produced in deep-tank liquid fermenters where pH, temperature and aeration can be controlled.

- Formulation involves concentration and processing of the microbial biomass produced in a fermenter to achieve a viable, high quality, genetically stable, cost effective and commercially useful product. The type of formulation selected will depend on the method of application. Easily handled products that can be applied with standard machinery have a distinct advantage over those that require a special applicator.
- Biological control agents have been formulated as dry products such as wettable powders, dusts and granules and in liquid forms as aqueous or oil-based products.
- Once a formulated product is available, its efficacy must be evaluated under a variety of conditions to ensure consistency of performance.

## Compatibility of different bio- agents

- Agriculture in modern era depends largely on the use of agrochemicals, for managing plant diseases and to enhance crop productivity. Some naturally occurring soil bacteria and fungi have shown great potential to inhibit plant pathogens, hence, biological control involving the use of such beneficial micro-organisms for plant protection is being considered as a viable substitute to reduce the use of agrochemicals in general and pesticides in particular.
- A large number of plant diseases have been successfully managed through fungal and bacterial antagonists. *Trichoderma* sp. have been used in the management of plant diseases. The duration and degree of active disease control can be extended by using chemicals and biological control agents (antagonists) together as a mixed formulation in integrated disease management system. In a mixed formulation even reduced amount of the fungicide may weaken the pathogen and render its propagules more susceptible to subsequent attack by the antagonists. Usually fungicidal resistant or tolerant isolates of bio-agents are readily screened and obtained through selection on pesticide containing media. *Trichoderma* is being used as a biological component in the integrated disease management of soil borne pathogen of cardamom (*Elettaria cardamomum* Maton.) viz. capsule rot and rhizome rot.

## Advantages of use of bio-agents

- These are environmentally friendly and no side effects on humans.
- Comparatively cheaper than agrochemicals as insecticides and pesticides.
- Easy to use, easily available and can be used in any season.
- Reduces the use of chemicals.

## Disadvantages

- Causes a significant and noticeable deterioration in quality of produce.
- Eradicate all the pests and pathogens when used on large scale.
- Used against specific diseases.
- Slow effect in control of plant diseases.
- Shelf life is short.
- Less effective than fungicides.