REVIEW ARTICLE

Bio-control by using Antagonistic (Filamentous Fungi and VAM) and Bacteria against *Macrophomina phaseolina*

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Abstract

The excessive use of pesticides and fertilizers in the current agricultural system, which is done to increase production, completely eradicate plant pathogens, and reduce undesirable weeds, has a detrimental effect on soil quality, water body environment, animal and human health due to the toxicity, recalcitrance, and carcinogenic potential of many of these compounds. It has long been thought that biological plant disease control could replace current methods of prevention. Filamentous fungi (especially *Aspergillus* spp., *Trichoderma reesei*, and *Neurospora crassa*), bacteria (*Pseudomonas, Bacillus, Burkholderia, Lysobacter, Serratia,* and *Pantoea*), and Vesicular Arbuscular Mycorrhiza (VAM) (*G. mosseae, Glomus claroideum, Glomus aggregatum*) are employed instead of chemicals in biocontrol. *M. phaseolina* is a fungus that lives in the root soil and produces dry root rot/stem canker, stalk rot, and charcoal rot. The fungus *M. phaseolina* causes charcoal rot, stalk rot, and dry root rot/stem canker in plant roots. Melon, strawberries, and tomatoes are just a few of the horticultural crops that *M. phaseolina* has been discovered on throughout Europe, the US, Australia, Chile, and Israel. To manage *M. phaseolina*, various biocontrol agents (filamentous fungi, VAM, and bacteria) are used successfully and effectively.

Keywords: Alternative, bacteria, Antagonist fungi, Biocontrol, VAM, *M. phaseolina*.

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Introduction

The demand for agricultural products and food grains rises along with the population. The deliberate application of chemical fertilizers and pesticides has greatly risen in the modern global agricultural system. More than two billion metric tons of pesticides are used annually throughout the world. The use of fungicides eliminates plant infections caused by fungi, bacteria, viruses, and nematodes, bactericides, herbicides, insecticides, and other pesticides (Dean *et al*., 2012; Singh, 2014; O'Brien, 2017). Due to the toxicity, recalcitrance, and carcinogenic potential of many of these substances, excessive fertiliser use has a severe influence on soil quality, hydro - geological environment, both animal and human health. Pest biocontrol is now accepted as a practical alternative to pesticides. Around the world, fungi antagonists are used as biocontrol agents (BCAs) to protect plants from diseases and infections. As biocontrol agents, various antagonistic fungi (including filamentous fungus and VAM) and bacteria are used to prevent and treat diseases and enhance plant development and productivity.

Filamentous Fungi

Filamentous fungi (especially *Aspergillus* spp., *Trichoderma reesei*, and *Neurospora crassa*). It is well known that filamentous fungi secrete a large number of different hydrolytic enzymes, making them the main source of enzymes for the hydrolysis of lignocellulose (Stirling and Stirling, 1997). In the soil, filamentous fungi perform two key tasks: they break down organic materials and promote soil aggregation. Numerous fungi that are connected with plants are well known for promoting plant growth, and soil-borne microbes can enter roots and establish themselves as endophytes in plants (Sylvia *et al*., 2005).

The antagonists can be specialized in a variety of ways to control biological ailments, both directly and indirectly (Fig. 1). These mechanisms include antibiosis (in which the antagonist

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produces an inhibitory metabolite or antibiotic), mycoparasitism (in which the antagonist obtains all or the majority of its nutrients from the fungal host), induced resistance (induction of plant defence responses against plant pathogens), and growth promotion (BCAs promote plant growth while the effects of the disease are being reduced and also through microbial hormones such as indole acetic acid and gibberellic acid). Further activities

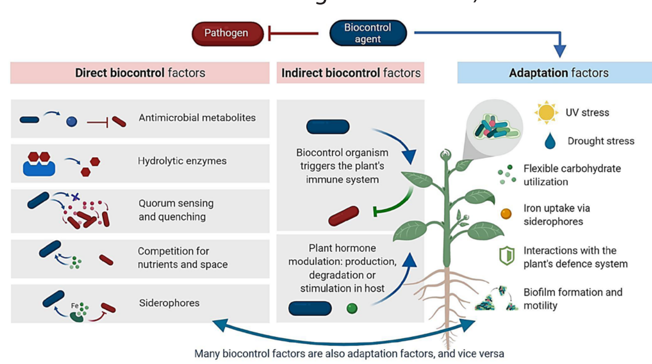


Fig. 1: Interaction of Pathogens and biocontrol agents.

extracellular hydrolytic enzymes secreted by the antagonist, microbes competing for nutrients and space, and pathogenicity detoxification are a few biological disease control strategies (Zhang *et al*., 2014; Deketelaere *et al*., 2017). With the exception of oomycetes, which contain cellulose in their cell walls but no chitin, many plant pathogenic fungus have chitin and - (1, 3)-glucan as their two main structural constituents.

Mechanism of Antagonist Fungi

It has been associated with the biological control of a number of soil-borne fungal infestations that microbes producing chitinases or glucanases exhibit antagonistic behavior toward fungus *in-vitro*. One of the few beneficial fungi that have been successfully used effectively as a biocontrol agent is the *Trichoderma* species. India, France, the UK, Switzerland, Sweden, Belgium, Chile, New Zealand, and the US have all recognized the microbe as a bio-fungicide; rules are still pending in a large number of additional nations.

Trichoderma often grows chemotropically in consequence to hyphal activation or gradients of the host's chemical production. The mycoparasite coils around the host or develops hook-like structures to adhere to it whenever it gets there. Sometimes during these interactions, the mycoparasite can enter the host mycelium by partially destroying it. The idea that *Trichoderma* spp. produce and secrete mycolytic enzymes that lead to a partial breakdown of the host's cell wall was based on microscopic findings. The six complementing modes of action of these enzymes, all of which may be necessary for maximal efficacy against chitin-containing plant pathogenic fungi, are what give rise to the appearance and diversity of the *T. harzianum* chitinolitic system.

Antibiotic-mediated Suppression

Antibiotics are microbial toxins that, in small doses, can harm or kill other microbes. Bacteria can create both volatile and nonvolatile antibiotics, such as diacetyl phloroglucinol (DAPG) and mupirocin, as well as phenazine derivatives including pyocyanin, phenazine-1-carboxylic acid (PCA), PCN, and hydroxyphenazines (Ahmad *et al*., 2008).

Competition

When a biocontrol agent reduces a certain substance's availability and consequently restricts the pathogen's ability to develop, this is known as biocontrol *via* nutrient competition. In particular, the biocontrol agents are more effective at utilizing the chemical than the pathogens are at absorbing it (Handelsman and Parke, 1989). Some bacteria, particularly *Fluorescent pseudomonads*, make siderophores with extremely high iron affinities that can snare this scarce resource from other microflora, inhibiting their growth.

Parasitism

The direct use of one organism as food by another occurs during this phase (Handelsman and Parke, 1989). Mycoparasites are fungi that feed off of other fungi and are commonly seen (Baker and Cook, 1974.). On a variety of fungi, several mycoparasites exist, and some of them have been suggested to be crucial in the prevention and treatment of disease (Adams, 1990). Saccardo, for instance, characterized *Darluca filum* (now *Sphaerellopsis filum*) as a parasite of several rust fungi, particularly Puccinia and Uromyces (Sundheim and Tronsmo, 1988).

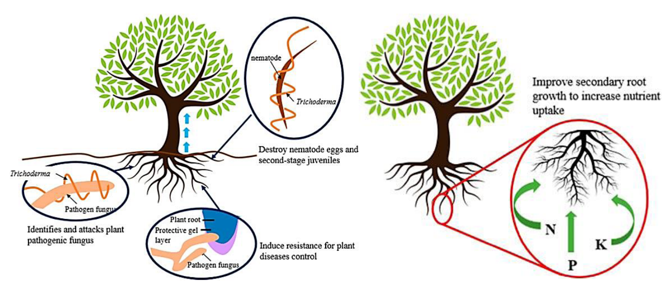
In general, *Trichoderma lignorum* (*T. viride*), which parasitizes *Rhizoctonia solani* hyphae, and suggestions of Mycoparasites can either coil around the host hyphae or grow beside it and release enzymes that attack the target fungus by dissolving its cell walls (Chet, 1987). These enzymes, such as chitinases and b-1,3-glucanase, may be involved in the breakdown of host cell walls and may be parts of complicated combinations of synergistic proteins that cooperate to combat pathogenic fungus (Di Pietro *et al*., 1992). Penetration is the ultimate phase. To puncture the target fungus' cell wall, the biocontrol agent creates structures resembling appressoria (Chet, 1987). A specific BCA that kills the pathogen or its propagules directly attacks it in hyperparasitism.

Induction of Systemic Resistance

Systemic acquired resistance (SAR) is the term used to describe the induced resistance of plants to different diseases. Inoculating plants with a necrogenic pathogen, a nonpathogen, or specific natural or manufactured chemical substances can cause SAR (Lam and Gaffney, 1993). The synthesis of various proteins, such as chitinases, glucanases, peroxidases, and other pathogenesis-related (PR) proteins, as well as the physical thickening of cell walls through lignification, callose deposition, accumulation of antimicrobial low-molecular-weight substances (such as phytoalexins), are examples of these defence responses (Hammerschmidt *et al*., 1984).

According to the host-parasite connection under consideration, different levels of hydrolytic enzymes are produced. This phenomenon is associated with the particular disease-controlling properties of each Trichoderma isolate. Mycoparasitism is considered to be one of the primary mechanisms underlying Trichoderma's antagonistic behavior as

Fig. 2: Mechanism of Biocontrol agents.



(Nur and Badaluddin, 2020)

Fig. 3: Role of biocontrol agents in disease management and soil health improvement.

a biocontrol agent, along with chemotropic growth, the release of extracellular enzymes, and host lyses (Fig. 2). Trichoderma's ability to biocontrol is most likely the result of several different processes (Howell, 2003). *In vitro* antibiotics that are effective against infections are produced by the well-known biological control agent Trichoderma (Howell, 2003). (Fig. 3).

Fungal Biocontrol Agents

The use of Trichoderma is completely safe for both humans and animals. The five main species utilized in the biocontrol of plant diseases are *Trichoderma harzianum*, *T. koningii, T. longibrachiatum, T. pseudokoningii*, and *T. viride*. Mycoparasitism is a difficult process with numerous steps. Trichoderma's connection with its host is distinctive. Numerous *Trichoderma* species have undergone significant research as biocontrol agents (Papavizas, 1985).

Bacterial Biocontrol Agents

Bacterial competition for nutrients and space, the avoidance of pathogen colonization of host tissues, the production of crop disease resistance in plants, and the accumulation of toxic metabolites (enzymes, antibiotics, and volatile organic compounds) are all causes of bacterial antagonism. By working through one or more routes, it has been discovered that the bacterial species belonging to the genera *Pseudomonas, Bacillus, Burkholderia, Lysobacter, Serratia,* and *Pantoea* are effective against a range of plant infections. The rhizobacterial species are effective biocontrol agents and plant growth stimulators, giving the treated plants a two-fold advantage (Table 1).

Table 1: Some bacterial biocontrol agents used against diseases

Biocontrol agents	*Disease*
Agrobacterium radiobacter	crown gall
Pseudomonas fluorescens	bacterial blotch, seedling diseases
Trichoderma viride	timber pathogens
Trichoderma spp.	root diseases
Trichoderma harzianum	root diseases, wood decay
Gliocadium virens	seedling diseases

VAM (Vesicular - Arbuscular Mycorrhiza)

Symbiotic connections develop between higher plants and mycorrhizal fungi. Endotropic or vesicular - arbuscular mycorrhiza (VAM) and ectotrophic mycorrhiza are the two forms of mycorrhiza that have been identified. The arbuscle is a dichotomously branching structure that is thought to be the site of plant-fungus nutrient exchange. The fungal partner in VAM is restricted to the cells of the plant cortex, where it grows both within and outside the cells. As a fungal partner, soil doesn't seem to have a distinct existence of its own. Vesicular-arbuscular mycorrhizas can directly improve the uptake of vital nutrients like as phosphorus, copper, and iron. Therefore, some plants are protected from these elements' harmful effects *via* mycorrhizal connections. Additionally, ectotrophic mycorrhizas consume more phosphorus, and by mineralizing organic nitrogen, they increase the availability of nitrogen to plants. They may also help plants absorb more water from the soil and defend their plant hosts from disease and heavy metal damage.

In symbiotic relationships with mycorrhizal fungi, around 90% of all plants produce hyphae networks. Mycorrhizae mostly draw phosphorus and other minerals, such zinc and copper, from the soil. The plant root provides the fungus with nutrients like glucose. This advantageous contact is known as a mycorrhizal network (Smith, 1997). Mycorrhizal fungi are responsible for the improved growth of host plant species due to increased nutrient intake, production of compounds that promote growth, tolerance to salt and drought, and synergistic interactions with other helpful bacteria (Maeder *et al*., 2002). Arbuscular mycorrhiza fungi can alter the soil's characteristics, promoting plant growth in both benign and adverse conditions (Mo *et al*., 2016). Arbuscular mycorrhiza fungi increase plant development and alter the morphological, nutritional, and physiological traits of plants to increase their ability to withstand abiotic stresses (Shekoofeh *et al*., 2012).

Table 2: Management of Macrophomina phaseolina pathogen by antagonistic fungi (filamentous fungi and VAM) and bacteria

Biocontrol agent	*Disease*	*References*
T. harzianum + G. mosseae	Reduced root-rot in geranium plants caused by *Macrophomina phaseolina*	Haggag and Abd-El latif (2001)
Pseudomonas aeruginosa, Bacillus subtilis, and *Burkholderia* species	Seed dressing and soil drenching with bacterial formulations of reduced dry root rot incidence of mungbean and urdbean.	Satya *et al*., 2011; Latha *et al*. (2017)
Trichoderma isolates	Mungbean dry root rot was reduced when cultivated in organic substrates such groundnut shell, coir pith, and press mud.	Raghuchander *et al.* (1993)
Trichoderma viride or *Trichoder maharzianum* with *Pseudomonas* fluorescens or *B. subtilis*	lower incidence of mungbean and urdbean dry root rot in greenhouse and outdoor settings.	Kumari *et al*. (2015); Shahid & Khan, 2016)
T. harzianum or *T. viride*	reduced dry root rot of mungbean and urdbean	Athira, (2017); Jamwal *et al*. (2016)
Trichoderma species, the arbuscular mycorrhizal species *Glomus claroideum*	reduced dry root rot of mungbean and	Chandra *et al*. (2007).
Glomus aggregatum	reduced dry root rot of urd bean in field trials	Chandra *et al*. (2007).
T.harzianum, T. Polysporum and *T. viride*	Soil application of talc-based formulation effectively controlled the eggplant's root rot (M. phaseolina) under field conditions.	Ramezani, (2008)

Macrophomina Phaseolina

Macrophomina Phaseolina results in root decay (Tassi) a number of crop plants are affected by the disease, which has a considerable economic impact (Jana *et al*., 2005). The fungus *M. phaseolina* causes charcoal rot, stalk rot, and dry root rot/stem canker in plant roots. Different physiological and ecological factors, such as heat, high temperatures, and low moisture content, may make it more common. In Australia (Gomez *et al*., 2020), Chile (Sánchez *et al*., 2013), Israel (Sánchez *et al*., 2013), Europe and the United States (Baggio *et al*., 2019), *M. phaseolina* has also been discovered on melon, strawberry, and tomato. (Chamorro and others, 2015a). Under drought conditions, it also threatens sunflower output in South Asia (Khan *et al*., 2017) and Italy (Manici *et al*., 1995).

The most prominent diseases that have an impact on sunflower plant growth are root rot and charcoal rot, both of which are caused by *Macrophomina phaseolina*. The biological control of soil-borne plant diseases and the stimulation of plant growth have both made extensive use of microorganism enrichment, conservation, and management. Although *Bacillus* and *Streptomyces* species have also been utilized, *Fluorescent pseudomonas* are the most frequently used bacteria for biological control and plant growth promotion. With soil-borne plant infections as well as pathogens on phylloplane, competition has been used as a biological control method. Increased growth response was observed in lettuce bean, cucumber, and pepper following the treatment of *Trichoderma* spp. in pot culture or field conditions. Trichoderma has the capacity to modify plant hormones and vitamins, which may have an impact on plant growth (Harman *et al*., 2004). It has been proposed that control of plant infections and induced resistance as indirect mechanisms for Trichoderma-mediated plant growth promotion.

Antagonistic fungi (filamentous fungus and VAM) and bacteria are used to manage the *Macrophomina phaseolina* pathogen (Table 2).

Conclusion

Overusing chemicals as pesticides, weed killers or to treat various plant diseases or increase productivity creates risks to the health of people, animals, plants, and the environment because of the residue problem. One ecofriendly alternative to fungicides is biocontrol agents (BCAs). In addition to bacteria, VAM is a biocontrol agent for filamentous fungi. Biocontrol-active microorganisms can work in a variety of ways to prevent fungus-related plant diseases, including hyperparasitism, predation, antibiosis, cross-protection, competition for location and resources, and generating resistance. Most biocontrol treatments are used to treat soil- and seed-borne fungal infections, like those that cause seed rot, damping-off, and root rot. Utilizing biological control is a viable technique for controlling plant diseases, boosting productivity, safeguarding the environment and biological resources, moving toward a sustainable agricultural system, and efficiently resolving issues with chemical hazards.

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