

Bio-control strategies

for management of plant diseases under sustainable agriculture

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Over the last 15 years, microbial biological control agents (MBCAs) have seen significant research and innovation, driven by the global climate crisis and the increasing demand for food. While extensive use of chemical pesticides have caused major and oftentimes irreversible damage to the soils and thereby its productivity, these agents represent a more sustainable alternative, which not only works to eliminate pathogens but also, provides additional advantages to the soil. In this article, we discuss the mechanism of action of MBCAs, their formulations and the future they represent for agriculture.

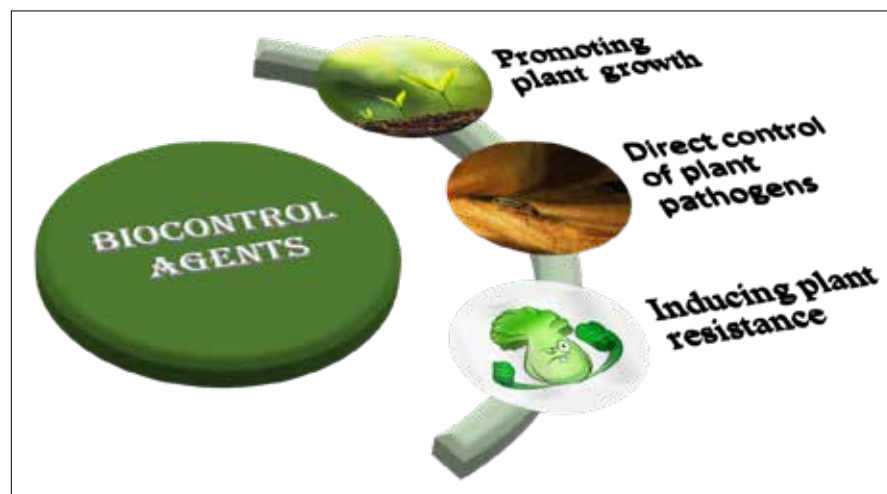
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A need for innovation in sustainable agricultural practices has been on the rise since the late 90s when issues regarding detrimental effects of conventional methods became more evident. These conventional methods were rampantly applied to various yield, production and pathogen related problems that cultivators often faced and for the most part included chemical solutions like fertilizers and pesticides. It is estimated that around 85% of all pesticides produced are used in the agriculture industry and while they have undoubtedly helped a number of economies reach their zenith, mostly in developed countries, with unparalleled benefits like protection of wood and timber, reduction in plant pathogens and resulting endemics, overall protection of farms and an all-round increase in productivity, the price that we still continue to pay for it weighs heavier each passing day. With the establishment of agriculture as a major economic factor, lesser-developed countries became a ready baked market for these chemical pesticides offering solutions to all

pest related problems in a bottle. These new markets offered another advantage, the chemicals that had been rendered harmful in developed nations could still be sold in these countries due to lack of proper regulations and comparatively lenient laws surrounding toxigenicity of these formulations. These dire situations were and continue to be worsened by the lack of proper knowledge and training regarding appropriate handling of these chemicals that result in higher incidences of pesticide poisoning

so much so that data estimates that about 99% of all cases of deaths due to pesticide poisoning occur in less affluent countries like Venezuela, India and Bangladesh.

The assumed causalities from these chemicals resulting from their ability to enter the food chain prove detrimental to humans. Such chemical treatments however do not discriminate in their prey and their unbridled use inevitably puts at risk a number of crucial insects among which are also the ones responsible for important processes



Roles of biocontrol agents

like seed dispersal, nutrient cycling, bioturbation and most important of all, insect pollinators which are responsible for seed production and quality improvement in about 88% of all flowering plants. Not only that, they have also been detrimental to the soil itself, depleting the biota and reserve of essential nutrients, disturbing the equilibrium of soil systems (physical, chemical & biological), subduing soil enzyme activity consequentially reducing long term productivity of soil and proving toxic to soil microbial communities that are responsible for the integrity of soil nutrients. These microbial communities play a pivotal role in stimulation of plant growth, fixation and mineralization of essential nutrients like phosphorus (P) and nitrogen (N), detoxification of harmful substances and suppression of disease-causing organisms. As a result of indiscriminate use of pesticides, soil productivity and yield has significantly decreased. Moreover, global population size is on the rise so much so that it is estimated that by 2050, an average farmer would have to produce 70% more to meet global food requirements. These, coupled with the global impact of climate change call for new and sustainable alternatives to these toxic compounds where Microbial Biological Control Agents (MBCAs) are one such alternative option.

MBCAs or Bio-control agents are described as a group of microorganisms that hold the ability to not only prevent plant diseases but, also aid in nutrient supplementation and promote plant growth. The first inklings to the phenomenon come from Hiltner who, in the year 1984, discovered that density of microbes was the highest in the soil surrounding the roots *i.e.*, the rhizosphere, as compared to the rest of the bulk soil. The presence of these microorganisms called rhizobacteria warrants multiple complex interaction not only among the organisms but also the plant root tip and between the epidermal cells, the indigenous microflora and microbiota like nematodes and protozoa. It is these varied

interactions that form the basis of applications of bio-control agents.

Mechanisms of action

As mentioned previously, the mechanism of a biocontrol agent depends upon interactions of the microflora, the microbiota and the subsequent effects it produces, resulting in the total positive sum of interactions. Microbial biological control agents employ various physiological, metabolic and even pathological routes to achieve optimum disease control. Various mechanisms through which these effects include indirect interactions (increasing plant resistance and competition) and direct methods (antibiosis via production of antimicrobial secondary metabolites and parasitism).

Competition

The plant pathogens, like any other microbe are dependent upon nutrients for their overall growth and survival. A large number of these plant pathogens rely on dead plant debris and other necrotising organic matter in early stages of their development. Successful host infection of most fungal necrotrophic pathogens also depends on exogenous nutrients during spore germination and formation of infection structures on host tissues whereas bacterial pathogens use the same exogenous nutrients to reach an optimum population necessary in order to attack and colonize the host. MBCAs consume these nutrients essential for survival of these pathogens and deplete the reserves leaving little to no opportunities for them to grow. Microbes including pathogens may also compete for specific stimulants of germination that may come from germinating seeds or growing roots. These factors may include fatty acids, or their peroxidation products or volatile components such as ethanol and acetaldehyde. These agents are able to do that because they can efficiently colonize these niches and pose a stark competition for the limited amount of nutrients.

Increasing plant resistance towards pathogens

Another indirect approach used by MBCAs during biological control is by enhancing a plant resistance towards a specific or a group of pathogens. This inducible resistance is also known as systemic acquired resistance (SAR) which may be induced by inoculating plants either with a neurogenic pathogen or non-pathogenic or with certain natural or synthetic chemical compounds. PGPRs have been used for the purpose of inducing systemic resistance in plants by using microbe associated molecular patterns or MAMPs which elicit a response from plant defences by activating the corresponding pattern recognition receptors (PRRs).

Antibiosis

Microorganisms have been known to secrete substances that either kill or stunt the growth of other microbes and ever since the discovery of penicillin; they have been actively used in therapeutics. These antibiotics act as toxins for other microbes having a fatal effect in their metabolism. PGPRs too exhibit antibiosis in select species and help regulate the growth of plant pathogens by producing these compounds formed as secondary metabolites after the organism is past its log stage of growth. Major contributor towards the production of antibiotics by organisms used for microbial biological control agents include *Agrobacterium*, *Bacillus*, *Pantoea*, *ratia*, *Stenotrophomonas*, *Streptomyces* and many other genera. One of the most extensively studied PGPR in this aspect is *Pseudomonas* sp., which produces a number of vital secondary metabolites including but not limited to DAPG, pyrrolnitrin and phenazine with potential to produce many more.

Parasitism

This method involves the action of microorganisms that act as parasites towards a particular species or various species. These microbes work by directly competing for nutrients with one species as

a host which is at an obvious disadvantage. This phenomenon is mostly seen in fungi and only occasionally in bacteria and thus the term may be interchangeably used with myco-parasitism, where the prefix 'Myco' signifies the exclusive involvement of fungi. The target fungi produce certain chemicals that attract the biocontrol agents, causing chemotaxis of the MBCAs towards the fungi producing the chemical stimuli. The biocontrol agent then identifies the target fungus, which involves Lectins of hosts (pathogens) and carbohydrate receptors on the surface of the biocontrol fungus. The interaction then progresses to the stage of gradual cell wall degradation by attachment of mycoparasites around the target fungi which is brought about by enzymes like chitinases and β -1, 3-glucanase.

Preparation of an effective bio-control agent

The formulation of a biocontrol agent corresponds to its use, organism involved, desired effect, expected prolonged shelf-life and activity along with ensuring safe handling of the product. A wide variety of formulation types have been developed for MBCAs involving both liquid and solid formulations. Liquid formulations when compared to their solid counterparts have been known to produce higher yield with less investment cost, whereas solid formulations improve the MBCA stability. These formulations in question may include the active ingredient (microorganism cells and/or secondary metabolites synthesized); carriers, formed by inert materials that support and deliver the active ingredient in the target site; and adjuvants, which include protectants, stabilizers, adherents, surfactants, additional nutrients *etc.* needed to sustain the function of the active ingredient by protecting it from high temperatures.

Liquid formulations: These consist of whole culture and cell suspensions in different carrier liquids. Depending upon the carrier liquid used, they may be:

- **Oil based-** When the culture is suspended in vegetable or mineral oil taking into consideration the potential toxicity of the oil towards the microorganism as well the receiving plant. For example, the formulation of the filamentous fungus, *Trichoderma harzianum*.
- **Soluble (liquid) concentrate-** Usually water-based and contain an active ingredient dispersed in water as a carrier. For this reason, they tend to have the lowest viscosity out of all three of the formulations.
- **Suspension Concentrate-** These formulations usually consist of a solid active ingredient suspended in water. Adjuvants may also be included for bio enhancement.

Solid formulations: These formulations are specifically designed according to the needs and behaviour of a particular microorganism in use. These are easy to use as compared to the liquid however elicit greater making cost due to the involved machinery. These formulations involve dried products that are efficiently dehydrated via processes like freeze-drying, spray drying, fluidized bed drying, and more recently encapsulation.

Successful examples of bio-control agents

Once a bio-control agent has shown reproducible activity in screening trials, methods for inoculum production, formulation and application needs to be considered in relation to the crop, disease and environment of use. Plethora of antagonistic fungi and

their products have been registered as commercial biopesticides globally. Different types of formulations of these antagonists are currently available in markets and are successfully used against various phytopathogens. *Trichoderma* spp., *Gliocladium* spp., *Coniothyrium*, and non-pathogenic strains of fungal genus are available as different formulations. Various bio-pesticide products (Binab-T, Bio-Fungus, Supresivit, Root Shield, T-22HB, T-22G, Trichodex, Trichoseal, Trichopel, Trichodowels, BioMax, BioVam, Trichoject, *Trichoderma* 2000) contain *Trichoderma* spp. to control a variety of pathogens, including *Fusarium*, *Botrytis*, *Gaeumannomyces*, *Rhizoctonia*, *Pythium*, *Sclerotinia*, *Verticillium*, *Sclerotium*, and wood-rot fungi.

A report from eastern India in 2020 highlighted biocontrol agents-based treatment recorded increased defense enzyme activities such as catalase, peroxidase, super oxide dismutase and α -amylase when compared to untreated control. This also clearly indicated that, grain discolouration can be efficiently managed using native bio-control agents which are well known for plant growth promotion and can induce systemic resistance (ISR) activities. In recent years, native strains of bio agents' viz., *Bacillus firmus* and *Trichoderma harzianum* have been isolated from arid soils. *Bacillus firmus* was found specific antagonist to *Macrophomina phaseolina* in various laboratory tests. In a similar effort, bio-formulated product of consortium demonstrated at 10 farmer's field by KVK Jodhpur

Table 1. Organisms used for preparing MBCAs and their target diseases

Organism used	Trade name	Target crop	Target disease
<i>Trichoderma harzianum</i> ATCC 20,476	Binab T	Ornamental, shade, and forest trees	Tree wound pathogens
<i>Trichoderma album</i>	Bio Zeid	Tomato	Tomato wilt
<i>Gliocladium virens</i>	SoilGard 12G	Canola and crucifer vegetable crops	Clubroot
<i>Chaetomium globosum</i>	Ketomium	Rice, black pepper, citrus, strawberry, tomato, corn	Rice blast, durian, and black pepper rot, citrus rot, strawberry rot, anthracnose
<i>Pseudomonas fluorescens</i> A506	Bligh Ban A506	Fruit crops, almond, potato, and tomato crops	Frostban Frost damage, fire blight, bunch rot

resulted in significant reduction in disease incidence resulting in 22.9% increase in seed yield of guar. This bio-formulated product also improved root colonization of *B. firmus* providing long duration of protection to host by pathogen.

The recent endeavours have also been towards incorporating nanobiotechnology into agriculture in the form of nano biocontrol agents. Encapsulation research has increased in recent decades in response to the growing need for biocontrol agents. These formulations can address the difficulties of free-form formulations. They can improve their efficacy by prolonging their shelf-life and offering the controlled release of biological components.

The use of nanoparticles (NP) in nanotechnology could be an efficient and effective way of controlling plant pathogens and diseases and improving growth in one goal. This is because most of the NPs are plant micronutrients with the ability to improve growth by enhancing nutrient uptake required by plants.

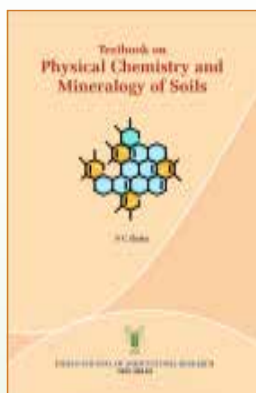
Future perspectives and conclusion

Microbial biological control agents represent the horizon of endless possibilities in the practice of sustainable agriculture with research paving the way for discovery and utilization of more effective strains each day. The abundant diversity of microbes with unique requirements and metabolisms provide unprecedented opportunities for

further breakthroughs. In a relatively short time, MBCAs have made significant impact owing to their targeted mode of action, desirable results, production of minimal/no harmful by-products, enhancement of beneficial indigenous flora and no detrimental effect to plant growth. Although a fair amount of research still needs to be done on the mechanisms and potential variation between laboratory and *in situ* test results, MBCAs provide a stable and much needed alternative to the toxic pesticides that continue to be in use till date adding to the deterioration otherwise preventable by the use of these beneficial microorganisms.

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TECHNICAL SPECIFICATIONS

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