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# Current Status and Future Strategies of Microbial Control of Fungal Diseases of Forest Trees

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## Abstract

Microbial control is one of the potential alternatives of chemical pesticides for plant disease management. Microbes like fungus, bacteria, virus, nematode *etc*. as bio-agent is widely exploited during the last two decades. However, inconsistency in their field performance is a matter of great concern and proving to be a major bottleneck in their large-scale application. These agents are capable of replicating in the environment, but require certain frequent manipulations to get activated/ multiplied after its application. Formulation for easy field application is its major requirement for optimization of its efficacy, stability and ease of application. Formulation is available in the form of water dispersible powder, granule, emulsion, *etc*. with carriers, diluents and surface-active agents, according to need of a final consumer product. Amongst different application methods seed treatment is found to be the best method in combating disease management as it protect the crop from both seed and soil borne pathogen.

**Keywords:** Disease management, Forest disease, Formulation, Microbial control

## Introduction

India has a total forest area of about 64 mha of which 80% are tropical mixed forests (Lal, 1989). Over 4 mha of land is under man made plantations of both exotic and indigenous species. The major forest tree species raised in plantations include gamhar, teak, sal, eucalyptus, pine, chopa, sisoo, nahar, *etc*. Tree pathogens are part of the natural forest ecosystem. Some of the diseases are known to cause significant losses through decay or detoriation, reduction in storability, damage to the seedlings in nurseries and regenerations thus eventually affecting the general health and productivity of forests (Table 1). Fungi are probably responsible for most of these losses although the damage may vary be area; tree species; general health of the tree, the type of the fruit and the seed; season of the year; weather conditions; fungal inoculum available.

## Disease Management Programme

In general, routine disease management programmes are not practiced in forest plantations except pesticide application in nurseries. It is a fact that unlike in the agricultural sector there is no social demand for plant protection in forest plantations as most of the plantations are under government sector. Moreover, very little attempt has been made for an economic analysis of the impact of disease problems and convince the forest managers the benefit of diseases control. As conversion of forest area for plantation activity is no longer permitted, attempts to enhance productivity through management of diseases is the only alternative to meet the ever-increasing demand for wood, industries and various domestic purposes.

Currently, around 200 distinct fungicides have been incorporated into global agriculture and horticulture. While chemical pesticides are typically cost-effective for disease control and have become integral to modern farming practices, many of these substances are also associated with ecological, environmental and human health issues (Economidis and Kessler, 2004). Several chemicals with

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Table 1: Pathogens causing diseases in various forestry species

| Host Plant | Disease | Pathogen |
|---|---|---|
| *Acacia auriculifonnis* | Leaf blight | *Colletotrichum gloeosporioides, Cylindrocladium quinqueseptatum* |
| | Leaf spot | *Exserohilum rostratum, Phomopsis* sp. |
| | Powdery mildew | *Oidium* sp. |
| | Pink disease | *Corticium salmonicolor* |
| *Acacia mangium* | Leaf spot | *Colletotrichum gloeosporioides, Cylindrocladium quinqueseptatum, Phomopsis* sp. |
| | Root and butt rot | *Ganodenna lucidum* |
| | Vascular wilt | *Fusarium solani* |
| | Pink disease | *Corticium salmoniclor* |
| *Ailanthus triphysa* | Seedling collar rot | *Rhizoctonia solani* |
| | Seedling blight | *Colletotrichum dematium* |
| | Leaf shot-hole | *Colletotrichum gloeosporioides* |
| | Bacterial leaf spot | *Pseudomonas solanacearum* |
| | Sooty mould | *Meliola ailanthii* |
| | Stem canker and die-back | *Botryodiplodia theobromae* |
| | Pink disease | *Corticium salmonicolor* |
| *Azadirachta indica* | Seedling blight | *Rhizoctonia solani, Sclerotium rolfsii* |
| | Leaf spot | *Colletotrichum gloeosporioides, Cylindrocladium scoparium, Cercospora subsessilis* |
| | Seedling wilt | *Fusarium solani* |
| *Bombax ceiba* | Collar rot | *Rhizoctonia solani* |
| | Seedling blight | *Sclerotium rolfsii* |
| | Leaf spot | *Colletotrichum gloeosporioides* |
| | Leaf rust | *Myrothecium roridum* |
| | Pink disease | *Cercospora bomacina, Uredo bombacis, Corticium salmonicolor* |
| *Bambusa bambos* | Seedling damping-off | *Rhizoctonia solani* |
| | Leaf blight | *Bipolaris maydis, Bipolaris urochloa, Exserohilum rostratum, Exserohilum holmii* |
| | Leaf spot | *Colletotrichum gloeosporioides, Dactylaria* sp., *Ascochyta* sp. |
| | Seedling rhizome rot | *Rhizostilbella hibisci* |
| | Rhizome bud rot | *Pythium middletonii* |
| | Culm rot | *Fusarium moniliforme var. intermedium, Fusarium equiseti* |
| | Branch die-back | *Fusarium pallidoroseum* |
| | Witches' broom | *Balansia linearis* |
| | Thread blight | *Botryobasidium salmonicolor* |
| | Leaf rust | *Dasturella divina* |
| | Leaf tar spot | *Phyllachora ischaemi, P. longinaviculata, P. shiraiana* |
| | Leaf spot | *Petrakomyces indicus, Phoma herbarum, Phoma sorghina, Phomopis, Chaetospermum carneum, Cerodothis aurea, Coccodiella* sp., *Rosenscheldiella* sp., *Alternaria alternata* |
| | Basal culm decay | *Ganoderma lucidum* |
| *Bombax insignae* | Seedling collar rot | *Rhizoctonia solani* |
| | Leaf blight | *Sclerotium rolfsii* |

Table 1: Continue...

| Host Plant | Disease | Pathogen |
|---|---|---|
| | Leaf spot | *Colletotrichum gloeosporioides* |
| *Calamus hookerianus* | Seedling damping-off | *Rhizoctonia solani, Fusarium oxysporufn, Fusarium longipes, Guignardia calami* |
| | Seedling blight | *Fusarium solani* |
| | Seedling wilt | *Colletotrichum gloeosporioides, Bipolaris ellisii* |
| | Leaf spot | *Corynespora cassiicola, Pestalotiopsis theae, Phomopsis* sp., *Curvularia lunata* |
| *Calamus thwaitesii* | Seedling damping-off | *Rhizoctonia solani, Fusarium oxysporum, Fusarium longipes, Sclerotium rolfsii* |
| | Seedling blight | *Guignardia calami, Sphaerodothis* sp. |
| | Seedling wilt | *Fusarium solani* |
| | Leaf spot | *Colletotrichum gloeosporioides, Bipolaris ellisii, Corynespora cassiicola, Pestalotiopsis theae, Phomopsis* sp., *Curvalaria lunata* |
| | Tar spot | *Phyllachora calamigena* |
| *Calamus travancoricus* | Seedling blight | *Guignardia calami* |
| | Leaf spot | *Colletotrichum gloeosporioides, Phomopsis* sp. |
| | Thread blight | *Pellicularia filamentosa* |
| *Casuarina equisetifolia* | Seedling blight | *Rhizoctonia solani* |
| | Seedling wilt | *Pseudomonas solanacearum* |
| | Stem canker | *Phomopsis casuarinae* |
| | Blister blight | *Trichosporium vesiculosum* |
| *Cupressus* sp. | Stem canker | *Corticium salmonicolor* |
| *Dalbergia latifolia* | Leaf spot | *Colletotrichum gloeosporioides, Physalospora dalbergiae* |
| | Leaf tar spot | *Phyllachora dalbergiae* |
| | Leaf rust | *Uredo sissoo* |
| *Dendrocalamus strictus* | Seedling damping-off | *Rhizoctonia solani* |
| | Leaf spot | *Bipolaris maydis, Colletotrichum gloeosporioides* |
| | Culm rot | *Fusarium moniliforme var. intermedium, Fusarium equiseti* |
| | Thread blight | *Botryobasidium salmonicolor* |
| | Leaf rust | *Dasturella diving, Phomopsis* sp. |
| | Leaf spot | *Amylosporus campbelli, Exserohilum rostratum, Dactylaria* sp., *Alternaria alternata, Ganoderma lucidum* |
| *Dioscorea pentaphylla* | Leaf spot | *Myrothecium roridum* |
| *Eucalyptus grandis* | Seedling damping-off | *Rhizoctonia solani, Pythium deliense, P. myrotylum, P. spinosum, Cylindrocladium ilicicola, C. quinqueseptatum, Fusarium oxysporum* |
| | Seedling blight | *Cylindrocladium ilicicola, C. floridanum, C. parvum, C. camelliae* |
| | Web blight | *Rhizoctonia solani* |
| | Seedling wilt | *Sclerotium rolfsii* |
| | Foliage blight | *Cylindrocladium theae, C. floridanum, C. quinqueseptatum, C. clavatum* |
| | Leaf spot | *Phaeoseptoria eucalypti, Coniella fragariae, Coniella castaneicola, Guignardia citricarpa, Phomopsis eucalypti* |
| | Stem canker | *Cryphonectria cubensis, C. havanensis, Valsa eucalypti, Cytospora eucalypticola, Corticium salmonicolor* |

Table 1: Continue...

| Host Plant | Disease | Pathogen |
|---|---|---|
| *Eucalyptus tereticornis* | Seedling damping-off | *Rhizoctonia solani, Pythium deliense, P. myrotylum, P. spinosum, Cylindrocladium ilicicola, C. quinqueseptatum, Fusarium oxysporum* |
| | Seedling blight | *Cylindrocladium ilicicola, C. floridanum, C. parvum, C. camelliae, Bartalinia terricola* |
| | Web blight | *Rhizoctonia solani* |
| | Seedling wilt | *Sclerotium rolfsii* |
| | Foliage blight | *Cylindrocladium theae, C. floridanum, C. quinqueseptatum, C. clavatum* |
| | Leaf spot | *Alternaria alternata, Phaeoseptoria eucalypti, Coniella fragariae, Coniella castanaecola, Guignardia citricarpa, Phomopsis eucalypti, Pestalotiopsis disseminata, P. neglecta, P. guepini, P. versicolor* |
| | Stem canker | *Cryphonectria cubensis, C. havanensis, Valsa eucalypti, Cytospora eucalypticola, Cytospora eucalypti, Macrovalsaria megalospora* |
| | Pink disease | *Corticium salmonicolor* |
| *Gmelina arborea* | Seedling damping-off | *Rhizoctonia solani* |
| | Seedling blight | *Sclerotium rolfsii, Fusarium solani* |
| | Collar rot | *Rhizoctonia solani, Sclerotium rolfsii* |
| | Leaf spot | *Glomerella cingulata, Colletotrichum gloeosporioides, Corynespora cassiicola* |
| | Leaf blight | *Pseudocercospora ranjita* |
| | Stem die-back | *Griphosphaerea gmelinae* |
| | Stem canker | *Thyronectria pseudotricha, Phomopsis gmelinae* |
| | Pink disease | *Corticium salmonicolor* |
| | Stem decay | *Lentinus squarrosulus* |
| *Holarrhena antidysentrica* | Leaf spot | *Myrothecium roridum* |
| *Holarrhena pubescens* | Leaf spot | *Myrothecium roridum, Colletotrichum gloeosporioides, Phomopsis sp.* |
| *Leucaena leucocephala* | Leaf spot | *Phomopsis leucaenae, Colletotrichum crassipes, C. gloeosporioides, Exserohilum rostratum* |
| | Stem canker | *Corticium salmonicolor, Hydnum subvinosum* |
| *Michelia champaka* | Leaf spot | *Phomopsis michaliae* |
| *Mimusops elengi* | Seedling collar rot | *Cylindrocladium quinqueseptatum, C. camelliae* |
| *achroma pyramidale* | Die-back | *Calonectria rigidiuscula, Fusarium moniliforme var. subglutinans* |
| *Pongamia pinnata* | Leaf spot | *Asperisporium pongamiae, Urohendersonula pongamiae, Cylindrocladium quinqueseptatum, Phomopsis* sp. |
| *Pterocarpus indicus* | Leaf spot | *Cylindrocladium quinqueseptatum* |
| *Pterocarpus santalinus* | Seedling blight | *Sclerotium rolfsii, Rhizoctonia solani* |
| | Leaf spot | *Phomopsis* sp. |
| | Pink disease, Stem canke | *Corticium salmonicolor* |
| *Pterocarpus marsupium* | Seedling blight | *Rhizoctonia solani, Sclerotium rolfsii* |
| *Swietenia macrophylla* | Collar rot | *Sclerotium rolfsii* |
| *Tectona grandis* | Seedling collar rot | *Rhizoctonia solani,* |
| | Seedling wilt | *Pseudomonas tectonae* |
| | Powdery mildew | *Uncinula tectonae* |

Table 1: Continue...

| Host Plant | Disease | Pathogen |
|---|---|---|
| | Leaf rust | *Olivia tectonae* |
| | Leaf spot | *Phomopsis variosporum, P. tectonae* |
| | Die back | *Colletotrichum gloeosporioides, Pseudoepicoccum tectonae, Sclerotium rolfsii* |
| | Stem canker | *Fusarium solani* |
| | Pink disease | *Corticium salmonicolor* |
| *Terminalia belle rica* | Leaf spot | *Glomerella cingulata* |
| *Terminalia catappa* | Stem canker | *Corticium salmonicolor* |
| *Terminalia paniculata* | Leaf spot | *Cylindrocladium quinqueseptatum, Colletotrichum gloeosporioides, Phomopsis* sp. |
| *Wrightia tinctoria* | Leaf spot | *Myrothecium roridum* |
| *Xylia xylocarpa* | Leaf spot | *Colletotrichum gloeosporioides, Phomopsis* sp. |

enhanced properties are available, but they are often financially out of reach for many farmers in developing nations. Hence, there is a clear necessity to explore alternative approaches that are both effective and cost-effective while minimizing harmful impacts (Economidis and Kessler, 2004). The World Health Organization (WHO) has estimated that there are approximately 25 million cases of acute occupational pesticide poisoning in developing countries, resulting in approximately 20,000 deaths worldwide each year (Jeyaratnam, 1990). Such reports, strongly advocate the use of alternative safe and secure means of pest eradication in post green revolution era.

**Current Need for Microbial Pesticides**

The increasing demand for food production at competitive rates has accelerated intensive agriculture leading to the outbreak of new pests and diseases. The changing dynamics of biotic stresses on one hand and the non-sustainability of chemical pesticides on the other, have projected microbials as potential alternatives. However, the current level of production of microbial agents is not commensurate with the potential demand in India. Considering the area under cultivation of different crops in India and projecting the target coverage of an ambitious 10% area with bio-control agents, the potential requirement of various microbial bio-pesticides is quite substantial. An alternative method to chemical pest control is using microbial pesticides. Numerous developing countries have cultivated research expertise in microbial pesticides, often equipped with a sufficient level of technology (Economidis and Kessler, 2004). Conversely, in many developed nations, there exists a gap between promising research concepts and the actual implementation and adoption of new technologies. Some developing countries are facing some challenges and limitations in their microbial pesticide research efforts. These challenges include a shortage of expertise during critical developmental stages, limited investment primarily from the public sector and a deficiency in the multidisciplinary knowledge needed to oversee the complete development process of a bio-pesticide. In conclusion, it is evident that targeted assistance in the form of specialized support, access to facilities and expertise on a multinational, multi-institute, multi-disciplinary basis is essential in developing countries. Such support is necessary to overcome the existing constraints and pave the way for the successful development and utilization of microbial pesticides in the future.

India boasts a diverse landscape of microbial pesticide production, with more than 23 private companies manufacturing various types (Subramanian *et al.*, 2006), primarily focusing on plant disease antagonists. Furthermore, there are an additional 32 central Integrated Pest Management (IPM) centers (Subramanian *et al.*, 2006), operating under the Department of Agriculture and Cooperation within the Ministry of Agriculture, Government of India, which are actively involved in producing specific biocontrol agents. Several state departments of agriculture and horticulture, particularly in states such as Tamil Nadu, Kerala, Karnataka andhra Pradesh and Gujarat, have taken the initiative to establish multiple biocontrol laboratories. These laboratories are entrusted with the task of producing specific microbial biocontrol agents, including NPV (Nuclear Polyhedrosis Virus) and *Trichoderma* spp., to promote sustainable pest management practices in their respective regions.

A few state agricultural universities and ICAR institutions are engaged in the production of limited quantities of microbial pesticides and are in the process to obtain the necessary registrations for their formulations from the Central Insecticides Board, ensuring the safety and efficacy of these products in pest management. But the production capacity of these companies and institutions with reference to most of the products against the potential need is rather meager. For example, the projected annual demand for *Trichoderma* is 22,038 metric tons but the total production capacity of the companies is only 8.4% (Table 1). The picture of NPV is rather dismal. The *HaNPV* with an annual market potential of Rs. 714.28 crores is being produced only to an extent of 0.7% of the potential requirement. Similarly, the *Sl* NPV has a potential market of Rs. 542.00 crores but the production capacity is only 0.8% of the potential need. With low market potential of entomofungal pathogens however, the capacity is somewhat satisfactory with *Metarhizium anisopliae* recording the highest value of 75.2%. The picture is still gloomy when the figure of actual quantity reduced and sold for the years 2003 and 2004 are considered. Hence there seems to be an enormous gap between the potential need of the production capacity of the companies *viz-viz* the demand for the microbial pesticides.

**Types of Formulations of Microbial Pesticides Available in India**

There are mainly three types of formulation of microbial pesticides in India. These are: a) Water dispersible powders (WDP), b) Granules and c) Emulsions.

*1. Water Dispersible Powder* (*WDP*)

Water dispersible powder is the most popular formulation for fungal and bacterial antagonists in India. It consists of the microbial agent along with diluents (*e.g*., talc, charcoal, molasses enriched charcoal powder, kaolin, lignite, *etc*.) and surface-active agents (*e.g*., carboxymethyl cellulose (CMC), gelatin, gur, gum, *etc*.) to ensure suspending and wetting properties and stickers (xanthan, gum *etc*.). Sometimes nutrient base (*e.g*., FYM powder, plant products) is added to support multiplication of bioagents after application. WDP formulations are generally used both for seed treatment and foliar spray. Population of fungal antagonists like *Trichoderma* should not be less than $10^6$ cfu $g^{-1}$ of formulation and of bacterial antagonists *(Pseudomonas, Bacillus*, *etc*.) or entomopathogens *(Beauveria, Metarhizium, Verticillium*, *etc*.) not less than $10^8$ cfu $g^{-1}$ of formulation. Moisture content of these formulations should not be more than 8% for fungi and 12% for bacteria (Anonymous, 1999). Shelf-life of WDP formulations of different microbial control agents vary between 3 to 6 months. However, this shelf-life estimate is based on cfu only.

*2. Granules*

Antagonists like *Trichoderma* are available in granular formulation. The granular formulations are made from the clay mineral lignite, attapulgite, montmorillonite and bentonite clays, granular diatomaceous earths and vermiculite. Granular formulations are used for soil application. These granules may also contain plant products.

*3. Emulsions*

Liquid formulations of microbial control agents are only recent introduction to Indian scenario. In formulations of this kind, microbial agents are suspended within an immiscible solvent, such as a petroleum derivative (like, diesel or mineral oils) or vegetable oils (such as groundnut oil), with the assistance of a surface-active agent (Kumar *et al.*, 2014). This mixture can be dispersed in water to create a stable emulsion. Emulsifiable concentrates necessitate a substantial concentration of an oil-soluble emulsifying agent to achieve immediate formation of a uniform emulsion upon dilution in water (Kumar *et al.*, 2014). Presently, formulations containing *Trichoderma*, *Pseudomonas* and *Beauveria* are being employed as foliar sprays.

**Solid vs. Liquid Formulations**

Majority of the microbial control products available in the market are in the form of solid formulations (*i.e.*, granular or water dispersible powder). Development of oil-based formulations on microbial control agent is recent area of research. The potential for the development of mycopesticides has received a boost due to the discovery that fungal conidia formulated in oils exhibit greater infectivity compared to traditional water-based suspensions. Oil-based (paraffinic petroleum oil) formulations of *Trichoderma harzianum* (TH), *Pseudomonas fluorescens* (PsF) or their mixture (TH + PsF) showed better efficacy against *Cochliobolus miyabeanus* (Ito & Kuribayashi), *Drechslera* (anamorph *Bipolaris oryzae* (Breda de Haan) Shoemaker) when used as foliar spray as compared to talc-based formulation of the same microbial control agents (Singh *et al.*, 2005). Shelf-life of oil-based formulation was >10 months against 4 months for talc-based formulation at room temperature (12-37 °C).

Oils are non-phytotoxic. They substantially enhance the efficacy of hyper parasitic fungi and their non-evaporative nature makes them highly compatible with ultra-low volume application techniques for spraying microbial pesticides, especially in conditions of low humidity.

The primary advantages of oil-based formulations for microbial agents include:

• *Enhanced Target Penetration*: Oils effectively adhere to leaf surfaces, facilitating greater penetration of the spray into the target area.

• *Drift Reduction*: These formulations produce uniformly sized droplets, reducing the occurrence of very fine droplets that can lead to drift.

• *Decreased Evaporation*: Spray oils coat the droplets, minimizing issues related to evaporation. This enables fungal spores to germinate and become active even under conditions of low atmospheric humidity.

• *Improved Spreading Capability*: Spray oils can address challenges associated with beaded droplets on waxy surfaces, ensuring that the spray forms a uniform, thin film across the entire treated surface.

• *Mitigation of Unfavorable Spray Conditions*: These formulations help mitigate issues associated with drizzle, wind and dew, improving the overall effectiveness of pesticide application.

Oil-based formulations are being developed with high population of *Trichoderma, Pseudomonas* or *Beauveria* (> $10^{10}$ cfu $ml^{-1}$ of water). Dose requirement of such formulations is much lower than WDP formulations. Unfortunately, till date little research work has been done in India on liquid formulations.

**Delivery of Microbial Control Agents**

The delivery system for microbial control agents represents a critical area of focus within the field of biocontrol research, as the most of the introduced organism faces a stiff competition from the resident soil microbes resulting loss of its efficacy against the soil borne plant pathogen. Indeed, it is imperative to establish an efficient, cost-effective and ecologically sustainable method for applying microbial control agents within the soil ecosystem (Mishra, 2002). In general, microbial control agents are applied as: (i) seed treatment, (ii) seedling root treatment, (iii) soil treatment and (iv) foliar application.

***1. Seed Treatment***

Dry seed treatment of antagonist in the spermosphere leads to proliferation of antagonist without affecting germination, thus creating a physical barrier against invasion by the pathogen. Seed treatment with antagonists is relatively a new concept in the approaches for biological management of plant disease and particularly more feasible and convenient approach for management of seed and soil borne diseases. Biological seed treatment has tremendous potential (Mukhopadhyay, 1994). Seed coating enables the biocontrol agents to be introduced to specific courts of infection where seedlings are most susceptible. Seed coating allows its use in small quantities and where involvement in larger quantities may not be economically feasible (De and Mukhopadhyay, 1994). The practice of inoculating seeds, corms, bulbs, tubers and similar planting materials with antagonistic agents serves to prevent seed or corm decay, seedling blight and effectively manages seed-borne pathogens, especially those that originate externally, thereby addressing diseases that can impact the plant's above-ground parts (Mishra, 2002). Seed application or seed pelleting is the cheapest method for the application of biocontrol agents. This method of application also takes care of soil-borne plant diseases also. Biocontrol agent propagules germinate on the surface of seeds and subsequently colonize the roots of germinated seedlings. For commercial applications, dry powders containing antagonists are used @ $3\times10^9$ kg of powder $kg^{-1}$ of seed, with the specific amount depending on the size of the seed and the formulation of the antagonist (Mishra, 2002; Mukherjee and Mukhopadhyay, 1995; Singh *et al.*, 2005). *Trichoderma hamatum*, *T. harzianum*, *T. virens* and *T. viride* are proven effective as seed protectants against *Pythium* spp. and *Rhizoctonia solani* pathogens (Das and Dutta, 1999; Dutta and Das, 1999a; Mishra, 2002; Mukherjee and Mukhopadhyay, 1995). The application of antagonists through seed treatment has demonstrated its effectiveness in suppressing a wide range of diseases, including those affecting seeds, seedlings, roots, stems, foliage and panicles (Dutta and Das, 1999b; Mishra, 2002; Singh *et al.*, 2005).

*Seed Bio-Priming*

Seed bio-priming, involves slurry treatment of seeds with microbial agents in the presence of gum arabica, jaggery (gur) and/or FYM powder and its incubation under high moisture for 24 to 48 h, results in improved performance of microbial control agents against seed and soil-borne diseases. Biocontrol agents like *Trichoderma* and *Pseudomonas* grow and form a layer around seed surface. Biopriming offers the advantage of reducing the quantity of microbial control agents required for seed treatment (Mishra, 2002). It is usually 2 to 5 g bioagent $kg^{-1}$ seeds against 4 to 10 kg bioagent $kg^{-1}$ seeds for normal seed treatment.

***2. Seedling Treatment***

Seedling roots can be effectively treated with spore or cell suspensions of antagonistic microorganisms. This approach is commonly employed in crops that involve transplanting as a regular agricultural practice (Mishra, 2002).

***3. Soil Treatment***

Numerous reports have documented the application of microbial control agents to soil and various growing media, either before or during planting. This approach has been effective in controlling a diverse array of soil-borne fungal pathogens (Baby and Manibhushanarao, 1996; Mishra, 2002). These applications are particularly well-suited for greenhouse and nursery beds, where precise control is more manageable. Due to the larger quantities needed, cost considerations and challenges associated with achieving uniform distribution, the feasibility of field-scale application is often limited. Direct application of *Trichoderma* and/or *Pseudomonas* to soil by drenching is being used widely but its performance had been found quite inconsistent. Soil application of local antagonists based formulations has been proven to be effective in managing soil-borne plant pathogens such as *Sclerotinia sclerotiorum*.

The application methods like seed treatment or root dip are most effective when used in combination with the soil application of microbial control agents within colonized compost (Dutta and Das, 1999a). When introduced via colonized-FYM, these microbial agents benefit from substrate colonization, which enables them to establish and thrive in the soil more effectively compared to their application through alternative methods.

***4. Foliar Application***

The success of any antagonist on leaf or sheath surfaces is heavily reliant on its capacity to colonize these surfaces (Mishra, 2002). Environmental factors such as humidity, temperature and sunlight play a significant role in colonization. Numerous reports have demonstrated the effectiveness of antagonists applied as foliar sprays against various plant pathogens, highlighting the potential of this approach in biocontrol. Oil-based liquid formulations and WDP are the available formulation which may be effectively used against foliage diseases.

In recent pot and field experiments conducted by Upamanya *et al.* (2020), to evaluate the effectiveness of six different Integrated Pest and Disease Management (IPDM) modules using prepared consortia, a combination of methods proved to be the most successful in managing diseases such as Phomopsis leaf blight, Alternaria leaf spot and Fusarium wilt. This comprehensive approach involved seed treatment + seed bed treatment in the nursery + soil application in the main field + seedling dip treatment with a consortium of biofertilizers (*Rhizobium* sp., *Azotobacter* sp. strain 52, *Azospirillum* sp. strain 71 and *Bacillus* sp. strain 5W) and spraying of the same consortium. Furthermore, this module was found to be effective in improving soil nutrient levels, with a significant increase in phosphorus (P) and potassium (K) content, as well as microbial biomass carbon in both years of experimentation (Upamanya *et al.*, 2020). Pending further confirmation in diverse agro-ecological conditions in Assam, this module holds promise for organic brinjal cultivation and can potentially be adopted by farmers (Upamanya *et al.*, 2020).

## Future Line of Work

In near future more concentration should be given on the following points: a) Selection of highly virulent strains of microbial control agents; b) Intensive R & D to create potent microbials using, (i) recombinant (ii) non recombinant DNA Technologies; c) development of stable formulations with good shelf life and high field efficacy and persistence; d) development of mixed formulations of plant disease antagonists; and e) development of efficient storage, packing and transport methods to avoid loss of virulence in storage and transport.

*Quality Assurance*

a) Establish quality standards for combination products, b) establishment of public sector quality control labs with trained technicians, c) Effective training of entrepreneurs and public sector quality control personnel, d) Strict in-house quality control, e) Strict enforcement of quality/ promotion of biological control, f) More aggressive Govt. policy to promote microbial control agents - particularly in organic farming g) Provide incentives and tax concessions to entrepreneurs, g) Encourage village level production of *Trichoderma* and *Pseudomonas* in farmyard manure, vermicompost, pressmud *etc*. by farm women for local consumption.

*Increase Awareness and Demand for Microbial Control Agents*

a) Area-wise farmer-participatory demonstrations/ front line demonstrations with good quality microbial control agents in the IPM mode to infuse faith on biological control, b) Education of all stake holders - farmers, extension workers (Public/ NGO, entrepreneurs, dealers, self help groups), c) Popularization through farmers' field school, melas, mass media *etc*. (including Kisan call centres).

*Regulation of Production and Distribution*

a) Develop market intelligence, b) Establish efficient distribution system and marketing outlets.

## Conclusion

The forest is an integral part of human life. Apart from the goods forest bestows upon us, it also combats desertification, conserves soil and water, regulates carbon sequestering and maintains the biological diversity. Therefore, it is essential to understand the forest ecology and to take up the necessary eco-friendly strategies to save the forest plantations from biological threats.

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