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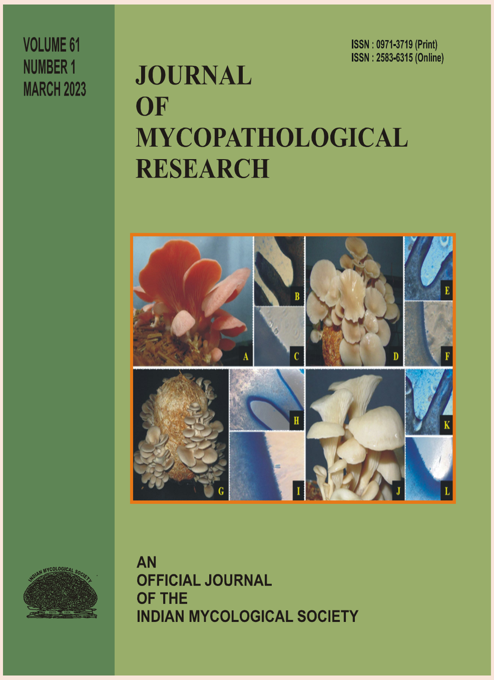

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REVIEW

Harnessing fungal biocontrol agents for the management of plant diseases

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Plant diseases are considered as one of the serious limiting factors for the successful cultivation/ plantation of various agricultural, horticultural and forestry plants. As the yield losses are being estimated in alarming economic threshold levels every year, it is imperative to use appropriate control measures to manage the plant diseases. Although, chemical control is being used extensively by the farmers, it is exhibiting serious demerits and hence biological control is gaining momentum. Various bacterial and fungal species were already identified as promising biocontrol agents and are being used for the management of plant diseases. Among them, *Trichoderma* spp. are the most promising biocontrol agents and they are being mass multiplied, formulated and commercially available for farm applications. Therefore, research efforts were made to identify a promising *Trichoderma*, develop into biofungicide formulation and distribute the farmers for the management of root diseases in different crops. An effective strain of *Trichoderma viride* was isolated from soil, multiplied using molasses yeast medium and formulated with talc powder as biofungicide. This formulation was distributed to the farmers in Andhra Pradesh and Karnataka states for the management of root diseases in different crops. Impact assessment study carried out with the farmers have revealed that the application of *T. viride* effectively controlled the soil-borne root diseases in cotton, chilli, sugarcane and acid lime. Distribution of *T. viride* in large scale made tremendous impact on farmers in Andhra Pradesh, who are at present coming forward to buy the commercial products of biocontrol agents from the local market.

Keywords: Biological control, *Trichoderma viride*, biofungicide, root diseases

INTRODUCTION

The crop losses due to various biotic and abiotic stresses are the serious concern world over and it is estimated from 20 to 40% worldwide in various crops due to pests and diseases (Anonymous, 2000; CABI, 2023). The biotic stresses like pathogens and insect pests are considered most important since they cause crop losses to the tune of Rs. 29,240 crores per annum in India (Saksena, 2001). As far as plant pathogens are concern, they cause about 30,000 diseases all over the world in crop plants and in India alone 5,000 plant diseases were recorded. Of which, some diseases occur in severe form and destructing the crop. Hence, there is a continuous need for development of suitable effective control measures for the management of crop diseases to minimize the yield losses.

Different practices such as cultural, chemical and biological are being recommended to the farmers for protecting the crops against diseases. The cultural practices include crop rotation, alteration of sowing/planting time, irrigation, mulching, solarization, sanitation and application of fertilizers. Each cultural practice can influence on plant pathogen and reduce the disease incidence in different levels. Though there is considerable reduction in the proliferation and attack of pathogens, but this method cannot ensure complete protection in various crops.

The use of chemical pesticides is one of the most important methods, which widely adapted to

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achieve high levels of crop protection. Several fungicides and bactericides are being used in agriculture for centuries for the control of various fungal and bacterial diseases. However, the constant and indiscriminate use of chemical pesticides create serious problems such as development of resistance to pesticides, resurgence of target pests, secondary pest outbreak, killing of non-target organisms, residual toxicity and environmental pollution (Urech *et al.* 2000; Aktar *et al*. 2009; Kalia and Gosal, 2011; Larramendy, 2014; Sharma *et al*. 2019; Tudi *et al*. 2021; WHO, 2022). Further, often the chemical fungicides failed to offer satisfactory control of root diseases caused by soil pathogens though they were effective against foliar diseases. The injudicious use of chemicals has to be curtailed because of their hazardous effects on non-target organisms and also the undesirable changes they inflict on the environment. With the growing concern over the adverse effects of chemical pesticides in the environment, there is an urgent need for alternative disease control strategies. Therefore, biological control assumes special significance because it is eco-friendly and cost effective control strategy. Further, biological control or biocontrol can be integrated with other disease management practices such as cultural and even partial chemical control to achieve higher levels of crop protection for sustaining the crop yields (Mathivanan *et al*. 2000 a; 2004; 2005). Further, it is increasingly becoming more popular as it is a potential non-chemical tool for crop protection against plant pathogens (Mathivanan *et al.* 2004; Pal and Gardener, 2006; O'Brien, 2017; Tariq *et al*.,2020; He *et al*. 2021; Collinge *et al*. 2022).

Biological control is the "control of plant diseases with microorganisms (antagonists) by destroying the existing pathogen inoculum, excluding the pathogen from the host plant or suppressing or displacing the pathogen after infection has occurred". However, the definition of biological control has further been elaborated as "the use of natural or modified organisms, genes or gene products to reduce the effects of undesirable organisms such as plant pathogens and to favour desirable organisms such as crops. In the last three decades, numerous microorganisms have been examined for their antagonistic activity against various bacterial and fungal plant pathogens, which resulted in increased use of microorganism based biopesticides for the management of various crop diseases (Jayarajan and Nakkeran, 2000; Kong, 2017; Babbal *et al*. 2017; Poveda *et al*. 2021; Bonaterra *et al*. 2022; Boro *et al*. 2022).

Many bacteria, fungi and actinomycetes are playing a vital role in biological control of plant diseases. Various microbial antagonists including the some species of bacterial genera *viz*., *Bacillus*, *Pseudomonas, Burkholderia, Agrobacterium* and *Enterobacter, Streptomyces* and fungi, *Trichoderma, Gliocladium* and *Coniothyrium* have shown enormous biocontrol potential against various foliar and root diseases in several crop plants (Jayarajan and Nakkeran, 2000; Prabavathy, 2005; Prabavathy *et al*., 2006; Mathivanan *et al*. 2005; Prashanth *et al.* 2006; Shanmugaiah *et al.* 2006; 2008; Srinivasan *et al.* 2006; Srinivasan and Mathivanan, 2009; 2011; Shanmugaiah *et al.* 2010; Robati and Mathivanan, 2013; Krishnaraj *et al.* 2014; Kong, 2017; Babbal *et al*. 2017; Chenniappan *et al*. 2019; Poveda *et al*. 2021; Bonaterra *et al*. 2022; Boro *et al*. 2022; Gusella, *et al*. 2022) and several commercial bioproducts have already been developed using bacterial and fungal biocontrol agents, evaluated against various plant diseases and currently available in the global market (Table 1) for farm use.

FUNGAL BIOCONTROL AGENTS

The antagonistic effect of different fungi is being exploited in the control of plant diseases. Some of the fungal biocontrol species used are, *Trichoderma*, *Ampelomyces*, *Coniothyrium*, *Trichothecium*, *Gliocladium*, *Talaromyces*, *Phlebiopsis*, *Tilletiopsis*, *Lactisaria*, *Penicillium*, *Aspergillus* and *Sporodesmia* (Mathivanan *et al*. 2006). Among them, *Trichoderma, Gliocladium* and *Coniothyrium* have been extensively exploited due to their high efficacy of broad spectrum of activity and amenable for large-scale multiplication and formulation (Mathivanan *et al*. 2000 b; de Vrije *et al*. 2001; Mcquilken *et al*. 2001; Whipps *et al*. 2008; Ojaghian, 2010; Agarwal *et al*. 2011; Zeng *et al*. 2012; Kumar and Ashraf, 2017; Mukhopadhyay

Table 1: Commercial products of biocontrol agents from fungi and bacteria

Product	Biocontrol agent	Target pathogen/ disease	Crop	Formulation
Antagon-TV	*T. viride*	Seed and soil-borne pathogens	Legume, cotton, vegetable crops, Sunflower, peanut, soyabean	Talc powder
Biocure-F	*T. viride*	Root diseases	Various crops	Talc powder
SunAgroderma	*T. viride*	Root diseases	Various crops	Talc powder
Eco-fit	*T. viride*	Seed and soil-borne pathogens	Legume, cotton, vegetable crops, Sunflower, peanut	Talc powder
Ecoderma	*T. viride*	Root diseases, sheath blight	Various crops	Talc powder
Bumika	*T. viride*	Root diseases	Various crops	Talc powder
Agnee	*P. fluorescens*	Root diseases, sheath blight	Legume, Rice	Talc powder
Biocure-B	*P. fluore scens*	Root diseases, sheath blight	Legume, Rice	Talc powder
Actinovate	*Streptomyces lydicus*	Root diseases	Nursery, Green house, turf grass	Granules
Bio-Fungus	*Trichoderma* spp.	*Sclerotina, Phytophthora, R. solani, Pythium* spp. *Fusarium*	Flowers, trees, strawberries, vegetables	Granules/ wettable powder
Binab T	*T. harzianum* and *Trichoderma polysporum*	Wilt, take-all, root rot and internal decay of wood	Flowers, fruits, ornamentals, turf, vegetables	Wettable powder and pellets
Biofox C	*F. oxysporum* (non-pathogenic)	*F. oxysporum, Fusarium monoliforme*	Basil, carnation, cyclamen, tomato	Dust or alginate granule
BlightBan A506	*P. fluorescens* A506	*Erwinia amylovora* and russet- inducing bacteria	Almond, apple, apricot, blueberry, cherry, peach, pear, potato, strawberry, tomato	Wettable powder
Companion	*B. subtilis GB03, B. licheniformis, B. megaterium*	*Rhizoctonia, Pythium, Pytophthora, Fusarium*	Green house, nursery	Liquid
Contans WG	*Coniothyrium minitnas*	*Sclerotinia sclerotiorum, S. minor*	Sunflower, peanut, soyabean, vegetables	Water dispersable granules
Deny	*Burkholderia cepacia, Psedomonas cepacia*	*Rhizoctonia, Pythium, Fusarium* and nematode diseases	Legume, cotton, vegetable crops and wheat	Peat based, aqueous
Epic	*B. subtilis*	*R. solani, Fusarium , Alternaria, Aspergillus*	Cotton, legumes	Dry powder
Fusaclean	*F. oxysporum* (non-pathogenic)	*F. oxysporum*	Asparagus, basil, carnation, tomato, gerbera	Spores, micro granule
Galltrol	*A. radiobacter*	*Agrobacterium tumifaciens*	Fruit, nut, ornamental, nursery	Agar grown culture
Intercept	*P. cepacia*	*R. solani, Fusarium, Pythium*	Maize, vegetables, cotton	-
Kodiak, Kodiak HB, Kodiak AT	*B. subtilis*	*R. solani, Fusarium, Alternaria, Aspergillus*	Cotton, legumes	Dry powder
KONI	*C. minitans*	*S. sclerotiorum, S. minor*	Green house crops	Granules
Mycostop	*Streptomyces griseoviridis* K61	*Fusarium, Alternaria, Phomopsis, Botrytis, Pythium*	Field, ornamental, vegetable crops	Powder
Nogall, Diegall	*A. radiobacter*	*Agrobacterium tumifaciens*	Trees	Washed plates, culture suspensions
Norbac 84C	*A. radiobacter* K84	*A. tumifaciens*	Fruit, nut, ornamental nursery stock	Aqueous suspension
Polygandron	*Pythium oligandrum*	*P. ultimum*	Sugar beet	Granule, powder
PreStop, PrimaStop	*Gliocladium catenulatum*	*Pythium, R. solani*	Greenhouse crops, ornamental, vegetable and tree crops	Wettable powder
PSSOL	*Pseudomonas solanacearum* (non-pathogenic)	*P. solanacearum*	Vegetables	-
Rhizo-Plus, Rhizo – Plus Konz	*B. subtilis* FZB24	*R. solani, Fusarium* sp., *Alternaria* sp., *Sclerotinia, Verticillium, Streptomyces*	Field vegetables and ornamental plants	Water dispersible granule
RootShield	*T. harzianum*	*Pythium, R. solani, Fusarium*	Trees, shrubs, plantations, vegetables	Granules, wettable powder
Rotstop	*Phlebia giganteana*	*Heterobasidium annum*	Trees	Spores in inert powder

Product	Biocontrol agent	Target pathogen/ disease	Crop	Formulation
Serenade	*B. subtilis*	Late blight, brown rot, fire blight	Peanut, pome fruits, stone fruits	Wettable powder
SoilGard (GlioGard)	*Gliocladium virens* GL-21	Damping-off, *R. solani, Pythium* spp.	Ornamental and food crops	Granules
System 3	*B. subtilis* GB03 and chemical pesticides	Seedling pathogens	Beans, cotton, peanut, pea, rice, soybean	Dust
T-22G, T-22 Planter box	*T. harzianum*	*Pythium, R. solani*, *Fusarium*	Trees, shrubs, plantations, ornamentals, cabbage, tomato, cucumber	Granules, wettable powder
Trichodex	*T. harzianum*	*Colletotrichum, Fulvia fulva, S. sclerotiorum*	Cucumber, soybean, strawberry, sunflower, tomato	Wettable powder
Trichopel, Trichojet, Trichodowels, Trichoseal	*T. harzianum* and *T. viride*	*Armillaria, Fusarium, Nectaria, Phytophthora, Pythium, Rhizoctonia*	Various crops	-
Trichoderma 2000 ("TY")	*Trichoderma* sp.	*R. solani, S. rolfsii, Pythium* spp., *Fusarium* spp.	Nursery and field crops	-
YieldShield	*Bacillus pumilis GB34*	Soil-borne fungi	Soybean	Dry powder

(Contd. part table 1)

and Kumar, 2020; Zin and Badaluddin, 2020; Sood *et al*. 2020; Rush *et al*. 2021; Sivagnanapazham *et al.* 2022; Tyœkiewicz *et al.* 2022).

They are reported to control collar rot, root rot, wilt and damping-off caused by *Sclerotium*, *Rhizoctonia*, *Fusarium*, *Phytophthora* and *Phythium,* respectively that affect crops like cotton, sunflower, pulses, vegetables, sugarcane, turmeric, ginger and plantation crops like pepper, cardamom, banana, etc. Granular formulations developed with biomass of *Gliocladium* and *Trichoderma* were effectively reduced the damping-off caused by *Rhizoctonia solani* (Arya 2001; Abbas *et al*. 2017; Amer *at al*. 2019; Kumhar *et al.* 2022).

Gliocladium virens Syn. *Trichoderma virens* (https://www.uniprot.org/taxonomy/29875 Accessed on 17.01.2023) was used to control damping-off diseases of vegetables and ornamental plant seedlings caused by *R. solani* and *Pythium* sp. (Jun and Kim, 2004) and chickpea wilt complex disease (Tewari and Mukhopadhyay, 2001). *G. roseum* was active against *B. cinerea* on strawberry. Biocontrol of *Botrytis* leaf blight of onion has been achieved using *Gliocladium* as spray. *Ampelomyces quisqualis* was the first biocontrol fungus developed specifically for controlling powdery mildew. AQ10, the product of *A. quisqualis* is a water disposable formulation used against powdery mildew of strawberry, tomato, grape, fruit trees and ornamental plants. *Coniothyrium minitans* was a mycoparasite to sclerotia of *Sclerotinia sclerotiorum* and *Sclerotinia minor* (Grendene and Marciano, 2003; Partridge *et al*. 2004; Li *et al*. 2006; Whipps *et al*. 2008; Ojaghian, 2010; Zeng et al. 2012; Jones *et al*. 2014; Sivagnanapazham *et al*. 2022). A white yeast *Candida oleophila*, is a biocontrol agent that reduces the rot diseases on citrus and apples and also prevents postharvest diseases caused by *Botrytis cinerea* and *Penicillium expansum* on apple and pear fruits (Lahlali *et al*. 2004; 2010). It also reduces green and blue mold caused by *Penicillium digitatum* and *Penicillium italicum,* respectively.

Species of *Fusarium viz., F. heterosporum, F. semitectum* var. *majus, F. decemcellulare, F. longipes, F. chlamydosporum* and non-pathogenic *F. oxysporum* have been reported as biocontrol agents against various plant pathogenic fungi (Mathivanan and Murugesan, 2000; Fravel *et al*. 2003; Kaur *et al*. 2010; Thongkamngam and Jaenaksorn, 2017; Mulero-Aparicio, 2019; Sajeena *et al*. 2020). Foliar application of conidial suspension and culture filtrate of *F. solani* isolated from the rust pustules on groundnut, markedly reduced the pustule formation on groundnut leaves. *F. solani* was found to produce extracellular chitinases, glucanases and cellulases and the crude enzyme preparation inhibited the spore germination and germ tube growth of *P. arachidis*

(Mathivanan, 2000). *F. chlamydosporum* has been reported as biocontrol agents for groundnut rust pathogen, *Puccinia arachidis* and it capable of degrading the uredospore and germ tube of the rust pathogen by secretion of extracellular chitinase (Mathivanan and Murugesan, 2000) and also inhibit the uredospore germination by production of antifungal metabolites. Interestingly, *F. oxysporum* as a root endophyte, can reduce diseases caused by *Verticillium dahlia*, *Pythium ultimum*, *Botrytis cinerea* and *Phytop-hthora capsici* (de Lamo and Takken, 2020).

Among the fungal biocontrol agents, members of the genus *Trichoderma* are considered the most important because they control various root diseases caused by a wide range of fungal pathogens (Mathivanan *et al,* 2000a; 2004; Mathivanan, 2004; Mathivanan and Manibhu-shanrao, 2004; Ryu *et al*. 2006; Kumar and Ashraf, 2017; Mukhopadhyay and Kumar, 2020; Zin and Badaluddin, 2020; Sood *et al*. 2020; Rush *et al*. 2021; Kumhar *et al*. 2022; Tyœkiewicz *et al*. 2022; Walid *et al*. 2022). Potential of *Trichoderma* as an effective biocontrol agent against soil-borne fungal pathogens has been demonstrated across the world. Biocontrol potential of *T. viride* was assessed against soft rot decay of wood under field trials. A talc-based formulation of *Trichoderma* significantly controlled the root diseases in field grown cotton, egg plant, okra and sunflower crops (Mathivanan *et al.* 2000a). Seed treatment or soil amendments of conidia or other formulations of *Trichoderma* reduced root and foliar diseases in many agricultural and plantation crops (Prasad *et al.* 2002; Batta, 2004; Mathivanan *et al.* 2004; 2005). Further, several commercial formulations of *Trichoderma* are available worldwide for the farmers' use (Whipps *et al.* 2001; Mathivanan, 2004).

Biological control using biocontrol agents for the management of soil-borne plant pathogens is a promising and viable supplement/alternative to chemical control. Several antagonistic fungi and bacteria are effective in controlling many soil-borne diseases. Among them, fungi in the genus *Trichoderma* are considered the most important. Many species of *Trichoderma* are effective in controlling several soil-borne diseases in various crops (Mathivanan *et al.* 2000a). A talc based formulation of *T. viride* was reported to control root rot disease in urd bean caused by *Macrophomina phaseolina*. At present, a number of commercial products of *Trichoderma* are available in many countries (Mathivanan *et al*. 2006; Cumagun, 2012; Kumar *et al*. 2014; Woo *et al*. 2014; Ghazanfar *et al*. 2018; Pranab *et al*. 2022).

Among different biocontrol agents, species of *Trichoderma* exhibited promising disease suppression in field conditions and they are suitable candidates for large scale commercial production as they have showed wide spectrum of activity against various fungal pathogens, non-pathogenic in nature, promoting plant growth, amenable for mass multiplication, eco-friendly and commercially viable. Therefore, efforts have been made to isolate, identify and develop *Trichoderma* based biofungicide formulation and supply to the farmers under the Department of Biotechnology, Government of India sponsored projects. The detailed are presented in this article.

Isolation, evaluation and mass multiplication of Trichoderma

Several strains of *Trichoderma* were isolated from native soil and evaluated for their bioefficacy against root pathogens *viz*., *Fusarium oxysporum*, *Rhizoctonia solani* and *Sclerotium rolfsii*. Based on

Table 2 : Crop-wise distribution of *T. viride* and area covered

Crop	No. of villages	No. of districts	No. of farmers	Area covered (ha)
Cotton-ST	218	7	4598	8898
Cotton-SD	8	1	50	20
Chilli-SA	83	4	1039	1169
Chilli-SD	2	1	4	4
Chickpea	NR	NR	NR	4
Sunflower	15	1	31	14
Turmeric	3	1	4	4
Sugarcane	55	1	274	670
Acid lime	24	1	50	1600 trees

ST: Seed treatment; SD: Soil drench; SA: Soil application; NR: List not received the performance in dual culture against root pathogens, a *T. viride* strain designated as NCC 34 was selected, multiplied using molasses yeast medium and formulated with talc powder as biofungicide.

Distribution of biofungicide (T. viride)

The biofungicide was distributed to the farmers in eight districts (Adilabad, Karimnagar, Warangal, Khammam, Krishna, Guntur, Prakasam, Nellore) in Andhra Pradesh state and Bellary district in Karnataka state for the control of soil-borne diseases in different crops. The entire quantities of *T. viride* produced at Nagarjuna Agricultural Research and Development Institute (NARDI), Hyderabad were distributed free to all the growers through different agencies *viz*., Farm management Services (FMS), Hyderabad, Gayathri Sugar Complex Ltd (GSCL), Nellore, Centre for World Solidarity (CWS), Hyderabad, Navajyothi, Ramayampet, Medak district, the erstwhile combined Andhra Pradesh (AP) state. About 10,783 ha area was covered with the biofungicide, spread over eight districts in AP and an adjacent Bellary district in Karnataka. The crop-wise details for the distribution along with total villages, districts and beneficiaries are presented in Table 2. The biofungicide has also been supplied to the acid lime growers in Nellore district in AP for the control of wilt disease.

DETAILS OF USE

Soil application/drench

Cotton

The year 1998 witnessed very heavy rainfall in most of the cotton growing tracts of AP. Many farmers in Warangal reported plant mortality due to root rot disease aggravated by constant soil moisture. To help these farmers, NARDI arranged to supply 50 kg of *T. viride* with the assistance of FMS. The

Table 3: Response of the cotton growers on the distribution of *Trichoderma viride*

Statements related to biological control	Farmers' response (%)
Farmer's attitude towards disease control	
Need based use of fungicides is better	71
Removal and destruction of infected plant parts reduce disease incidence	58
Follow seed treatment	78
Effectiveness of *T. viride* in farmers' fields	
Not effective	13
Effective for some extent	23
Moderately effective	36
Highly effective	28
Reasons for accepting *T. viride* by the farmers	
T. viride reduces disease incidence	75
Reduction of fungicide drench	73
Increase in yield	68
No health hazards	83
Prevents pollution	73
Farmers' attitudes to buy the commercial product of *T. viride*	
If available free	19
If available at reasonable price	81

biofungicide was supplied to 50 select farmers in 8 villages to cover 20 hectares (Table 2). The farmers mixed one kg of formulation in a drum containing 200 L of water. This was drenched near the root zone of cotton plants. This method of application was reported to give satisfactory control of the root disease.

Chilli (nursery and main field)

Table 4: Problems faced by the farmers for using *Trichoderma viride*

Problems for using *T. viride*	% response
Distance to production centres	46
Lack of adequate knowledge	38
Difficulties in consulting the extension personnel when needed	31
No immediate control like fungicide	29
Short shelf life	20
Delayed supply	17

Successful production of chilli is hampered by the damping-off in nursery caused by *Pythium aphanidermatum.* After planting, the crop is affected by *Rhizoctonia* root rot. Farmers use COC for drenching the nurseries and main field to control these pathogens. Alternatively, was recommended applying *T. viride* in soil both in the nursery and transplanted crop. The biofungicide formulation of *T. viride* was distributed free to 1039 farmers to cover 1169 hectare spread over four districts *viz*., Khammam, Krishna and Prakasam in AP and adjacent Bellary district in Karnataka (Table 2).

Chilli and turmeric

Root rot caused by *R. solani* is the major constraint for successful chilli cultivation in AP. Rhizome rot is a serious problem in turmeric caused by *Pythium*. In Warangal, the Marketing Division of Nagarjuna Fertilizers and Chemicals Limited distributed 20 kg of biofungicide formulation for soil drenching to eight farmers (Table 2). Soil drenching of *T. viride* gives quick remedy of root pathogens as compared to soil application mixed with FYM.

Sunflower

A group of 31 farmers from Karimnagar district visited NARDI in the second week of November 1999. They reported collar rot and root rot incidence caused by *Sclerotium rolfsii* and *R. solani*, respectively in sunflower. Thirty five-kg of biofungicide was distributed to these farmers along with instructions on the method of usage.

Acid lime

The Venkatagiri taluk in Nellore district of AP is a specialized acid lime production centre. Root diseases caused by *Fusarium, Phytophthora* and *Rhizoctonia* affected several trees in this area. The farmers have also reported unsatisfactory control with chemical fungicides. Therefore, *T. viride* formulation was distributed to citrus growers in this region and the farmers were asked to apply 500 g formulation mixed with farmyard manure to each tree. Among 1600 trees applied with *T. viride* (Table 2), most of the partially affected trees were recovered.

Sugarcane

Sugarcane is affected by seedling rot (*Pythium aphanidermatum, P. catenulatum* and *P. graminicolum*) and red rot (*Colletotrichum falcatum*). Planting an acre of sugarcane requires about 30,000 setts. It is not possible to treat all the setts with chemicals or *Trichoderma* formulation. Hence, soil application is recommended either before planting along the planting lines or immediately after planting. The Gayathri Sugar Complex Limited, Nellore, AP came forward to distribute *T. viride* to their registered growers for soil application. About 670 ha are covered with the application of *T. viride* till January 2000 (Table 2).

Seed treatment

Cotton

Cotton is afflicted by damping off/root rot and wilts caused by *Rhizoctonia solani/R. bataticola* and *Fusarium oxysporum*, respectively. In traditional cotton areas of AP, these diseases cause 20-30% mortality of plants at various crop stages. Common method adopted by growers to control these diseases is to drench the plants with copper oxychloride (COC) at 3 g /liter of water. Alternatively, the biofungicide (*T. viride*) is recommended as seed treatment at 50 g/kg seeds for the control of soil-borne diseases. The approximate quantity of seeds required to grow hybrid cotton for one acre is 450-500 g. Therefore, 25 g packets of biofungicide formulation were distributed, which enabled the farmers to treat seeds required for one acre. Up to May 2001, about 4598 cotton farmers were supplied with biofungicide to cover 8,898 ha of cotton spread over 218 villages in seven districts (Adilabad, Guntur, Karimnagar, Khammam, Krishna, Prakasam and Warangal) of AP (Table 2).

Chickpea

The Centre for World Solidarity, Hyderabad arranged distribution of 13 kg of *T. viride* for prophylactic use as seed treatment in chickpea to control wilt disease caused by *Fusarium oxysporum* in four hectares.

General performance of T. viride

Seed treatment

This method is prophylactic and requires minimum quantity of 50 g to treat one kg of cotton seeds. Treated seeds germinated better than untreated seeds. In addition, it was also effective against pre- and post-emergent damping-off and root rot caused by *R. bataticola* and *R. solani*. The cotton farmers have expressed their satisfaction on the performance of *T. viride*.

Soil application and drench

Application of *T. viride* along with FYM and also drenching with water in different crops effectively controlled various root diseases. Field visits to *Trichoderma* applied plots revealed that *Trichoderma* formulation was effective against pre- and post-emergent damping-off, root rot and wilt in cotton, damping-off and root rot in chilli and seedling rots in sugarcane.

IMPACT ASSESSMENT ON THE DISTRIBUTION OF *T. VIRIDE* IN COTTON FIELD

Farmers' attitude towards disease control

In general, most of the farmers have expressed that the use of fungicides gave appreciable control of plant diseases. About 71% farmers opined that the need based spraying is better than prophylactic application of fungicides. Seed treatment with chemical fungicides is a common practice followed by 78% farmers. About 58% farmers were aware that the removal and destruction of infected plant parts would reduce the occurrence of diseases (Table 3).

Effectiveness of T. viride in farmers' fields

Many farmers (36%) expressed that *T. viride* is moderately effective for the management of soil-borne diseases (Table 3). About 28% farmers revealed that *T. viride* was highly effective in controlling cotton diseases. Only 13% farmers were not fully satisfied with the performance of *T. viride* as they responded for "not effective".

Reasons for accepting T. viride by the farmers

After using *T. viride* formulation, most of the farmers (68-83%) have expressed positively to accept *T. viride* as an alternative to chemical fungicides for different reasons. They believed that the use of *T. viride* certainly reduced the disease incidence (75%), fungicide usage (73%), health hazards (83%) and environment pollution (73%). In addition, 68% farmers agreed that the use of *T. viride* increased the crop yield (Table 3).

Farmer's attitudes to buy the commercial product of T. viride

About 81% farmers were interested to buy the commercial product of *T. viride* at reasonable rate (Table 3). Only 19 % were not ready to buy, however, they were willing to use the biocontrol agent, if available free of cost.

Farmers' constraints for the use of T. viride

The distance of production units is one of the constraints as informed by 46% farmers in procuring the *T. viride*. Lack of adequate knowledge and difficulties in consulting with extension personnel are also major hindering factors for not using the biocontrol agent. Some of the farmers have reservation on the use of *T. viride* because of its delayed response in controlling diseases as compared to chemical fungicides, short shelf life and delay in supply of the biocontrol agent (Table 4).

The role of biocontrol agents particularly *Trichoderma* in controlling soil-borne diseases has been well documented and also established both by research and demonstration trials all over the country. The use of biocontrol agents is cost effective and can be easily adopted by the farmers as prophylactic measure. Various institutions have standardized the production technologies of these bioagents, but the adoption rate by the farmers compare to chemical fungicides was found to be low.

The use of biocontrol agents was not very popular among farmers for the control of diseases due to several reasons. Realizing the gaps and deficiencies in promoting these agents, research efforts were focused to improve upon these technological aspects particularly with reference to the mass production of *Trichoderma*, effective packaging, timely delivery and guidance for effective use by the farmers. Under a scheme sponsored by the Department of Biotechnology, GOI, NARDI has made sincere and systematic efforts to popularize the technology in nearly eight important districts of erstwhile combined Andhra Pradesh state. Within three and half years, NARDI supplied the quality inputs to the farmers, besides working out the commercial and economic potential. In addition to the distribution, a number of trials were conducted in the farmers' fields to demonstrate the efficacy of biocontrol agents. Several farmers' meets and field days were organized in different regions of AP to popularize the use of biocontrol agents at a large scale.

The impact assessment surveys conducted by NARDI on the distributions of *T. viride* have indicated that majority of the farmers are willing to use the biocontrol agents. They are also eager to adopt this technology and incorporate it in their regular package of practices, if suitable arrangements exist for their regular production and supply at economical costs. The awareness on biocontrol agents has increased several folds among farmers since 1998 due to constant efforts taken by NARDI with the help of other distributing agencies. Distribution of *T. viride* in a wide coverage of area within short period has made tremendous impact on the farmers in AP, who are now coming forward to buy the biocontrol agents from local market at this regions to use for controlling insect pests and diseases.

TRICHODERMA STRAIN IMPROVEMENT BY PROTOPLAST FUSION

The protoplast fusion is considered as an important technique for improvement of fungal strains by bringing genetic recombination, which leads to the development of hybrid strains (Lalithakumari, 2000; Prabavathy *et al*. 2006 a; b). Protoplasts fusion has improved the enzyme production and biocontrol potential in fusant *Trichoderm* strains (Prabavathy *et al*. 2006 a; b; Srinivasan et al. 2009). Therefore, interspecific protoplast fusion between *Trichoderma harzianum* and *T. viride* has been carried out with the objective of enhancing the chitinase production and the antagonistic potential of the fusant hybrid strains. The wild *T. harzianum* MML3001 and *T. viride* MML3116 were selected as parent strains based on chitinase production and fungicides tolerance, respectively. Protoplasts were isolated *T. harzianum* MML3001 and *T. viride* MML3116 and their viability was estimated as 81% and 78%, respectively. Inter-specific protoplast fusion between *T. harzianum* MML3001 and *T. viride* MML3116 was carried out and six fusants were selected. Among six fusants, *Th+Tv1, Th+Tv4* and *Th+Tv14* showed good antagonistic activity against *R. solani, M. phaseolina, F. udum* and *F. oxysporum.* However, maximum antagonistic activity was observed with *Th+Tv1*. Among six fusants, *Th+Tv1* produced highest extracellular chitinase than the others. Genomic DNA was isolated from two *Trichoderma* parents and six fusants and the ITS region was amplified using specific primers. RFLP was carried out with EcoR1, HindIII, Bam, SalI, Sma, Sau3a, MboI and XbaI restriction enzymes. However, DNA restriction was observed only with EcoR1. The ITS sequences of the parent and fusant *Trichoderma* strains were deposited in the NCBI Genbank database with the accession numbers GU084409, GU084410, GU084412, GU084413, GU084414, GU084415 and GU08416. Phylogenetic tree was constructed and analysed the relationship between the parent and fusant strains.

Talc and liquid formulations with parent and fusant *Trichoderma* strains were developed and the population of *Trichoderma* was checked up to 90 days. The parent and fusant *Trichoderma* strains exhibited good viability in liquid formulation than in talc formulation. Treatment of talc and liquid formulations of parent and fusant *Trichoderma* strains enhanced the seed germination and growth parameters and reduced the *Fusarium* wilt in black gram (*F. udum*), *Macrophomina* root rot in green gram (*M. phaseolina*), *Rhizoctonia* root rot (*R. solani*) and *Fusarium* wilt of tomato (*F. oxysporum*) incidences in the above crops in glasshouse experiment. Similar results were obtained in the field experiment with enhanced yield parameters.

CONCLUSIONS

With the growing concern on the environment especially pollution due to indiscriminate use of chemical pesticides in agriculture, biological control is gaining momentum for the management of various plant diseases. Several biocontrol agents have been identified with excellent biocontrol potential/activity against various plant pathogens. Furthermore, the efficiency of fungal biocontrol agents such as *Trichoderma* can be enhanced by protoplast fusion technique. Although several formulations of biocontrol agents are available commercially, it is necessary to identify newer microorganisms as biocontrol agents for the management of crop diseases and their potential might be exploited judiciously, which could significantly bring down the indiscriminate use of chemical pesticides.

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