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Effect of bio-control agents on soil borne pathogens: A review

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Abstract
Research in the past few years has clearly shown that selected strains of the fungi and bacteria provide an eco-friendly well balanced plant health management system through systemic resistance to diseases of biotic and a biotic nature, increasing seed vigour, improving nitrogen fertilizer use efficiency, enhancement of root area. Bio-control association also stimulated plant defensive mechanisms induction of resistance metabolism similar to the hypersensitive response, systemic acquired resistance and induced systemic resistance in plants. There is a growing demand for biologically based soil borne pathogen management practices.

Keywords: Bio-control, pathogen and eco-friendly.

Introduction
In view of the adverse effect of fungicides to the environment and increasing interest in sustainable agriculture, biological control has been tried as an attractive possibility for management of soil borne plant pathogens (Bapat and Shar, 2000). The value of the global biopesticide market is expected to reach $4,556.37 Million by 2019, at a compound annual growth rate of 15.30% from 2014 to 2019. The reasons for such growth can be found in: the increasing concerns on the impact of residues and overuse of synthetic chemical pesticides and the increasing relevance of pests and pathogens due to growth in food demand, the withdrawal of several chemical pesticides including soil fumigants, the appearance of new invasive species and pesticide resistant strains of pests, the effect of climate change and the specialised monoculture.

The decrease of the cost of several biopesticides, the introduction of technologically advanced products, the increase knowledge of farmers and the strict thresholds in pesticides residues set by many food retailers are also promoting the switch form chemical to biopesticides. Kumar and Dubey (2001) [19] reported that among three Isolates of bio control agent *Trichoderma viride, T. harzianum* and *Gliocladium virens* were screened against *Fusarium solani* f.sp. *pisi* causing collar rot of pea through dual culture and production of volatile and non-volatile antibiotic substances *in vitro*. The Ranchi isolate of *T. harzianum* and *G. virens* showed superiority over other isolates with respect to inhibition of mycelial growth of the pathogen. Singh *et al.* (2002) [28] reported that supplementing the soils with fungal or bacterial antagonists reduced incidence of *Fusarium* wilt. Soil amendment with *Trichoderma harzianum* at all pathogen levels has been reported to give a disease control of 22.1 – 61.5 per cent (Prasad *et al.*, 2002). Chaudhary and Prajapati (2003) [9] studied the efficacy of six bio-agents against *Fusarium udum* Butler in dual culture and found minimum colony growth of *F. udum* with *Gliocladium virens* and *T. viride* and observed mycoparasitism such as coiling, twisting and lysis of wilt pathogen by *T. harzianum*, *T. viride* and *G. virens.* All the six bio-agents reduced pigeonpea wilt and *F. udum* population in the soil in variable degrees. Gupta *et al.* (2003) [12] reported that *Trichoderma* spp. native isolates, exhibited the maximum inhibition 70.5% of radial growth of *F. udum* in dual culture. Khan and Khan, 2003 [17] found that application *Trichoderma harzianum* decreased the incidence and severity of *Fusarium* wilt by 43% and 48%, respectively in comparison to control, other bio-agents were also found effective in decreasing the incidence and severity of wilt by 42% and 39% in case of *T. virens,* 27% and 30% in case of *P. fluorescens,* and 22% and 17% in case of *Bacillus subtilis.*The yield increased by 41% due to seed treatment with *T. virens* and *P. fluorescens* and by 35% due to *T. harzianum* and by 21% due to *Bacillus subtilis.* Dayaram *et al.* (2004) [10] reported that among 3 bio-control agents *i.e. Trichoderma viride, Aspergillus flavus* and *A. niger*, *Trichoderma viride* was found most effective and inhibited the growth of test pathogen *in vitro.*

Anjaiah *et al.* (2003) [3] reported that inoculation of incidence of *pigeonpea and chickpea* f *seeds with Pseudomonas aeruginosa significantly reduced the Fusarium* wilt in naturally infested soil. Bajwa *et al.* (2004) tested five *Trichoderma* spp. (*T. viride, T.harzianum, T. koningii, T. aureoviride* and *T. pseudokoningi*) and three *Aspergillus* spp (*A. fumigates, A. giaucus* and *A. oryzae*) for their antagonistic potential against *Fusarium solani* causal agent of shisham (*Dalbergia sissoo)* wilt *in vitro.* Among the *Trichoderma* spp., *T. harzianum* showed the best antagonistic effect followed by *T. viride, T. aureoviride, T. koningii* and *T. pseudokoningii* resulting in 52.4, 24.0, 13.7, 9.0 and 2.0 % reduction in colony growth of test pathogenic fungus, respectively. Similarly, there was 23.0, 20.0 and 7.5 % reduction in radial growth of *Fusarium solani* due to antagonistic effects of *A. fumigates, A. glaucus* and *A. oryzae,* respectively. Kaushik *et al.* (2005) tested six biological agents e.g. *Trichoderma harzianum, T. viride, Gliocladium virens, Bacillus subtilis, B. ceres (B. cereus)* and *Pseudomonas fluorescens* against *Fusarium solani, F. moniliforme* and *Fusarium oxysporum* f.sp. *dalbergiae in vitro* and *in vivo* conditions. Results indicated that *G. virens* and *B. subtilis* were highly effective antagonists that significantly controlled the disease.

Mukhopadhayay (2005) reported that root colonization by *Trichoderma* spp. frequently enhanced root growth and development, crop productivity, resistance to a biotic stress and uptake and use of nutrients. Root fungus association also stimulated plant defensive mechanisms induction of resistance metabolism similar to the hypersensitive response (HR), systemic acquired resistance (SAR) and induced systemic resistance (ISR) in plants. Jayalakshmi *et al.* (2005) reported that all the isolates of *Trichoderma* in dual culture inhibited the mycelia growth of the wilt pathogen (*F. udum)* but the native isolate of *Trichoderma* inhibited maximum (84.92%) mycelia growth of *F. udum*. Bohra *et al.* (2005) studied the efficacy of different plant growth promoting rhizobacteria (PGPR) to control *Fusarium* wilt in the nursery. Seed treatment with *Pseudomonas* significantly reduced disease severity in Soybean as compared to control. Micro-organism resulted in changes in activities of Phenyl alanine ammonia lyase, chittnase and phenoloic contents of the pulse crop. Numerous rhizobacteria have been exploited as bio control agents (Siddiqui *et al*., 2005; Siddiqqui and Shakeel, 2007) [27]. Yadav *et al.* (2005b) [29] reported that inhibition of mycelia growth of *F. udum* due to *T. viride* was 14.6 and 70.5% as compared to *T. harzianum* (8.5 and 62.5%) at 4 and 7 days of incubation, respectively. Soil application of *T. viride* and *T. harzianum* showed disease control by 83.3 and 66.7% respectively at 30 and 60 days after sowing while 70.0 and 53.3% disease control were obtained by use of *T. viride* and *T. harzianum* respectively as seed treatment at 60 days of sowing. Soil treatment of bio agents was more effective in managing *F. udum* as compared to seed treatment. Jha and Jalali (2006) [15] evaluated the fungal isolates of pea rhizosphere for their biological control potential against *Fusarium solani* f. sp. *pisi* under *in vitro* study (dual culture method) and sick soil (pot) condition. Under *in vitro* study, isolate *Trichoderma viride* showed the strongest antagonistic activity towards *Fusarium solani* f.sp. *pisi* in dual culture followed by *Aspergillus niger, A. terreus, A. sydowii, A. flavus and Spicaria silvatica.* The two highly efficient antagonists, i.e. *T. viride and A. niger,* were studied individually and in combinations in relation to the biological control of pea root rot under pathogen sick soil (pot) condition. Both the antagonists controlled the disease more efficiently when used individually than those used in combinations. *T. viride and A. Niger* applied individually at 15 g/5 kg sick soil recorded the disease severity of 33.3 and 40% respectively, while the disease severity under untreated sick soil was 96.6%.

Sagar *et al.,* (2007) [25] evaluated nine biological control agents under *in vitro* conditions against *Pythium aphanidermatum* and *Fusarium solani* which cause rhizome rot of ginger (*Bacillus subtilis, Pseudomonas fluorescens, Pseudomonas fluorescens* (Sirsi isolate), *Trichoderma koningii, Trichoderma virens (Gliocladium virens), Trichoderma viride*, *Trichoderma harzianum, Trichoderma harzianum* (Sirsi isolate) and *Trichoderma harzianum* (Dharwad isolate). In *Pythium aphanidermatum*, the maximum inhibition of mycelial growth was noticed with *T. harzianum* (Sirsi isolate) (77.77%), which was *at par* with *T. harzianum* (Dharwad isolate) (76.40%). Among the nine biological control agents evaluated against *F. solani*, *T. harzianum* (Sirsi and Dharwad isolates) inhibited the maximum mycelial growth (78.51 and 76.29%), which was at par with *T. virens* (77.03%). Kumar and Upadhyay (2007) [20] reported that all the three species of *Trichoderma viride, T. harzianum* and *T. virens (Gliocladium virens)* inhibited the growth of *F. udum in vitro. T. viride* was found more potential in inhibiting the radial growth of *F. udum. T. virens* was more effective in inhibiting the growth of Fu-61. Minimum disease incidence of 26.4 % and maximum disease control of 56.0 % was observed in combined application of *T. harzianum* and *T. viride* as seed treatment and soil application followed by 54 % disease control in *T. harzianum* seed treatment and soil application. Chandal and Tomer (2008) reported *T. harzianum* to be superior over *T. viride* and *T. virens* in managing the wilt disease of carnation. Under field conditions all the treatments had a positive effect on the plant growth parameters. Arshad (2008) reported that *Trichoderma viride, T. harzianum, T. aureoviride, T. koningii, T. pseudokoningii, Aspergillus fumigates, A. Glacus* and *A. Oryzae* were effective in controlling *in vitro* the growth of the wilt pathogen (*Fusarium solani).* Gupta and Mishra (2009) [11] reported that *Aspergillus niger* was found superior in inhibiting growth of *Fusarium solani* in dual culture techniques. In field evaluation of bioagents, the best growth was recorded in plant treated with *Aspergillus niger* to control wilt in guava.

There have been extensive efforts to commercial *Trichoderma* products for managing plant diseases in the field in Viet Nam. *Trichoderma* product can be used in many ways *viz.* seed treatment, applied direct to the soil before planting and added to organic fertilizers (Ha, 2010). An attempt was made by Mallesh and Narendrappa (2010) [21] to manage *Fusarium solani* and *Rhizoctonia solani* in glasshouse and field conditions by using different microbial antagonists. Out of seven bio control agents tested under laboratory condition in dual culture, *Trichoderma viride* and *T. virens*, maximum inhibition of both the pathogens followed by *T. harzianum* and *T. hamatum*. Abdel Monaim (2011) [1] tested seven bio control agents, namely *Bacillus subtilis, B. megaterium, B. cereus*, *Trichoderma viride, T. harzianum*, *Aspergillus* sp., *Penicillium* sp. isolated from chickpea rhizosphere, for their antagonistic action against the tested pathogens. *B. subtilis* isolate BSM1, *B. megaterium* isolate TVM5, *T. viride* isolate TVM2 and *T. harzianum* isolate THM4 were the most antagonistic ones to the tested fungi *in vitro*, while the other isolates were moderate or weak antagonists. The most antagonistic isolates as well as the commercial biocide Rhizo-N were applied as seed treatment for controlling damping-off, root and/or stem rot diseases caused by the tested fungi under greenhouse conditions. The result showed that all tested antagonistic isolates were able to cause significant reduction in damping-off, root and/or stem rot diseases in chickpea plants. *T. viride* (isolate TVM2) and *B. megaterium* (isolate BMM5) proved to be the most effective isolates for controlling the diseases. Muneeb *et al*., 2011 isolated *Fusarium oxysporum* f. sp. *ciceri, F. solani* and *Rhizoctonia solani* from the wilted chickpea (*Cicer arietinum*) plants. *In vitro* evaluation of biological control agents revealed the superiority of *Trichoderma viride.*

Soil drench with bio-agents was evaluated by Abdel-Kader *et al*., (2012) [2] against root rot incidence of cucumber, cantaloupe, tomato and pepper in pot experiments. The pathogenic fungi, *Fusarium solani, F. oxysporum, Rhizoctonia solani, Sclerotium rolfsii, Sclerotinia sclerotiorum, S. minor, Macrophomina phaseolina, Alternaria solani* and *Pythium* sp. as well as bio-agents, *T. harzianum, T. viride, Bacillus subtilis* and *Pseudomonas fluorescens* were used in the study. The obtained results revealed the efficacy of bio-agents as soil drench against tested vegetables root diseases incidence at both pre-, and post-emergence growth stages comparing with untreated control. *T. harzianum* either alone or combined with chemicals were superior for reducing root rot disease for all tested vegetable plants followed by *B. subtilis* treatments.

Kumar (2012) [18] screened the bacterial and fungal isolates for their antagonism against *Fusarium solani*, an associated pathogen of root rot disease of mulberry. Screening revealed that eight bacterial and two fungal isolates were highly antagonistic to *F. solani*. Nine bacterial isolates and one fungal isolate showed moderately and the rest were less or not antagonistic to *F. solani*. All the bacteria which showed highly antagonistic reactions to *F. solani* were Gram negative except Bacillus sp. Of these, four bacterial species were identified as *Pseudomonas*, three *Bacillus* and one *Azotobacter*. The fungi were identified as *Trichoderma harzianum* and *Trichoderma virens.* Among the bacterial isolates, highest inhibition of pathogen growth was shown by *Pseudomonas* strain-2 (65.96%) and *Pseudomonas* strain-1 (48.26%) followed by *Bacillus* strain-2 (42.61%). Similarly, among fungi the highest inhibition of pathogen was shown by, *T. harzianum* (47.74%). However, *T. virens* (23.70%) showed less inhibition.

Different group of bio-control agent

General characteristics of *Trichoderma* spp.

Trichoderma viride: *Trichoderma viride* conidiophores and side branches are long and slender without sterile hyphal elongation, phialides not croweded, rather slender, colonies yellowish, bright, dull to dark green, floccose or with compact conidophore tufts. Conidia are roughened.

Trichoderma harzianum**:** Colononies growing rapidly with most isolate 6-9 cm in diameter after 5 days. Aerial mycelium floccose, white to grayish or rarely yellowish. Hyphae hyaline, smooth walled mostly 4-6 mm in diameter or submerged mycelium occasionally up to 12 mm in diameter. Conidiophores are highly branched with primary branches arising at nearly right angles usually in whorls of 2 or 3 phialides ampulliform or subglobose to ovoid, apex broadly rounded, base more narrowly rounded and surface is smooth walled.

Mechanism of Trichoderma spp. as a fungal antagonist

In-vitro by dual technique and *in-vivo* proved that *Trichoderma spp.* is strong antagonist against mostly soil born pathogen. *Trichoderma* may directly kill or suppressed the growth of pathogen by mycoparasitism and antibiosis. It may adversely affect the growth and development of the pathogen either by antibiosis or by competing for nutrient, space for oxygen. Indirectly, it may contribute by promoting plant growth resistance to biotic and a biotic stresses and changes in the nutritional status of the plant. Many soils borne fungal pathogen like, *Rhizoctonia, Sclerotinia, Sclerotium, Macrophomina* etc. form hard resting structure called sclerotia. These sclerotia play vital role in long term survival of these pathogen in soil. It is difficult to kill these sclerotia using fungicides. In general *Trichoderma virens* colonizes and kill these sclerotia, wheres as *Trichoderma harzianum* primarily attack hyphae.

General characteristics of *Gliocladium catenulatum*

Gliocladium catenulatum is a naturally-occurring saprophytic fungus which is widespread in the environment.The mode of action is reported to be by an enzymatic mechanism. There are no reports indicating that *Gliocladium catenulatum* produces any toxins or antibiotics of concern.

General characteristics of *Pseudomonas* spp.

Pseudomonas spp. is aerobic, gram-negative bacteria, ubiquitous bacteria in agricultural soils and has many traits that make them well suited as biocontrol agents of soilborne pathogens. Tremendous progress has been made in characterizing the process of root colonization by pseudomonads, the biotic and abiotic factors affecting colonization, bacterial traits and genes contributing to rhizosphere competence, and the mechanisms of pathogen suppression.

Mechanism of action

The traits related to biocontrol include the ability to rapidly utilize seed and root exudates, colonize and multiply in the rhizosphere and spermosphere environments and in the interior of the plant, produce a wide spectrum of bioactive metabolites (i.e., antibiotics, volatiles, siderophores, and growth-promoting substances), compete aggressively with other microorganisms; and adapt to environmental stresses.

General characteristics of *Bacillus* spp.

Bacillus strains are effective against a broad spectrum of plant pathogens and they can be used either as foliar application or root application before trasplanting. *Bacillus* genus is among the beneficial bacteria mostly exploited as biopesticides to control plant diseases but also nematodes. The most known species hosting biocontrol agents are *B. subtilis* and *B. amyloliquefasciens,* but also *B. firmus* and *B. pumilus*. They are Gram positive rod-shaped bacteria that can form a protective endospore that can tolerate extreme environmental conditions.

Streptomyces spp.

General characteristics of Streptomyces spp.

The active ingredients, *Streptomyces* spp. and *S. lydicus*, are ubiquitous and naturally-occurring
bacteria that are commonly found in soil environments.

Fig 1: Mash Multiplication of *Trichoderma viride*

Fig 2: Different strain of *Pseudomonas* fluoresces

Different mechanisms of biocontrol agent against pathogen

- As a Mycoparasitism
- As a antibiosis
- Bio-control agent produce a Metabolite subtance
- Competition with pathogen and bio-control agent

Ecology of antagonistic microbes

Ecology factors play vital role in the activity of bio-control agent.

- The distribution of fungal pathogens and their antagonists in the environment
- The optimum conditions in which bio-control microorganisms exert their suppressive capacities
- The response of native and introduced populations to different management practices
- The determinants factor of successful colonization and expression of bio-control traits
- The components and dynamics of plant host defense induction

Application of bio-control agent

Application of bio-control agent in a different form in soil and plant

1. Seeds treatment
2. Foliar spray with solvent in water
3. Soil application
4. Root dip methods
5. As a bio-fertilizers

Conclusion

Due to the serious environmental and health problems that wide spread use of chemical pesticides has created in the world, search for alternative safe methods is unavoidable. Bio-control agent frequently enhanced root growth and development, crop productivity, resistance to a biotic stress and uptake and use of nutrients. Root fungus association also stimulated plant defensive mechanisms induction of resistance metabolism similar to the hypersensitive response (HR), systemic acquired resistance (SAR) and induced systemic resistance (ISR) in plants. There is a growing demand for biologically based pest management practices. Recent surveys of both conventional and organic growers indicate an interest in using bio-control products suggesting that the market potential of bio-control products will increase in the future. Clearly, the future success of the biological control industry will depend on innovative business management, product marketing, extension education and research.

Future thrust in bio-control agent

- **Enhance the shelf life:** Greatest limiting factor is very short shelf 3-6 months.
- **Adaptability to survive in extreme environment:** Sensitiveness of pH factor and higher temperature is very important limiting factors restricting its application.
- **Compatibility with other substances and microbial agents:** Bio-control agent compatibility to another microorganism, organic amendment and plant extracts for the pathogen suppressed and cheeked the growth are need for integrated diseases management for sustainable agriculture.
- **Genetic variability:** Bio-control agents are directly affected to genetic variability.

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