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Trichoderma as an Efficient Biological Control Agent to Manage Disease in Organic Farms of Nepal

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Abstract

Disease management caused by fungi, bacteria, virus or nematodes in the organic farming system of Nepal is major concern. Different biological control methods are being implemented for disease control and higher yield in these farms, and one of the effective biological control agents (BCA) is Trichoderma spp. Therefore, in this paper, we are presenting brief outlook on Trichoderma species as a potential biological control agent against several pathogens infecting vegetable species, and cash crops of organic farms in Nepal.

1. Introduction

Use of chemical fertilizers and pesticides to meet up growing food demand has raised public concern on natural resource declination and environmental problems. Realizing the fact, agriculture development in many countries, shifted from mere increased production and productivity towards sustainable and environmental friendly production system, based on tomorrow's ecology is more important than today's economy. In the global context, the organic movement was started and widened in 1972 in Europe and USA (Raynolds. 2000).Individual countries have been implementing their own initiatives in this area at a range of social levels. Government of Nepal has instigated the term "Organic Farming" in the 10th five year plan, 2002-2007 (Commission, 2009).

In Nepal, commercial organic farming is gradually increasing in recent years due to its good nutrient value and high market value. According to the Nepal Fertilizer Use Baseline Study Volume 1, more than 90% farmhouse use chemical fertilizer whereas almost all farm household use certain amount of organic fertilizers as soil amendments. Since, cattle rearing are common in Nepal; every farm fields receive certain quantity of organic supplements in field. According to MoAC 2011, the organic farming in Nepal is growing by 20% annually. Government subsidy program for organic agriculture farming and organic fertilizer manufacturing has attracted more individuals in organic farming sector. At present, some organic products such as coffee, tea, honey, large cardamom, ginger etc are exported to international market (Tamang et al., 2011).

Even before institutionalization of "organic farming", Nepalese farmers raised maintained and flourished agricultural crops in mountainous regions in traditional way. Almost every farmer used to keep a few pair of goats and chickens, a pair of bullock, one

or two cows or buffaloes integrated with crop farming. International Federation of Organic Farming Movement has reported 1247 number of farms in Nepal, and the area under organic management is 1000 ha. Reports have shown that since 26% of total agricultural land of Nepal is free from the synthetic pesticides and fertilizers; the potential of transforming conventional farming to organic farming is high. In addition majority of farmers in the mountainous regions of Nepal do not use any chemicals. However, crop loss due to pests, diseases and nematodes are the major constrains in agriculture.

Plant diseases are of significantly greater concern in organic farming than in conventional farming as they are a greater threat without the use of chemical fertilizers and pesticides. To address this problem farmers in Nepal prepare bio-pesticides utilizing locally available plant material like mug-wort, *Justicia*, *Lantana*, red pepper, red chilli and neem plant, by extracting and fermenting local plants in bovine urine for several days called *Jholmal.* Microbial and biological control agents for plant diseases are being considered valid alternatives to synthetic pesticides. Microbial control agents an indispensable component of integrated pest management strategy which includes bacteria, fungi and viruses for the reduction of plant pathogens and pests. The development of fungi for the biocontrol of pests, weeds and diseases has received a significant amount of interest in recent years. Fungal biological control agents like *Trichoderma* can be considered a new milestone in organic farming as they are known to have several mechanisms of action like mycoparasitism, antibiotic production which allow them to control pathogens (Brimner & Boland. 2003) against wide range of plant pathogens. The decades of laboratory research has promoted for commercial application of *Trichoderma* worldwide. This has introduced more than 50 different commercial formulations comprising of fungi and bacteria.

2. *Trichoderma* as Biocontrol

Trichoderma spp. are soil borne saprophytic fungi specially found in temperate and tropical soil, forest humus layer, decomposing organic matter and agricultural soils. At in-vitro condition *Trichoderma* isolates shows rapid fungal colony growth, and stable morphological features can be obtained by point or streak inoculation on Potato Dextrose Agar (PDA), Malt Extract Agar (MEA), oatmeal or cornmeal, and incubation for 5 or a few more days at a temperature of 20–25°C (P.KUBICEK. 2002) as shown in Figure 1. They are reported circular as a ring, regular or irregular, powdery like dark green in the edge, white as cotton, powdery in colour. The reverse side of colonies are often uncolored, buff, yellow, amber or yellow-green and many species produce thick walled spores (chlamydospores) (Howell. 2003). A taxonomic approach to *Trichoderma* strains were first attempted by Rifai and Webster in Exeter (UK) who were

successful in defining nine species aggregates (P.KUBICEK. 2002)

The co-existence of biocontrol agent with plant in nature has been present since the emergence of agricultural evolution, though it took us a while to notice. Plants harboring microbes that secrete secondary metabolites were in a way destined to provide growth hormones and enhance transfer of minerals to rhizosphere in a synergistic manner. They are also believed to produce or release a variety of compounds that induce localized or systemic resistance responses.

The potential of *Trichoderma* species as biocontrol agents in plant disease was first recognized in the early 1930s (Weindling. 1932). *Trichoderma sp.* are conveyed as inexpensive biocontrol agent that lives in harmony with beneficial micro-organisms, combat plant pathogen and has moderate effect on soil balance. For instance *T. harzianum*, an effective biocontrol agent in both natural and controlled environments, do not accumulate in the food chain and so is considered safe to human health and wild life as well.

Rhizospheric green *Trichoderma* fungi are well known for their antagonism against wide variety of soil-phytopathogens including fungi, invertebrates, and bacteria. Akin to most fungal BCAs, *Trichoderma* spp. have been the focal point of many researchers for fungal based BCAs have gained wide acceptance next to bacteria because of their broader spectrum in terms of disease control and production yield (Brimner & Boland. 2003) ; (Verma et a. 2007). *Trichoderma spp.* alone or in combination with chemical adjuvant has been used in control of several plant diseases. They can be efficiently used as spores (especially conidia) during product formulation and field use as it is more tolerant to adverse environmental conditions compared to their mycelial and chlamydospore forms as microbial propagules (Yedidia et al., 2001). Various mechanisms have been proposed for *Trichoderma* which makes it potential biological control agent such as:

1. Mycoparasitism by cell-wall degrading enzymes

Trichoderma spp. parasitize peripheral pathogens and inhibit growth of pathogen (Benítez et al., 2004) by secretion of lytic, proteolytic enzymes, diffusible or volatile or secondary metabolites and ABC transporter membrane pumps that disrupts the host cell wall and cytoplasm (P.KUBICEK. 2002). Physiological activities of *Trichoderma* such as releasing the complex group of extracellular enzymes have been reported to be a key factor in pathogen cell wall lysis during mycoparasitism (Verma et al., 2007).

2. Production of antibiotics

Survival for existence or say, to complete ecological niche, *Trichoderma* secretes more than hundred metabolites with antibiotic activity. Secretion includes polyketides, pyrones, terpenes, metabolites derived from amino acids, and polypeptidesb. (Sivasithamparam and Ghisalberti, 1998).One of the first characterized

secondary metabolites of *Trichoderma* spp. was the peptide antibiotic paracelsin (Bruckner and Graf. 1983). Gliocladium (*Trichoderma*) virens (GV-P) produces gliotoxin that strongly inhibits *Pythium ultimum* and *Phytophthora* species.

3. Competition for the substrate, rhizosphere competence

Trichoderma spp. are successful colonizers of their habitat in rhizosphere reflected both by their efficient utilization of the substrate at hand as well as their secretion capability for antibiotic metabolites and enzymes. In-vitro experiment. they propagate readily along with the developing root system in rhizosphere by suppressing the growth of competitors simultaneously. They respond to their environment by regulation of growth, condition, enzyme production, and hence adjust their lifestyle to current conditions, which can be exploited for the benefit of mankind (Schmoll & Schuster. 2010) and is shown in Figure 2. This is dependent on their potential to defend their ecological niche and to thrive, and prosper despite the competition for nutrients, space and light.

4. Induction of defense response in plants.

Trichoderma species releases a variety of compounds that induce localized or systemic resistance responses. Association of *Trichoderma* in rhizosphere and lytic enzymes induced by plant defense system destroys pathogen cell wall which later becomes nutrient for plant (Benítez et al., 2004).

3. *Trichoderma* based products available in Nepal

Many Nepalese farmers are being aware about the use of biocontrol agents to combat agricultural pests in their farms. Chemical fungicides in combination to biopesticides are also been used to develop integrated approaches to diseases. Different organizations including community, government and private sectors are involved in production and research of *Trichoderma species* as shown in Table 1. In addition to this, various *Trichoderma* based products are being available in Nepalese market as shown in Figure 2

Use of *Trichoderma species* singly or in combination as Integrated Pest Management has been used in Nepalese agricultural fields as effective biocontrol agents against various commercial crops. The successful application of *Trichoderma spp.* to control soil borne fungal diseases was reported by farmers in Nepal as enlisted in Table 3. Among the various isolates of *Trichoderma* such as *T. viride*, and *T. harzianum*, are used against the management of various crop plants. Successful *In-vitro* and field test with *Trichoderma* based products against wide variety of plants have promoted government and private organizations for domestic market as enlisted in Table 1.

4. Conclusion and Recommendations

There has been increasing awareness in farmer's community to switch for organic farming in Nepal. Therefore, disease management problems would definitely arise in this system giving us challenges to control them. With regards to the researches reviewed, it can be concluded that *Trichoderma* as bio-control has been a most studied subject in world and has emerged as effective biological control agent in organic farming system. However, there lies a disconnection between the research and the implications in the real Nepalese farmlands. This may be due to lack of awareness in local farmers about its beneficial use and market reach of the bio-pesticides. Moreover, its use on natural conditions or agricultural fields does not show the effectiveness (disease suppression) to the extent it would in the greenhouse. We can recommend that the various GOs, NGOs and INGOs working on uplifting organic agriculture system of Nepal, should rather focus on conducting researches based on field trails followed by bridging the piled up technologies/strategies by conducting various educational activities. Additionally, disease management of organic farming system of Nepal could be obtained by commercial production and implementation of *Trichoderma* spp. in field infested with diseases. Therefore, *Trichoderma* spp. could be promising biological control agent to control different plant pathogens in organic farms of Nepal.

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Table 1 Names of organizations involved in production and research of Trichoderma spp. in Nepal

Name of Organization	Address	Name of Bio-pesticides	Sectors
Regional Plant protection Laboratory	Sundarpur, Kanchanpur	*Trichoderma viride*	Government
Community Resource Center	Kushadevi, Kavrepalanchowk	*Trichoderma viride*	Community
Community Resource Center	Banke	*Trichoderma viride*	Community
Community Resource Center	Kailali	*Trichoderma viride*	Community
Agricare Nepal Private Limited	Bharatpur-6, Chitwan	*Trichoderma viride*	Private
Praramva Biotech	Ramkot-6, Kalanki	*Trichoderma viride*	Private

Table 2 Trichoderma based products available in Nepalease market

S.N.	Trade Name	Common Name
1	Nirpot	*Trichoderma harzianum*

2	Bio Cure- F	*Trichoderma viride*
3	Nisarga	*Trichoderma viride*
4	Sanjeevni	*Trichoderma viride*
5	Carrier	*Trichoderma viride*
6	Nicoderma	*Trichoderma viride*
7	Astan-TV	*Trichoderma viride*
8	Bhoparistricho	*Trichoderma viride*

Source: Pesticide Registration & Management Section, 2071

Table 3 Use of Trichoderma species in Nepalese Organic farms

S.N.	*Trichoderma sp.*	Disease	Fruit/Vegetable	Location	In text citation
1	*Trichoderma harzianum*	Rhizome rot	Ginger	National Ginger Research Program (NGRP), Kapurkot Salyan	(Acharya & Regmi 2015)
2	*Trichoderma harzianum*	Black Scurf Disease (*Rhizoctonia solani*)	Potato	Mainapokhar, Bardiya	(Sharma & Kc 2007)
3	*Trichoderma harzianum*		Onion (Nasik-53 variety)	Banke	("Farmers' consultation workshop at project site of AgLEARN project | FORWARD Nepal", 2016)
4	*Trichoderma viride*	Tomato late blight	Tomato	Plant Pathology	(Shrestha & Ashley 2007)

		(*Phytophthora infestans*)		Division, Khumaltar	
5	*Trichoderma viride*	Clubroot disease (*Plasmodiophora brassicae* Woronin)	Cauliflower (Rami variety)	Palung and Bajrabarahi, Makawanpur	(Timila 2011)
6	*Trichoderma viride*	Botrytis gray mold (BGM)	Chickpea	Rampur and Tarahara	(Chaurasia & Joshi 2001)
7	*Trichoderma viride*	Stem rot (*Macrophomina phaseolina)*	Jute	Jute Research Program, Itahari	(Acharya & Prasad 2013)
8	*Trichoderma viride*	Stemphylium blight (*Stemphylium botryosum* Walr*)*	Lentil		(Subedi *et al*. 2015)

9	*Trichoderma harzianum*	Late Blight (*Phytophthora infestans*)	Tomato (Shrijana variety)	Bageswori and Sudal VDC, Bhaktapur	(Adhikari *et al.* 2015)
10	*Trichoderma viride*	Clubroot disease (*Plasmodiophora brassicae* Woronin)	Cauliflower and Cabbage	Kathmandu Valley and Palung/Daman area of the Makwanpur District	(Timila, Correll, & Duwadi, 2008)

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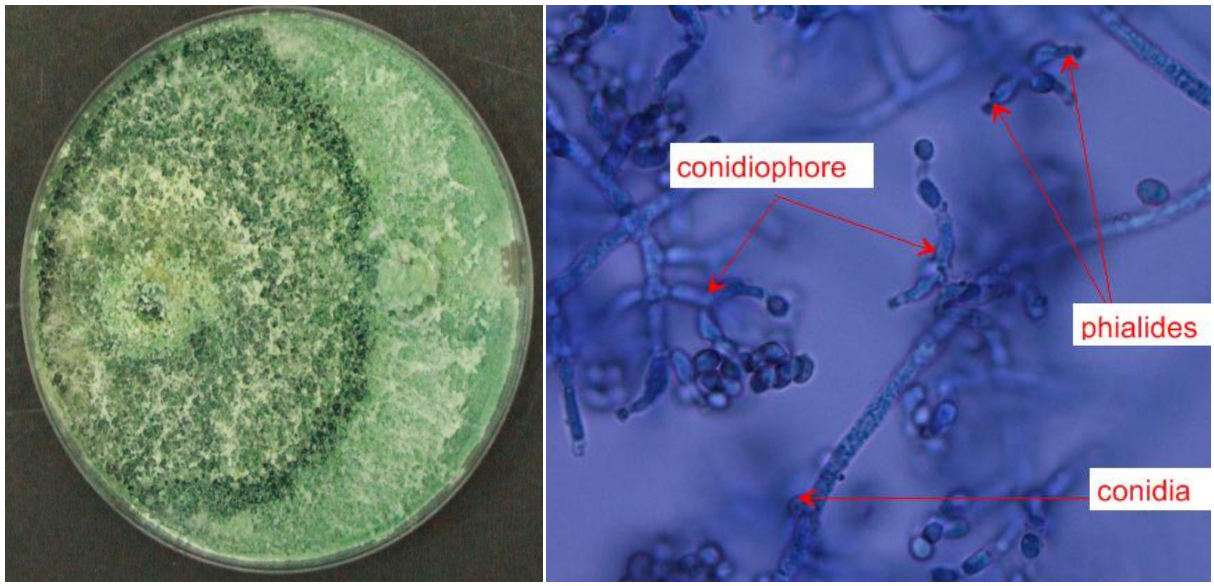

Figure 1 Morphology and microscopy of *Trichoderma sp.*

Figure 2 Overgrowth and growth inhibition of *Trichoderma* isolate against *Sclerotinia minor*.

Figure 3 Commercially available Trichoderma based products in Nepal.