

## In-vitro Evaluation of Bio-control agents against Soil Borne Plant Pathogens

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### ABSTRACT

Biocontrol is an important aspect of disease management for plant pathogens, especially for the soil borne fungi. Trichoderma species is the most exploited biocontrol agent in recent years. The soil specific nature of Trichoderma species is a well-known fact and hence native Trichoderma isolates should be more emphasized for control of plant pathogens. Fifty soil samples from rhizosphere of various agricultural crops were collected for isolation of Trichoderma sp. Ten isolates of Trichoderma were tested in dual culture with soil borne pathogens *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* in an in vitro assay. All of the test isolates were found to be significant in terms of mycelial inhibition growth as compared to control. However, varying degrees of antagonism by different Trichoderma isolates were observed for above mentioned soil borne pathogens. The isolate (T363) was found to exhibit more than 80% inhibition of *S. sclerotiorum* while the isolate T357 was found to control *F. solani* by more than 80%. For the control of *R. solani*, six of the tested Trichoderma isolates showed more than 80% inhibition of its radial colony growth. The Trichoderma isolates seen effective in this study need to be tested in pot and field experiments for exploiting the use and benefits of biocontrol.

**Keywords:** Biocontrol, Rhizosphere, Soil borne pathogens, Trichoderma

### सारांश

रासायनिक विषादीको अत्याधिक प्रयोगले मानव लगाएत अन्य विभिन्न क्षेत्रहरूमा देखापर्ने समस्या वारे चेतना बढ्दै जाँदा वाली रोग व्यवस्थापनको लागि जैविक नियन्त्रणको प्रयोगले अहिले धेरै महत्व बोकेको छ । ट्राइकोडर्मा हाल जैविक नियन्त्रणका लागि सबैभन्दा बढी प्रयोगमा आएको फाइदाजनक दुसी हा। ट्राइकोडर्माका विभिन्न प्रजातिहरू हुन्छन् र ती सबै एकै प्रकारको माटो वा बातावरणमा बाँच्न र फस्टाउन सक्दैनन् । त्यस कारण बाहिरबाट आयातित ट्राइकोडर्मा भएका जैविक विषादी नेपालको परिपेक्षमा प्रभावकारी नहुन सक्छन् । नेपालकै रैथाने र यहीं माटोमा भएको ट्राइकोडर्मा प्रयोगशालामा हेरी प्रभावकारी प्रजाति छान्ने उद्देश्यले यो परिक्षण गरिएको थियो । विभिन्न ठाउँहरूबाट माटोका नमुना संकलन गरी माटोबाट ट्राइकोडर्मा निकालियो र जम्मा १० वटा ट्राइकोडर्माका प्रजातिहरू लाई तीन विभिन्न माटोजनित रोगको जीवाणुहरूसंग जैविक दुसि र रोग कारक दुसि एकैसाथ वृद्धि गर्ने प्रविधि (ड्यूल कल्चर टेक्निक) बाट परिक्षण गरियो । ट्राइकोडर्मा को प्रजाति T ३६३ ले ८०% भन्दा बढिले स्क्लेरोटिनिया दुसीको वृद्धि रोक्यो भने अर्को प्रजाति T ३५७ ले पनि ८०% ले फ्युजारियम दुसीको वृद्धिलाई रोक्न सफल भयो । त्यस्तै राइजोक्टोनिया दुसी नियन्त्रणका लागि ६ वटा ट्राइकोडर्मा प्रजातिहरू प्रभावकारी देखिए । प्रयोगशालामा राम्रो पाइएका यी ट्राइकोडर्माका प्रजातिहरूलाई गमला तथा खेतवारीमा परिक्षण गरी जैविक नियन्त्रणको उपयोगिता र प्रयोगमा व्यापकता जरुरी छ ।

### INTRODUCTION

Plant pathogens are major constraints for increased crop yields. Among the pathogens, soil borne plant pathogens viz *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* are ubiquitous in nature and have wide host range. Biological control of soil borne pathogens is an environmentally sound method and avoids other threats posed by the use of chemicals such as health hazards and development of resistance

in pathogens (Kumar et al 2017). The antagonistic activity of *Trichoderma* was demonstrated more than eight decades ago (Weindling 1934) and today it is the most extensively used biocontrol agent (Mukherjee et al 2012). *Trichoderma* are free living microorganisms which are abundant in nature and can be isolated from soil, plant roots, decaying wood or other organic matter (Motlagh et al 2013). More than 100 species of *Trichoderma* have been recorded worldwide (Pandya et al 2011), many of which are potential biocontrol agents (Benitez et al 2004). *Trichoderma* are mainly used for the control of soil borne pathogens as these biocontrol agents inhabit soil (Ha 2010). Many commercial biopesticides with *Trichoderma* sp as are available in developed countries (Junaid et al 2013, Kumar et al 2017). However, it should be clearly understood that not all *Trichoderma* are effective in controlling pathogens and therefore it is essential to find out the *Trichoderma* with biocontrol potential which is possible through in vitro tests subsequently followed by pot and field experiments. Native *Trichoderma* can have significant biocontrol potential as they are adapted to the environment while exotic *Trichoderma* introduced to a new ecological niche can have problems of climatic adaptability and colonization in soils (Kamala and Devi 2012, Ommati and Zaker 2012). Therefore, this study was carried out to identify native *Trichoderma* isolates with biocontrol potential against the very common soil borne plant pathogens.

## MATERIALS AND METHODS

### Isolation of *Trichoderma* Isolates

Soils were collected from rhizosphere of various agricultural crops from different locations in the year of 2017 and plated on PDA media. Serial dilutions were followed for the isolation of *Trichoderma* from 50 soil samples. For each soil sample, three dilutions of  $10^1$ ,  $10^2$  and  $10^3$  were followed. Plates were incubated at 25°C until observation of *Trichoderma* colonies/mycelia and hyphal tip culture of the *Trichoderma* isolates was done on PDA plates to obtain their pure cultures. Altogether, ten *Trichoderma* isolates (*T. harzianum* and *T. viride*) were successfully isolated from 10 soil samples, the details of which are presented in Table 1.

**Table 1. Details of place of collection and the host plant for *Trichoderma* isolation**

<b>Trichoderma isolate</b>	<b>Host plant</b>	<b>Place of soil collection</b>	<b>GPS coordinates of the location</b>	<b>Elevation</b>
T153	Eggplant	Bhokrah, Sunsari	26.6140° N, 87.0924° E	352 masl
T154	Avocado	Bandipur, Tanahun	27.9170° N, 84.4068° E	1030 masl
T282	Rice	Naikap, Kathmandu	27.4227° N, 85.1805° E	1300 masl
T283	Rice	Manthali-1, Ramechhap	27.2318° N, 86.3473° E	1533 masl
T285	Chilli	Nala	27.6171°N, 85.5504°E	984 masl
T338	Sugarcane	Jitpur, Bara	27.1042° N, 84.4316° E	111 masl
T354	Garlic	Dang	27.9903°N, 82.3017°E	603 masl
T357	Soybean	Dakshinkali, Kathmandu	27.4227° N, 85.1805° E	1292 masl
T362	Broad leaf musard	Dhading besi, Dhading	27.9110° N, 84.8919° E	609 masl
T363	Ginger	Kapurkot, Salyan	28.2231°N, 82.9423°E	1530 masl

### Culture of Plant Pathogens

Three soil borne plant pathogens viz; *Fusarium solani*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* were isolated from diseased roots of tomato, broad leaf mustard and cauliflower respectively. For isolation of the pathogens diseased samples were cut into 1 cm pieces and surface sterilized in 1% sodium hypochlorite solution for 1 minute followed by rinsing with distilled water thrice. Samples were placed on blotting paper and dried inside laminar flow. Then they were transferred to water agar plates and incubated at 25°C. The growth of the pathogen was observed on second day of plating and the mycelia were transferred to PDA plates for pure culture of pathogens.

### Dual Culture of *Trichoderma* and Pathogens

The experiment was performed in November 2018 in the laboratory of Plant Pathology Division, Lalitpur. One week old cultures of *Trichoderma* isolates and plant pathogens were used for the study. Five millimeter disc of above mentioned soil borne pathogens and bio-control agents were placed apart on solidified PDA medium in an equal distance and incubated. Three plates for each treatment for each pathogen were used as three replicates and the experiment was set in completely randomized design (CRD). The plates inoculated with pathogens without *Trichoderma* isolates served as control. Radial colony growth of *Trichoderma* and pathogens was measured every day for a week. Percent inhibition of the pathogen growth was calculated by using the formula developed by Vincent 1947.

$$I(\%) = \frac{C-T}{C} \times 100$$

where, C= radial growth in control plate (without *Trichoderma*)

T= radial growth in *Trichoderma* inoculated plate

### Statistical Analysis

MSTATC was used for the analysis of variance (ANOVA) to test the significance of treatment effect on colony growth of different pathogens. Duncan's multiple range test (DMRT) was used to compare the values of significant treatment means at 1% level of significance.

## RESULTS

All of the *Trichoderma* isolates exhibited significant inhibition of the three pathogens as compared to control. However, the tested *Trichoderma* isolates differed in their abilities to suppress various pathogens. T363 isolate was found as best for inhibition of *S. sclerotiorum* (87.81%), while T357 proved to be most effective for *F. solani* control (84.76%). As for *R. solani*, six of the tested isolates (T153, T283, T285, T357, T362, and T363) exhibited more than 80% reduction in growth (Table 2). Some *Trichoderma* isolates were found to overgrow the pathogens completely after a week of inoculation.

**Table 2. Effect of *Trichoderma* isolates on radial colony growth of soil borne plant pathogens**

Treatment ( <i>Trichoderma</i> isolates)	<i>S. sclerotiorum</i>		<i>F. solani</i>		<i>R. solani</i>	
	Radial colony growth (cm)	Inhibition %	Radial colony growth (cm)	Inhibition %	Radial colony growth (cm)	Inhibition %
T153	4.05 (2.01)bc	42.1	5.31 (2.30)a	24.19	1.29 (1.13)d	81.55
T154	2.85 (1.68)f	59.24	2.27 (1.50)b	67.62	1.42 (1.19)d	79.76
T282	4.37 (2.09)b	37.52	2.96 (1.72)b	57.71	3.71 (1.92)c	47.02
T283	3.92 (1.97)bcd	44.00	2.16 (1.46)b	69.14	1.33 (1.15)d	80.95
T285	3.15 (1.77)ef	55.05	1.87 (1.36)bc	73.33	1.38 (1.17)d	80.36
T338	3.60 (1.89)cde	48.57	2.40 (1.54)b	65.71	4.25 (2.06)b	39.29
T354	2.93 (1.71)f	58.1	1.89 (1.37)bc	72.95	1.50 (1.22)d	78.57
T357	3.87 (1.96)bcd	44.76	1.07 (1.03)c	84.76	1.29 (1.13)d	81.55
T362	3.36 (1.83)def	52.00	1.84 (1.35)bc	73.71	1.29 (1.13)d	81.55
T363	0.85 (0.92)g	87.81	2.00 (1.41)bc	71.43	1.38 (1.17)d	80.36
Control	7.00 (2.64)a	0.00	7.00 (2.64)a	0.00	7.00 (2.64)a	0.00
LSD	0.02 (0.15)		0.11 (0.34)		0.006(0.07)	
P-value	***		***		***	
CV%	4.58		11.54		3.43	

Values are means of three replicates. For radial colony growth, figures in parenthesis represent square root transformed values. Means followed by the same letter in a column are not significantly different (DMRT,  $P=0.01$ ).

## DISCUSSION

Variation in the antagonistic activity by *Trichoderma* species has been explained by several authors (Bell et al 1982, Gveroska 2013, Hima and Beena 2016, Thavasvelvi et al 2016). The intensive development of

Trichoderma gives it a significant advantage in competition with pathogens for nutrients and space, besides the production of mycotoxins (Howell 2003). The mechanism of Trichoderma to control pathogens may be by attacking and binding the pathogenic organisms by sugar linkage and begins to secrete extracellular protease and lipase (Agrawal and Kotasthane 2012). Trichoderma sp grown over the pathogenic fungal hyphae coils around them and degrades the cell walls (Reddy et al 2014). This action of parasitism restricts the development and activity of pathogenic fungi (Asad et al 2014).

Reduced sclerotia formation of *S. sclerotiorum* and *R. solani* in dual culture with Trichoderma isolates has been observed by previous authors. Abdulla et al 2008 observed that local *T. harzianum* isolate reduced sclerotia formation of *S. sclerotiorum*. Seema and Devaki 2012 also found decreased sclerotia formation of *R. solani* by Trichoderma species in an in vitro assay. Sclerotia are the resistant structures of the aforementioned pathogens which serve as means of survival during unfavourable conditions. Hence reduction of sclerotia formation can have a significant effect on minimizing inoculum levels in fields (Agrios 2005). Ommati and Zaker 2012 reported biocontrol effect of *Trichoderma longibrachiatum* and *T. brevicompactum* against *Fusarium solani* in pot culture. Timila and Manandhar 2016 also found local *Trichoderma harzianum* isolate to control Phytophthora blight of pepper in field conditions. Similarly, efficacy of various Trichoderma species against different pathogens has been well documented (Ha 2010).

Various Trichoderma species have different ecological adaptability and hence it is wise to test native Trichoderma isolates for biocontrol of pathogens and exploit them in commercial formulations rather than to import biopesticides from other countries (Elad et al 1980). There are many evidences of Trichoderma being excellent antagonists against pathogens in laboratory and greenhouse conditions while they cannot perform well in fields (Glare et al 2012). In many cases, the exotic isolates cannot adapt to the new soil and ecology and hence cannot colonise soils. Therefore it is important to test the native isolates and try them in similar ecological niches. An in vitro screening facilitates for selection of effective isolates and hence eliminates the need to test a large number of Trichoderma isolates which would otherwise require a lot of space and time for field experimentations and applications both. The effective isolates identified from the present study can be used for further trial in pots or fields.

## CONCLUSION

The tested *Trichoderma* isolates (*T. harzianum* and *T. viride*) showed varying degrees of antagonism against the soil borne plant pathogens. This reveals the pathogen specificity of the *Trichoderma* isolates. The effective isolates concluded from the study should be tested for their efficacy in *in vivo* conditions. A close linkage between research and biopesticide companies is necessary so as to use the identified potential biocontrol agents in commercial formulations.

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