

In Vitro MICROBIAL CONTROL OF PATHOGENIC *Colletotrichum lindemuthianum* USING ANTAGONISTS

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ABSTRACT

In this study, pure cultures of 3 antagonist's fungi, *Trichoderma*, *Penicillium* and *Aspergillus* species and a fungal pathogen *Colletotrichum lindemuthianum* were obtained after inoculation on Potato Dextrose Agar (PDA) which was fortified with antibiotics to prevent bacterial contamination. Pathogenicity tests were carried out when the antagonistic isolates were inoculated on PDA. Of the three fungal antagonists evaluated for inhibitory efficacy, *Aspergillus niger* was the most effective antagonist as it exhibited the greatest inhibition to *C. lindemuthianum*. The result showed that the extent of inhibition by the fungi provides the use of potential antagonists capable of controlling the pathogenicity of *C. lindemuthianum* in crops for sustainable agriculture.

Keywords: Antagonists, Pathogen, microbial control, percentage inhibition.

INTRODUCTION

Microbial antagonists have caught the attention of scientists in recent times (Erkol et al, 2011). A biocontrol agent may act against pathogens by using one or more of the following mechanisms: competition, antibiosis and parasitism as well as activating host defense mechanisms (Papavizas, 1980). In fact several fungi have been reported to be effective bio-control agents of *R. solani* on potato.

Among these are species of *Gliocladium* (Van den Boogert, 1996), *Trichoderma* and *verticillium* (Turhan, 1990). *Chaetomium olivaceum*, *Cylindrocarpon destructans*, *Epiocum*, *nigrum*, *Fusarium culmorum*, *Fusarium moniliforme*, *Gliocladium viride* (Syn. *Gliocladium deliquescens*), *Gliocladium roseum*, *Penicillium cyclopium*, *Penicillium nigricans*, *Trichoderma harzianum*, and *Trichothecium roseum* were frequently isolated from *Sclerotia* of *R. solani* (Chand, et al., 1984).

Fungal, isolates such as *Alternaria*, *Aspergillus*, *Cladosporium*, *Coniothyrium*, *Curvularia*, *Gliocladium*, *Fusarium*, *Metarhizium*, *Penicillium*, *Phoma*, *Phytophthora* and *Trichoderma* genera were found to be effective bio-control agents (Feng, 2008).

Biological control of soil borne plant pathogens by the addition of antagonists microorganisms to the soil is a potential non-chemical means for plant disease management.

Microbial antagonists inhibit the growth of other microbes. The antagonists act with the aid of specific metabolites. These include production of antibiotics or parasitism or complete lysis of the pathogen (Campbell, 1989).

The conventional use of fungicides to stop their activity is being discouraged due to the harmful effect on the environment (Nwachukwu, 2003). This present study investigated the potentials of some antagonistic fungi in controlling the growth of *Colletotrichum lindemuthianum*.

Among the soil microorganisms, there are forms that inhibit the growth of other microbes. These are called antagonists (Campbell, 1989). They act with the aid of specific metabolites contained in their metabolic products. Substances formed by inhibitors are called toxins or phytoxins and substances produced by antagonists are called antibiotics.

The antagonists in biological control of plant pathogens include bacteria, actinomycetes, fungi, virus, higher plants and predatory microfauna such as protozoa, nematodes, rotifers, collembolan and mites (Baker and Cook, 1974). Antagonism on plant surface involves mainly competition, antibiosis and mycoparasitism (Cook and Baker, 1983). Each of these mechanisms has been demonstrated by careful observation and experimentation, sometimes with the use of electron microscopy, cyto-chemistry and in the case of antibiosis, molecular biology (Cook, 1991). These are referred to as resident antagonists, which preclude organisms taken from one environment and used for disease control in another environment (Baker and Cook, 1974).

Biological control is becoming a necessary component for safe and effective plant disease management. It has the potential to fill the gap created by the appearance of chemical pesticides (Campbell, 1989).

MATERIALS AND METHODS

The antagonists were obtained from the rhizosphere by serial dilution of the soil using potato Dextrose agar. The substrate Potato Dextrose agar (PDA) was the main substrate. It was sterilized in the autoclave at a temperature of 121°C (1.1kg/Cm² pressure) for 15 minutes. Pure cultures of the antagonist were maintained in pure cultures in agar slants. The antagonists were inoculated in agar plates. For the dixenic culture, a sterile cork was used to excise the antagonist and

placed at four equidistant points of the periphery of the plates (Singh, 1991) 5mm (five millimeter) agar disc of the *Colletotrichum* Sp. was placed in the middle of each petri-dish.

Zones of inhibition were noted while the size of each colony of the antagonists and *C. lindemuthianum* were noted. The size of the control cultures (without the antagonists) were noted. Percentage inhibition or efficacy of inhibition were measured using the method of Wokocha and Okereke (2005).

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

Where:

I = Inhibition of fungal growth

C = Growth in Control

T = Growth in treatment

Treatment means were compared using the Least Significant Difference (LSD) test at $P \leq 0.05$

RESULTS AND DISCUSSION

The result of Tables 1, 2 and 3 showed a high percentage of inhibition. The percentage inhibition for *Trichoderma harzianum*, *Penicillium notatum* and *Aspergillus niger* are presented in Tables 1-3.

The percentage inhibition for *T. harzianum* ranged from 41.37 to 60.58. For *Penicillium notatum*, percentage inhibition ranged from 17.00 to 49.23, while for *Aspergillus niger*, percentage inhibition was from 48.74% to 73.98. Of the three antagonists, *Aspergillus niger* had the highest percentage inhibition on *C. lindemuthianum*.

Recent work indicates that *Trichoderma* species may provide an effective controlling effect on some fungal pathogen (Elad et al., 1983).

The work agrees with the work of Bosah et al (2010) who controlled *S. rolfsii* using antagonist, *Trichoderma*, *Penicillium* and *Aspergillus niger*.

Table 1: Inhibitory effects of *Trichoderma harzianum* on the radial growth of *Colletotrichum lindemuthianum*

Treatment (Antagonist Vs Pathogen)	Day after Inoculation/Radial mycelia growth (cm)				
	2	3	4	5	6
<i>Trichoderma harzianum</i> (in <i>T. harzianum</i> Vs <i>C. lindemuthianum</i>)	1.39a	1.39a	2.91a	4.91a	8.00a
<i>C. lindemuthianum</i> (in <i>T. harzianum</i> Vs <i>C. lindemuthianum</i>)	0.51b	0.92b	1.00b	1.34b	2.31b
Control (<i>C. lindemuthianum</i> alone)	0.87b	1.83a	2.20a	3.40a	4.60b
LSD (0.05)	0.52	0.47	1.90	3.55	3.4
% Inhibition	41.37	49.72	54.54	60.58	49.78

Table 2: Inhibitory effects of *Aspergillus niger* on the radial growth of *Colletotrichum lindemuthianum*

Treatment (Antagonist Vs Pathogen)	Day after Inoculation/Radial mycelia growth (cm)				
	2	3	4	5	6
<i>Aspergillus niger</i> (in <i>A. niger</i> Vs <i>C. lindemuthianum</i>)	1.92a	2.71a	3.00a	6.28a	9.19a
<i>C. lindemuthianum</i> (in <i>A. niger</i> Vs <i>C. lindemuthianum</i>)	0.20b	0.45c	0.94c	1.47c	2.19c
Control (<i>C. lindemuthianum</i> alone)	0.58b	1.73b	2.42b	3.62b	4.25b
LSD (0.05)	1.32	0.90	0.72	2.64	4.92
% Inhibition	65.51	73.98	61.15	59.39	48.47

Table 3: Inhibitory effects of *Penicillium notatum* on the radial growth of *Colletotrichum lindemuthianum*

Treatment (Antagonist Vs Pathogen)	Day after Inoculation/Radial mycelia growth (cm)				
	2	3	4	5	6
<i>Penicillium notatum</i> (in <i>P. notatum</i> Vs <i>C. lindemuthianum</i>)	1.30a	1.81a	2.20a	3.31a	5.00a
<i>C. lindemuthianum</i> (in <i>P. notatum</i> Vs <i>C. lindemuthianum</i>)	0.33b	0.55b	0.83b	1.53c	2.84c

lindemuthianum)					
Control (C. lindemuthianum alone)	0.65b	1.81a	1.00b	2.11b	3.50b
LSD (0.05)	0.65	-1.25	1.35	1.1	1.4
% Inhibition	49.23	32.09	17.00	27.40	18.85

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