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Antagonistic Potentiality of *Trichoderma harzianum* Against *Cladosporium spherospermum, Aspergillus niger* and *Fusarium oxysporum*

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Abstract
Many species of genus *Trichoderma* are used as an important source of biological agents. The potential efficacy of *Trichoderma harzianum* against the pathogenic fungi like *Cladosporium spherospermum, Aspergillus niger* and *Fusarium oxysporum* was evaluated on the fungal growth by culture pattern in which radial growth extension rates of two categories of fungal colonies were analyzed. All the fungal species were isolated from the rhizosphere of *Juglans regia* L. and cultured on the separate sterilized potato dextrose agar (Hi Media). Antagonism of *T. harzianum* was observed when all the fungal isolates were grown on the same PDA petri-plate *in vitro* by using the dual culture techniques. *Trichoderma harzianum* had a discernible inhibitory effect on the growth of pathogens in dual culture. The mycelial growth of pathogenic isolates was noticeably constrained after a period of 10 days at the temperature of 25^0C and pH of 5.6. *T. harzianum* caused the maximum growth inhibition in *A. niger* (75%) followed by *C. spherospermum* (72.2%) and *F. oxysporum* (25%) at the specific temperature and pH, which justifies that *T. harzianum* is a promising biological agent for restricting the wilt and other fungal diseases.

Keywords; *Trichoderma harzianum*, pathogenic fungi, antagonism, radial growth

1. Introduction

In contemporary era, there has been a global swing to exploit eco-friendly methods for shielding the crops from pests and diseases (Rao *et al.,* 1998). Biological control of plant diseases particularly soil borne plant pathogens by microorganisms has been considered as environmentally acceptable alternative to the existing chemical treatment methods (Eziashi *et al.,* 2007).

Trichoderma is a prevalent filamentous imperfect fungi (Deutromycetes), found more or less in every soil. The distribution of genus *Trichoderma* is worldwide due to the high degree of ecological adaptability and survival shown by its strains under varied environmental conditions and substrates. Several *Trichoderma* species reduce the incidence of soil borne plant pathogenic fungi under natural conditions (Sivan and Chet, 1986; Calvet *et al*., 1990; Spiegel and Chet, 1998; Elad, 2000); nevertheless the effectiveness of this depends mainly on the physical, chemical and biological conditions of the soil. Antagonistic interactions have been recognized as one of the excellent mechanisms for biological control of pathogenic fungi (Khara and Hadwan, 1990). The antagonistic potentiality of *Trichoderma* species as bio control agents for plant diseases was first recognized in the early 1930^s (Howell, 2003). *Trichoderma harzianum* is an efficient bio-controlling agent commercially produced to thwart the development of several soil born pathogenic fungi (Shalini *et al.,* 2006).

Cladosporium spherospermum, Aspergillus niger and *Fusarium oxysporum* are considered as saprophytes which turn into plant pathogens under various stress conditions (Ellis, 1971; Despoulain *et al.,* 1990). They produce disquieting diseases in a number of indispensable fruit crops, viz., pear, grapes, cherry, resins and figs, besides damaging corn and rye. The pathogenic strain of *F. oxysporum* has been known to mankind since last 100 years. The host range of this strain is extremely broad, including animals, humans and plants (Nelson *et al.,* 1994) and is recurrently recognized for vascular wilt disease and affects a wide range of herbaceous plants. All these saprophytes are therefore, contributing significantly towards the heavy economic and productivity losses. Based on these specifics, soil fungi were isolated to scrutinize the antagonistic activity of *T. harzianum* against various pathogenic saprophytes *in vitro.*

2. Materials and Methods

To analyze the antagonistic activity of pathogenic fungi, isolates of *Trichoderma harzianum, Cladosporium spherospermum, Aspergillus niger* and *Fusarium oxysporum* were obtained from the rhizosphere of *Juglans regia* L. from the northern Kashmir, India, by serial dilution method to get more manageable results (Aneja, 2005). All the isolates were grown on sterilized standard PDA (potato dextrose agar) medium (Riker and Riker, 1936) at 25ºC in an incubator for 5 days in order to obtain juvenile colonies for the studies of antagonism. Dual culture testing (Skidmore and Dickinson, 1976) was followed wherein all the isolates were transferred to the PDA medium separately for the period of 5 days at 25^0C temperature. After the incubation period of 5 days, *T.*

harzianum was transferred to the center of PDA petri-plate (100mm × 15mm) and on the same day sterilized needle was utilized to transfer the spores of *C. spherospermum, A. niger* and *F. oxysporum* at a distance of 3cm away from the *T. harzianum* and 2cm away from the periphery of the petri-plate. Control was also maintained in which both the groups of fungi were grown independently on the sterilized PDA medium. The effect of *T. harzianum* against the pathogenic fungi was tested for 10 days until the fungi had achieved equilibrium, beyond which there was no further alteration in the mycelial growth. The assessment was made for both the groups of organisms and soon after the radial growth of fungal colonies was measured by digital milimetric paquimeter. The percentage inhibition of growth was calculated as follows:

Percentage inhibition of growth = $r\text{-}r_1/r\times100$ (Behzad *et al.,* 2008).

Where r = radial growth of fungus measured from the centre of the colony towards the centre of the petri-plate without antagonistic fungus.

r_1 = radial growth of fungus measured from the centre of the colony towards the antagonistic fungus in the centre of the petri-plate.

The interaction between the test pathogens and the antagonistic fungi was assessed following the model proposed by Porter (1924) and Dickinson and Broadman (1971). Five types of interaction grades as proposed by Skidmore and Dickinson (1976) have been used.

The types are as follows:

1. Mutual intermingling without any macroscopic sights of interaction– Grade 1.
2. Mutual intermingling growth, where the growth of fungus is ceased by the growth of opposed fungus– Grade2
3. Intermingling growth, where the fungus under observation is growing on the opposed fungus either above or below– Grade 3.
4. Sight inhibition of both the interacting fungi with narrow delineation line– Grade 4.
5. Mutual inhibition of growth at a distance of >2mm– Grade 5.

3. Results and Discussion

High cost associated with the use of fungicides to control the diseases caused by soil borne fungi is a restraining factor in the profitability of crop production. Biological control may well be the best alternative against the soil borne pathogens. In this study, the petri-plate containing two types of fungal isolates illustrates the exemplary antagonistic effect of *T. harzianum*. The petri-plate having *T. harzianum* colony in the center along with *C. spherospermum, A. niger* and *F. oxysporum* in the vicinity was observed for 10 days. *Trichoderma* species grew considerably faster on PDA than other isolates in the similar conditions of culture (temperature and pH). *T. harzianum* when observed for longer periods produced inhibition halos and sporulated over the colonies of *C. spherospermum, A. niger* and *F. oxysporum.*

The types of interaction depicted in 'Table 1' between the *T. harzianum* and soil pathogens like *C. spherospermum, A. niger and F. oxysporum* was observed and the results are as follows:

1. Mutual intermingling without any macroscopic sights of interaction between the *F. oxysporum* and *T. harzianum*.
2. Mutual intermingling growth, where the growth of *C. spherospermum* was ceased by *T. harzianum*.
3. Intermingling growth, where *T. harzianum* attacks the *A. niger*.

The radial growth of pathogenic fungi in the control and test petri-plate is shown in 'Table 2' and 'Fig. 1'. *F. oxysporum* shows 20mm radial growth extension in control and 15mm towards the antagonistic fungus. *C. spherospermum* shows 18mm radial growth in the control and 5mm towards the antagonistic fungus. In addition, *A. niger* shows 40mm radial growth extension in the control and 10mm towards the antagonistic fungus during the screening period of 10 days under the same culture conditions. *T. harzianum* induced the maximum inhibition of growth in *A. niger* (75%) followed by *C. spherospermum* (72.2%) and *F. oxysporum* (25%). This disparity exhibited by pathogenic fungi may be due to the genetic potentialities to tolerate a particular antibiotic substance with inimitable chemical properties.

As revealed in 'Plate 1' the presence of *T. harzianum* affects the growth of all the adjoining fungal isolates. The halos formed around the colonies of both the groups of fungi indicate a boundary which cannot be traversed by the two groups in question. The formation of halos is an indicator of production of antibiotic substances either by the pathogenic fungi to prevent itself from the attack of antagonistic fungus or vice versa. Earlier the effect of volatile metabolites from *Trichoderma* species against *C. spherospermum, A. niger* and *F. oxysporum* was tested in the assemblage described by Dennis and Webster (1971a). It is imperative to mention that *Trichoderma* species are renowned to produce a range of antibiotics such as trichodernin, trichodermol and harzianolide (Dennis and Webster, 1971b; Howell, 2003; Kucuk and Kivanc, 2004) in addition to some cell wall degrading enzymes such as glucanase and chitinase which break down the polysaccharides and chitins, thereby obliterating the cell wall integrity (Elad *et al.* 1983; Elad, 2000). The plausible mechanism of antagonism employed by *Trichoderma* species include nutrient and niche competition, antibiosis by producing volatile components and non-volatile antibiotics (Harman and Hadar, 1983; Dennis and Webster, 1971b; Behzad *et al.,* 2008) that are

inhibitory against a range of soil borne fungi, over and above parasitism (Dennis and Webster, 1971a). It is commonly acknowledged that environmental parameters such as soil type, soil temperature, soil pH, water potential and biotic factors like plant species, microbial activity of the soil as well as other factors such as method and timing of applications may have substantial impact on the biological control efficacy of *Trichoderma* species (Fravel, 1988; Behzad *et al.,* 2008). *T. harzianum* was capable of influencing the growth of all tested pathogens in dual culture and as a result may well be used as a broad spectrum bio- controlling agent for curbing various fungal diseases.

4. Conclusion

Trichoderma harzianum is the promising bio-controlling agent commercially produced to avert the development of several soil born pathogenic fungi. *In vitro* potential of *Trichoderma harzianum* was evaluated against soil borne phytopathogenic fungi by dual culture techniques and was found to be more efficient in influencing the growth of all tested pathogens through the production of volatile and non-volatile inhibitors under controlled conditions with marked inhibitory effect on mycelial growth of the pathogens. There is an apparent indication that the fungi differed in their growth rates, however each species kept its characteristic existence intact. The presence of antagonistic fungus, *T. harzianum*, in the soil thus seems to be exclusive in nature for keeping the population of other detrimental fungi under check.

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Table 1. Pathogenic fungi showing the type of interactions in grading system with *T. harzianum*

Pathogenic soil fungi	Type of interaction with *T. harzianum* (Grade)
Fusarium oxysporum	Grade 1
Cladosporium spherospermum	Grade 2
Aspergillus niger	Grade3

Table 2. Pathogenic fungi showing the radial growth extension and percentage inhibition by *T. harzianum*

Pathogenic fungi	Radial growth in control (r)	Radial growth in the test petri-plate (r_1)	Growth inhibition (%)
Fusarium oxysporum	20mm	15mm	25
Cladosporium spherospermum	18mm	5mm	72.2
Aspergillus niger	40mm	10mm	75

Plate 1. *In vitro* antagonistic study between *T. harzianum* (T), *A. niger* (N), *C. spherospermum* (C) and *F. oxysporum* (F).

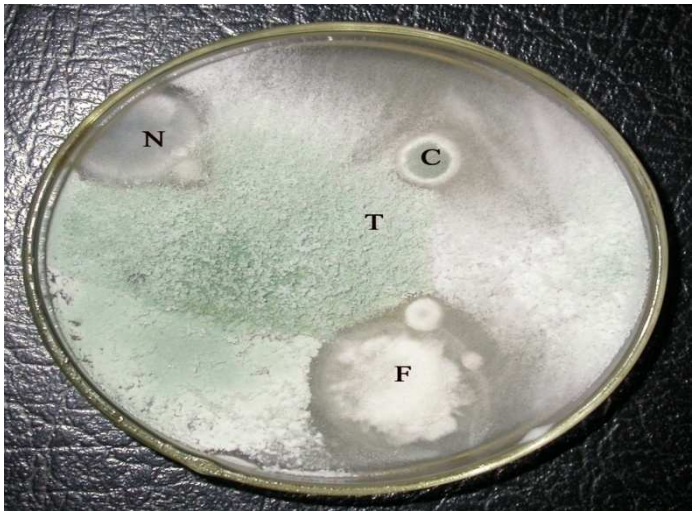

Figure 1. Radial growth extension shown by the pathogenic fungi in control (without antagonistic fungus, r) and test petri-plate having antagonistic fungus (r_1).