



Research Article

Evaluation of *Trichoderma harzianum* as a potential bio-control option for fungal root disease of Seabuckthorn

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Abstract: Biocontrol involves harnessing disease suppressive micro-organisms to improve plant health. Owing to the shortage of available fungicides for economic management of soil borne diseases, alternative techniques such as biological control are increasingly being sought for disease management. The present work was carried out to study the potential of *Trichoderma harzianum* isolate screened from the rhizospheric soil of seabuckthorn growing areas in Uttarakhand. Antagonistic activity of isolate was observed against *Fusarium oxysporum* causing damping off disease in seabuckthorn. Under *in vitro* conditions *Trichoderma harzianum* significantly reduced the growth of pathogenic *Fusarium oxysporum*.

Keywords: Antagonistic, disease, *Fusarium*, isolate, rhizosphere, soil

Introduction

The northern part of India harbours a great diversity of medicinal plants because of the majestic Himalayan range. So far about 8000 species of angiosperms, 44 species of gymnosperms and 600 species of pteridophytes have been reported in the Indian Himalaya, of these 1748 species are known as medicinal plants (Kala and Mathur, 2002). The World Health Organization (WHO) estimated that 80% of the population of developing countries relies on traditional medicines, mostly plant drugs, for their primary health care needs (Sekar and Kandavel, 2010). Seabuckthorn is one of the main plants collected and exploited unscientifically from Uttarakhand due to its use as food, medicines and cosmetics. It is also known as “wonder plant” due to its multifarious benefits.

Seabuckthorn is also affected by many soil borne pathogenic fungi which effects its production and yield. Soil borne pathogenic fungi are responsible for numerous economically important root diseases. Root rot diseases are more difficult to manage than many foliar diseases because they often are not detected before substantial damage has already occurred (Harveson & Rush, 2002; Harveson *et al.*, 2002a). Phytopathogenic microorganisms have a great impact on crop yields and can significantly reduce plant performance and crop quality. They are serious threat to crop production and ecosystem stability. Different methods have been used to control plant pathogens, being the most used cultural practices, resistant cultivars, chemical and biological control. A major factor influencing plant growth and health is the microbial population living both in the rhizosphere and as endophytes within healthy plant tissue. Even though, some success has

been achieved in controlling crop pathogens using bio-control options. Biological control agents support plant health via the suppression of plant pathogens (Dalal and Kulkarni, 2013). Biological control is a natural and specific way to control pathogens and enhance crop yield by growth promoting attribute of environment friendly microorganisms.

The present study aims to evaluate the role of rhizosphere associated fungus *Trichoderma harzianum* as a biocontrol option for fungal root disease i.e. damping off in seabuckthorn. There is an urgent need to develop effective and more consistent biocontrol agents in order to increase survival and growth of medicinal plants under field conditions. Though much information is available on activity of soil micro organisms and plant growth promotion for annual crops, very limited information is available in respect of biocontrol agents associated with rhizosphere of medicinal plants such as seabuckthorn.

Materials and Methods

Isolation and Identification of pathogen

Diseased samples were collected from different locations of Chamoli district in Uttarakhand. Diseased roots of *Hippophae salicifolia* were taken into the laboratory and washed thoroughly with running tap water to remove the surface dirt. The roots were cut into small pieces using sterile scalpel blades and kept in sterile petri dishes after surface sterilizing with 0.1 percent mercuric chloride for about one minute followed by two changes of sterile water. Further isolation of the fungus was done by performing moist chamber incubation

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method (Shutleff and Averre, 1997). These surface sterilized pieces were then placed between blotting papers and aseptically inoculated onto petridishes containing Potato Dextrose Agar media. The plates were incubated at $25\pm 2^{\circ}\text{C}$ for 5 to 6 days, and the growth of fungal colonies were recorded every day. Fungal colonies were isolated after 5-6 days and pure cultures were transferred to Potato Dextrose Agar slant. The mycelia and spore characters of the fungi were studied under microscope. Fungal isolates were identified on the basis of cultural, morphological and microscopic characteristics viz. Mycelium, sporangiophore, spore bearing organ, spore structure etc. and were identified following Barnett and Hunter (1972) and Gilmann (1967). Pure culture of *Fusarium oxysporum* was stored at 5°C in culture tubes and petri plates containing PDA.

Isolation of rhizosphere associated fungal isolate:

Rhizosphere soil samples of seabuckthorn were collected from some selected sites of district Chamoli in Uttarakhand. Soil samples were collected in sterile polythene bags. The isolation of rhizosphere fungi from these was done by dilution plate method of Wakesman (1927) and Warcup (1950). This method includes shaking of 1 g soil in 100 ml of distilled water followed by further dilutions under aseptic conditions. Various dilutions were cultured on Potato Dextrose Agar medium and Czapek's Dox medium (Raper and Thom, 1949). The colonies with characteristic growth of *Trichoderma* spp were observed under the microscope and growth from such colonies was sub cultured on agar slants and the fungi were identified following Barnett and Hunter (1972) and Gilmann (1967).

In vitro dual culture assay:

Dual culture method (Rao, 2003) was employed to evaluate the antagonistic activity of *T. harzianum*. Four mm diameter mycelial disc from the periphery of 10 day old culture of bioagent was placed on the petri plate at four locations, approximately 3cm from the center. A loopful of actively growing *Fusarium oxysporum* isolate (Plate 2) was placed opposite to *T.harzianum* isolate on the plate. Plates inoculated with *Fusarium oxysporum* but not with *T. harzianum* were used as control. All *in vitro* tests of antagonism were performed three times. All plates were incubated at 27°C for 7 days. Visual observations of growth inhibition were recorded every two days and the final measurements were recorded at the 7th day for incubation. Degree of antagonism was determined by measuring the radial growth of pathogen, (radial mycelia growth reduction) *Fusarium oxysporum* by *Trichoderma harzianum* in dual culture in relation to growth of the control. Three replications were maintained for each treatment. Percentage inhibition of pathogen was calculated by the following formula (Fokkema, 1973). $R1-R2/R1 \times 100$, where R2 denotes the radial growth of the pathogen towards the opponent antagonist and R1 denotes the radial growth of the pathogen towards opposite side.

Results and Discussion

Isolation of *Trichoderma harzianum*

A number of colonies were observed in PDA plate after 3-7 days when serially diluted samples were placed on PDA media. Colony that produced green colour conidia was picked, observed under microscope by staining with lactophenol cotton blue stain. The microscopic analysis of the mycelium with spore revealed that the isolate was *Trichoderma harzianum* (Plate 1). The isolate was sub cultured and stored in PDA slants at -20°C .

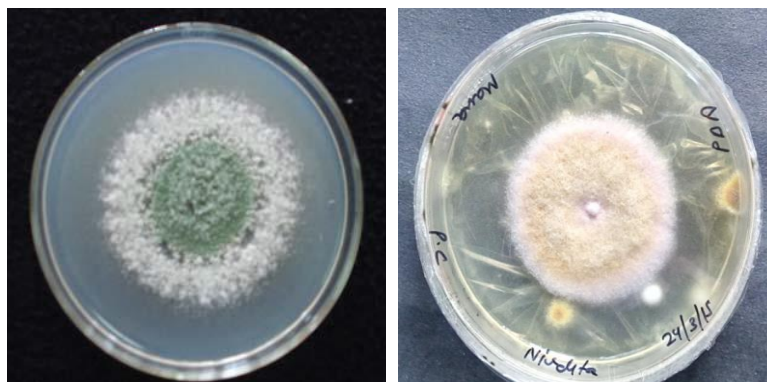


Plate 1. Colony and microscopic morphology of *T.harzianum*

Plate 2. Colony and microscopic morphology of *F.oxysporum*

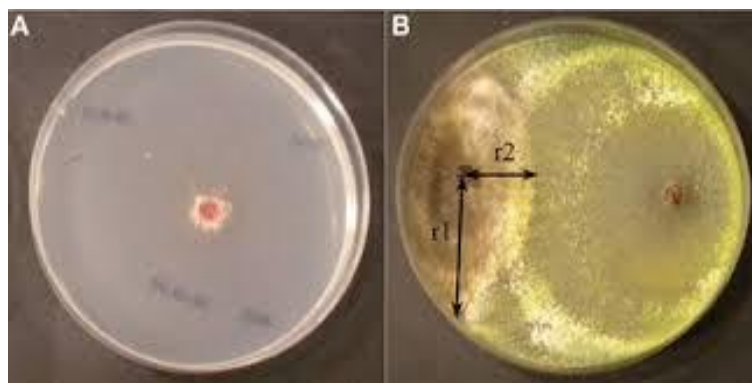


Plate 3. Antagonistic potential of *T. harzianum* against *Fusarium oxysporum*
a) Control b) Dual Culture

Trichoderma harzianum was evaluated for its antagonistic activity against *Fusarium oxysporum* in Petri dishes containing PDA medium. Results showed that *T. harzianum* inhibited the growth of pathogen. Growth inhibition in the pathogen differed significantly. Seven days of incubation showed different degrees of mycelia growth inhibition of *F.oxysporum* (Plate 3). Initially very less inhibition was observed near the intermingled contact zone of *F.oxysporum* and *T.harzianum*. However, after 4 days of incubation there was suppression of growth on antagonized portion of hyphae. *T.harzianum* completely overgrew the pathogen with percentage inhibition of 70%.

The genus, *Trichoderma* is ubiquitous which has been extensively studied for their biological control of plant diseases. Strains of *Trichoderma* are strong opportunistic invaders fast growing, prolific producers of spores and powerful antibiotic producers. The antagonistic activity of *Trichoderma* species depends on multiple synergistic mechanisms. According to Papavizas and Lumsden (1982); Devaki *et al.*, (1992), the mechanisms involved in the control of pathogens by *Trichoderma* spp. are probably due to antibiosis, lysis, competition and mycoparasitism. The species of *Trichoderma* have shown efficiency in the biological control of many foliar diseases. The *Trichoderma* may suppress the growth of the pathogen population in the rhizosphere through competition and thus reduce disease development. It produces antibiotics and toxins such as trichothecin and a sesquiterpine, Trichodermin, which have a direct effect on other organisms. The antagonist (*Trichoderma*) hyphae either grow along the host hyphae or coil around it and secrete different lytic enzymes such as chitinase, glucanase and pectinase that are involved in the process of mycoparasitism.

Conclusion

Treatment of valuable plant wealth is possible through the use of various pesticides, insecticides and other chemicals. The intensive use of fungicides for the control of diseases has resulted in the accumulation of toxic chemicals which are

hazardous to human beings and to the environment. Chemical treatments are not ecofriendly and, therefore, effective bio-control mechanisms have some hope to treat such plants in a healthy vision. The residual chemical pesticides on various parts also seriously affect the marketing potential of medicinal plants and their formulations. The increasing awareness of fungicide related hazards has emphasized the need for adopting a safer biological method but unfortunately very little work has been done to search for microbial antagonists which could serve as biocontrol agents against *Hippophae salicifolia* diseases. For this purpose *Trichoderma harzianum* was screened out for its antagonistic activity against *Fusarium oxysporum*. Fortunately, it proved to be a success acting as strong antagonist against the pathogen.


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