In vitro Antagonism of Trichoderma viride against Fusarium oxysporum strains

Pakkala Abhiram and Harison Masih

Abstract

Fusarium oxysporum is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, causes major economic losses by inducing necrosis and wilting symptoms. Trichoderma viride tested against Fusarium oxysporum strains under in vitro conditions. The results revealed that Trichoderma viride showed maximum inhibition 71.00% over Fusarium oxysporum strain (E) and minimum inhibition 62.50% over Fusarium oxysporum strain (D) in dual culture plate technique. Trichoderma viride showed maximum inhibition 45.27% over Fusarium oxysporum strain (E) and minimum inhibition 14.72% over Fusarium oxysporum strain (C) in sealing agar plate method. It is concluded that the T. viride has found to be a potential biocontrol agent against Fusarium oxysporum phytopathogenic strains. It may be therefore a promising ecofriendly bio controlling sources and cost effective for the safe agricultural practices as well as to farmers.

Keywords: Fusarium oxysporum, Trichoderma viride, inhibition, dual culture plate technique, sealing agar plate method

Introduction

Fusarium oxysporum is a soil borne fungal pathogen that attacks plants through roots at all stages of plant growth, causes major economic losses by inducing necrosis and wilting symptoms in many crop plants with a great overall impact on productivity. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and root rot minimal or absent crop yield. Fusarium oxysporum found in its many pathogenic forms, is the most damaging species of the genus where in plants are concerned. Recently a number of new disease reports on fusarium have been submitted to the literature pool on agricultural research (Bokhariand Perveen 2012) [2] Fusarium oxysporum is the causal agent of vascular wilt, a disease that affects a large variety of economically important crops worldwide (Ortoneda et al., 2004) [10]. Identification of Fusarium species by its morphology is notoriously difficult. Especially its conidiogenesis can be easily changed by environment particularly in the composition of the culture medium. Generally the appearance of a fungal culture which results from its metabolism is regulated by pH in association with the nitrogen source in the medium (Kwasna and Bateman 2005) [7]. Trichoderma is a filamentous fungus which has attracted the attention because of their multi prong action against various plant pathogens (Harmam et al., 2004). Several modes of action have been proposed to explain the biocontrol of plant pathogens by Trichoderma, these include production of antibiotic and cell wall degrading enzymes, competition for key nutrients, parasitism, stimulation of plant defense mechanisms and combination of those possibilities Trichoderma spp. generally grows in its natural habitat on plant root surface and therefore it controls root diseases in particular.

Many pathogenic microorganisms have developed resistance against chemical fungicides. This seriously hinders the management of diseases of crops and agricultural plants. Considering the deleterious effects of synthetic fungicides on life supporting systems, there is an urgent need for alternative agents for the management of pathogenic microorganisms. Biological control is still in its research phase with few studies reported for bacterial wilt (Messiha et al, 2007) [8]. Disease control is currently based on heavy uses of many neuro toxic fungicides, which are damaging the environment and /or pose a threat to public health via food residues, ground water contamination or accidental exposure. The problem caused by fungicides and their residues have amplified the need for effective, biodegradable fungicides with greater selectivity alternative strategies have included the investigation for new type of fungicides, and the re-evaluation and use of bio control agents for disease control.
Materials and Methods
Collection of pathogenic and antagonistic microorganisms
The Bio-control agent used in this study i.e. Trichoderma viride and five strains of pathogenic Fusarium oxysporum were obtained from microbial culture collection bank (MCCB), Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering.

List of Fusarium oxysporum strains

<table>
<thead>
<tr>
<th>Fusarium oxysporum strains</th>
<th>Source of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>MCCB 0068</td>
<td>From soil (A)</td>
</tr>
<tr>
<td>MCCB 0356</td>
<td>From rhizospheric soil of chickpea (B)</td>
</tr>
<tr>
<td>MCCB 0364</td>
<td>From rhizospheric soil of lentil crop (C)</td>
</tr>
<tr>
<td>MCCB 0412</td>
<td>From rhizospheric soil of moong bean (D)</td>
</tr>
<tr>
<td>MCCB 0455</td>
<td>From rhizospheric soil of chickpea (E)</td>
</tr>
</tbody>
</table>

In vitro evaluation of Trichoderma viride against Fusarium oxysporum strains by dual culture plate technique
The above mentioned fungal bio-agent was evaluated in vitro for their antagonistic effect against F. oxysporum by dual culture technique (Dennis and Webster, 1971a) [4, 5] on PDA medium.

15ml of PDA medium was poured into sterile Petri plate and allowed for solidification. Seven days old 5 mm disc of F. oxysporum strains were cut with a sterile cork borer and placed near the periphery on one side of PDA plate. A plate without antagonist was maintained as control. The inoculated plates were incubated at 28 °C for seven days. Each treatment was replicated.

The antagonistic activity of Trichoderma viride was screened in vitro against Fusarium oxysporum spp. by dual culture plate technique. The antagonistic efficacy against test pathogens was evaluated on PDA medium. Both pathogen and antagonists were grown on sterilized PDA plates separately for 7 days. For testing antagonism in dual culture method, a mycelial disk of 5 mm in diameter of antagonist was excised from the edge of an actively growing 7 day old culture plate and inoculated opposite to the pathogenic fungi in the same plate 1cm away from the edge inoculated similarly. For each treatment two replicates were maintained and incubated at 26 ± 2°C. The test pathogen was inoculated in the middle of the plate in duplicates these paired cultures of antagonist and test pathogen were placed equidistant from the periphery so that they would get equal opportunity for their growth. After the incubation period, the radial growth of Fusarium oxysporum strains. In control, as well as in treatment plate was measured and the per cent inhibition was calculated using the formula (Rehman et al., 2013) [9].

\[
I = \frac{(C - T)}{C} \times 100
\]

Where,

\( I \) = per cent inhibition
\( C \) = Growth of the pathogen in control plate (mm)
\( T \) = Growth of the pathogen in sealed plate (mm)

Results and Discussion
In vitro evaluation of Trichoderma viride against Fusarium oxysporum strains by dual culture plate technique.
The antagonistic activity of Trichoderma viride was screened in vitro against Fusarium oxysporum strains by dual culture plate technique on PDA media for 7 days. Trichoderma viride tested against five strains. The results revealed Trichoderma viride was shown maximum inhibition 71.00% over Fusarium oxysporum strain (E) and minimum inhibition 62.50% over Fusarium oxysporum strain (D) (Mean = 66.92%). (Plate 1), (Table 1).

![Plate 1: Antagonistic efficacy of Trichoderma viride against Fusarium oxysporum strains by dual culture plate technique](image)

Fig 1: Treatment of strain A with Trichoderma viride, Fig 2: Treatment of strain B with Trichoderma viride, Fig 3: Treatment of strain C with Trichoderma viride, Fig 4: Treatment of strain D with Trichoderma viride, Fig 5: Treatment of strain E with Trichoderma viride,
In vitro evaluation of Trichoderma viride against Fusarium oxysporum strains by sealing agar plate method.

The antagonistic activity of Trichoderma viride was screened in vitro against Fusarium oxysporum strains by Trichoderma viride on PDA media for 8 days incubation. Trichoderma viride was tested against five strains of Fusarium oxysporum by sealing agar plate technique. The results of Trichoderma viride showed maximum inhibition 45.27% over Fusarium oxysporum strain (E) and minimum inhibition 14.72% over Fusarium oxysporum strain (C) (Mean = 33.21%). (Plate 2), (Table 2).

Table 1: The effect of Trichoderma viride on mycelial growth (mm) of Fusarium oxysporum strains by dual culture plate technique.

<table>
<thead>
<tr>
<th>Fusarium oxysporum strains</th>
<th>Mycelial growth (mm)</th>
<th>Percent (%) inhibition over control.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>32.1</td>
<td>64.33</td>
</tr>
<tr>
<td>B</td>
<td>27.5</td>
<td>69.44</td>
</tr>
<tr>
<td>C</td>
<td>29.4</td>
<td>67.33</td>
</tr>
<tr>
<td>D</td>
<td>33.75</td>
<td>62.50</td>
</tr>
<tr>
<td>E</td>
<td>26.1</td>
<td>71.00</td>
</tr>
<tr>
<td>Control</td>
<td>90</td>
<td>-</td>
</tr>
</tbody>
</table>

Plate 2: Antagonistic efficacy of Trichoderma viride against Fusarium oxysporum strains by sealing agar plate technique

Fig 1: Treatment of strain A with Trichoderma viride, Fig 2: Treatment of strain B with Trichoderma viride, Fig 3: Treatment of strain C with Trichoderma viride, Fig 4: Treatment of strain D with Trichoderma viride, Fig 5: Treatment of strain E with Trichoderma viride.

Trichoderma viride was tested for their efficacy against Fusarium oxysporum strains came into contact with the pathogen in 2 days that infers the biocontrol agent is growing rapidly in dual cultures and occupies the space. The clear zone of inhibition was observed in between antagonist and pathogen in plates indicates that Trichoderma spp. restrict further growth of Fusarium oxysporum strains. T. viride overgrew partially over the Fusarium oxysporum strains in 7 days. The fast growing antagonists caused more growth inhibition of the pathogens may be due to mycoparasitism and competition for space and nutrients. Fusarium oxysporum strains were comparatively less inhibited by Trichoderma viride in the Sealing agar plate method.

Similarly, Bardia and Rai (2007) [1] showed antagonistic effect of Trichoderma viride and Trichoderma harzianum against Fusarium oxysporum f. sp. cuminis by 51.15% and 58.41% inhibition of mycelial growth respectively. Rehman et al. (2010) [9] showed efficacy of Trichoderma viride and Trichoderma harzianum against Fusarium oxysporum f. sp. Ciceris by inhibition of mycelial growth 81% and 83.33% respectively. Cherukupally et al. (2017) [3] evaluated the efficacy of Trichoderma viride and Trichoderma harzianum against Fusarium oxysporum f. sp. Melongenae by inhibition of mycelial growth 78.88% and 81.11% respectively.

In agriculture, farmers depend on the use of chemical fungicides to control plant diseases caused by pathogenic fungi which constrain the yield. However, overuse of these synthetic chemicals causes hazardous to both environment and health. The alternative method for replacement of chemical fungicides has led to the use of biological control agents. Microorganisms that grow in the rhizosphere are ideal for use as biocontrol agents. The studies proved that Trichoderma spp. have the potential to control Fusarium oxysporum strains under in vitro to the extent of 71.00% by dual culture plate technique and 45.27% by sealing agar plate method. It proves that dual culture plate technique found to be more appropriate rather than the sealing agar plate technique for in vitro studies, T. viride has found to be a potential biocontrol agent against Fusarium oxysporum strains. It may be therefore a promising ecofriendly bio controlling sources and cost effective for the safe agricultural practices as well as to farmers.

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References


