SCREENING OF ANTAGONISTIC TRICHODERMA FOR BIOCONTROL ACTIVITIES ON PHYTOPATHOGENS

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Abstract

Biological control, the use of specific microorganisms that interfere with plant pathogens and pests, is a nature-friendly and ecological approach to overcome the problems caused by standard chemical methods of plant protection. Trichoderma species are known as biocontrol agents against plant phytopathogens. This finding is a consequence of several key factors, such as faster metabolic rates, anti-microbial metabolites and physiological conformation. In this study, in vitro potential of selected isolates strains of Trichoderma were evaluated against well known and virulent phytopathogens, such as Rhizoctonia solani, Fusarium graminearum, Sclerotinia sclerotiorum, Botrytis allii, Botryris cinerea and Pythium debaryanum. The selection of bioantagonistic microorganisms was carried out using dual culture method observing the pathogen growth inhibition by biocontrol agent. The inhibition extent varies from strain to strain. Trichoderma T27 strain presented the highest bioantagonistic activity and the most sensitive pathogens were Fusarium graminearum, Botryris cinerea and Pythium debaryanum. The optical microscopy observations revealed changes of morphological characters at pathogens due to biocontrol agent activity. Full exploitation of the biocontrol potential of Trichoderma spp. could easily provide growth enhancement of domestic plants, green house plants, and agricultural crops.

INTRODUCTION

Plant pathogens produce significant losses to agricultural products. Traditional chemical control methods are not absolutely efficient to minimize these effects. Biological control of plant pathogens can be highly effective especially with potentials of antagonists on pathogenic fungi. Trichoderma spp. is the most widely studied biocontrol agents (BCAs) against plant pathogens. The genus comprises a great number of fungal strains that act as biological control agents [1, 2, 3]. In the present study, in vitro potential of four selected isolates strains of Trichoderma were evaluated against well known and virulent phytopathogens, such as Rhizoctonia solani, Fusarium graminearum, Sclerotinia sclerotiorum, Botrytis allii, Botryris cinerea and Pythium debaryanum. The selection of bioantagonistic microorganisms was carried out using dual culture method observing the pathogen growth inhibition by biocontrol agent.

MATERIAL AND METHOD

Microorganisms
Potential biocontrol agents Trichoderma strains (T27, T36, T57, T83) were cultured on solid PDA medium.
Phytopathogen strains: Rhizoctonia solani, Fusarium graminearum, Sclerotinia sclerotiorum, Botrytis allii, Botryris cinerea and Pythium debaryanum. Strains were grown for 7 days on potato dextrose agar (PDA) at 28°C.

Dual solid culture assay
The antimicrobial capacity of the selected strains was evaluated by dual solid culture assay in Petri plates. Agar dextrose-potato-agar medium was inoculated with culture solid disks from biocontrol agent and from fungal pathogen. After 3-5 incubation days, fungal growth was evaluated by measuring the clear inhibition zone around the disks.

The formula to determine the antagonism level is:

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\[ X = \frac{i_A}{i_B} \times \frac{e_B}{e_A} \]

where \( i = \) inner radius; \( e = \) outer radius; \( A = \) fungus test; \( B = \) fungus antagonist; \( X < 1 \) – antagonism present; \( X > 1 \) – antagonism absent.

**Morphological analysis**

The effect of biocontrol action against pathogens was observed using optic microscope Olympus BX 51 (40x photos).

**RESULTS AND DISCUSSIONS**

In vitro potential of selected isolates strains of *Trichoderma* were evaluated against virulent phytopathogens, such as *Rhizoctonia solani*, *Fusarium graminearum*, *Sclerotinia sclerotiorum*, *Botrytis allii*, *Botrytis cinerea* and *Pythium debaryanum*. Among the four isolated strains only *Trichoderma* T 36 and T 27 were been of interest as potential biocontrol agents. These strains were evaluated by dual solid culture method (Table 1.).

<table>
<thead>
<tr>
<th>Biocontrol</th>
<th>Phytopathogen</th>
<th>Antagonism evaluation (X)*</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Trichoderma</em> T36</td>
<td><em>Rhizoctonia solani</em></td>
<td>0.30</td>
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<tr>
<td></td>
<td><em>Fusarium graminearum</em></td>
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<td></td>
<td><em>Sclerotinia sclerotiorum</em></td>
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<tr>
<td></td>
<td><em>Botrytis allii</em></td>
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</tr>
<tr>
<td></td>
<td><em>Botrytis cinerea</em></td>
<td>0.27</td>
</tr>
<tr>
<td></td>
<td><em>Pythium debaryanum</em></td>
<td>0.19</td>
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<tr>
<td><em>Trichoderma</em> T27</td>
<td><em>Rhizoctonia solani</em></td>
<td>0.46</td>
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<tr>
<td></td>
<td><em>Fusarium graminearum</em></td>
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<td><em>Sclerotinia sclerotiorum</em></td>
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<tr>
<td></td>
<td><em>Botrytis allii</em></td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td><em>Botrytis cinerea</em></td>
<td>1.07</td>
</tr>
<tr>
<td></td>
<td><em>Pythium debaryanum</em></td>
<td>1.01</td>
</tr>
</tbody>
</table>

* Each value is an average of five replicates.

From the results of dual culture assay it was found that *Trichoderma* T 27 inhibited pathogen more than the other tested strain.

*Trichoderma* T 27 was particularly very active against *Fusarium graminearum* \((X=1.05)\), *Botrytis cinerea* \((X=1.07)\) and *Pythium debaryanum* \((X=1.01)\). For both *Trichoderma* strains, *Botrytis allii* presented higher resistance to growth inhibition. In this case, the antagonism level expressed as \( X \) was 0.17 for *Trichoderma* T 36, and 0.23 for *Trichoderma* T27, respectively.

The visual observations of solid dual cultures in Petri places are presented in Fig. 1 and Fig. 2. From Fig. 1 it can be seen that *Trichoderma* T 36 presents higher inhibition against *Fusarium* strain (Fig. 1 b).

The photographs presented in Fig 1 and 2 were taken 5 days after inoculation.
CONCLUSIONS

This study demonstrated the efficacy of *Trichoderma* T27 in controlling several virulent pathogens, such as *Fusarium graminearum* (X=1.05), *Botrytis cinerea* (X=1.07), and *Pythium debaryanum* (X=1.01). The result implied that the extent of inhibition by the fungi provides the use of potential antagonists capable of controlling the pathogenicity for sustainable agriculture.

ACKNOWLEDGMENTS

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REFERENCES


