Biocontrol Efficiency of Trichococcus spp., Against Seed-borne Fungal Pathogen of Peanut

K.C. Pushpalatha

P.G. Department of Microbiology, Mangalore University, P.G. Centre, Madikeri, India.

Abstract

Peanut or groundnut is a major oil crop grown in India. It contains about 48-52% of oil. Quality and quantity of peanut is mainly affected by seed-borne fungal pathogens, because many of them are efficient producers of mycotoxins. Hence, the management of fungal pathogens of many crops including peanut is gaining lots of important in agriculture research. Among the management approach, biological method is the promising one which is cheap and eco-friendly. In present investigation, peanut pathogens were isolated from the peanut seed samples collected from various places of Karnataka and disease incidence was recorded. Disease incidence of seed-borne fungal pathogens was varied between 88-92%. The isolated fungal pathogens ware identified as Penicillium sp., A. niger, A. flavus and Fusarium sp. However, maximum DI was recorded with the pathogen of A. niger. Biocontrol agent, Trichococcus sp., was cultured and crude and partial purified metabolites were used to evaluate the antifungal efficiency against these pathogens. The seed samples of peanut were treated with antifungal metabolites and % germination, vigour index, radical growth were recorded. Further, inhibition assay was confirmed Biocontrol effectiveness of Trichococcus against seed–borne fungal pathogens peanut.

Keywords: Trichococcus, seed-borne fungus, Biocontrol agent.

1. Introduction

Oil seeds are one of the economic crops of India. Oil extracted from these seeds forms an essential fat constituent in the diet of the Indian people. The major oil seeds include...
groundnut, sesame, safflower, castor seed, rape seed, linseed, soybean, sunflower etc. Groundnut contains 46-52% of oil. The high oil content and protein content makes it an important food ingredient. It is also a valuable source of vitamin E, K and B. (Rangaswami 1996). Seed borne diseases are commonly observed in cereals and pulses and the reduction in the yield of peanut is dependent upon several factors, among which the diseases have major share. (Khurana, 1998). Fungi form a major group of pathogens that can be seed borne or transmitted through seeds(Agarwall, 1997).

Biological methods mainly consist of using microorganisms to control harmful microorganisms causing plant disease without disturbing the ecological balance. Biological control practices in which microbial antagonists use often involve competitive interaction between antagonists and the pathogen in the competition for nutrients. The antagonistic microorganisms release antibiotic or other chemicals which are harmful to the pathogens or other organisms and inhibit its growth (Kerr, 1980).

A number of chemical agents are used to control these pathogens but management of diseases using biocontrol agents is crucial for the future crop cultivation. Uses of biocontrol agents reduce the environmental pollution and are eco-friendly. Since most the chemicals are implicated in modern environment and human health problems, genetically modified organisms provide alternative but this raises the new question of biological risk in association with their use (Spurrier, 1990). Hence, in the present study, seed-borne fungal pathogens were isolated from peanut and attempts have been made to develop biocontrol management strategy using *Trichococcus spp*.

2. Materials and Methods
2.1 Collection of Samples
Peanut samples were collected from different places namely Mangalore, Madikeri, Mysore, Udupi Bangalore and Kundapur and were used for the present study.

2.2 Screening of Samples for fungal pathogens
Collected seed samples were screened for fungal pathogens using agar plate method.

2.3 Agar Plate method
100 seeds were taken from each sample randomly and surface sterilized using 0.5% of sodium hypochlorite solution for five minutes and seeds were washed with sterile distilled water. Further seeds were blotted using blotting paper and seeds were placed on petri plates containing PDA medium. 10 seeds of each sample were plated using sterile forceps in equidistance and plates were incubated at 25±2°C for 7 days and percentage of Disease Incidence (DI) was recorded.

2.4. Extraction of metabolites from *Trichococcus spp*
*Trichococcus spp.*, culture was grown using nutrient broth for 48 hrs at 37°C for extraction of Trichococcus metabolite. The broth was filtered was using Whatmann No.1 Filter paper and collected supernatant was used extraction of metabolites.
Biocontrol Efficiency of Trichococcus spp., Against Seed-borne Fungal Pathogen

Metabolite of Trichococcus spp., was partial purified using saturated Ammonium sulphate and absolute alcohol. The supernatant obtained was treated with saturated ammonium sulphate and absolute alcohol was kept for 24hrs at 8°C for complete precipitation of the metabolites. It was centrifuged at 5000 rpm for 20min and the pellets were dissolved in phosphate buffer of 0.1M at pH7.0. The seeds were treated with the suspension for different time intervals and plated by Moist Chamber Technique as mentioned earlier and Disease Incidence was recorded.

2.5. Inhibition assay for Penicillium spp against Trichococcus metabolite
The Penicillium spp isolated from the samples was used for inhibition assay. 1 ml of Penicillium suspension was poured into sterilized petri plates and PDA media was poured mixed thoroughly and allowed to solidify. Trichococcus spp., metabolites treated peanut seed at different time intervals were plated in equidistance using sterile forceps. Plates were observed for inhibition zone.

Different sets of seed were taken and treated with Trichococcus spp., metabolite and different time intervals seeds washed with sterile distilled water and blotted. Then the seeds were plated by Moist Chamber Technique. After 7 days mean of root and shoot length and vigour Index was recorded for each treatment.

3. Results and Discussion
The seed samples collected from various paces showed varied between 88-92% and minimum of 92% in sample collected from Mangalore. Aspergillus niger was found as dominant pathogen when compared to other pathogens and disease incidence varied from 36 to 52% in seed samples collected from different palces of Karnataka. Penicillium sp. and Fusarium sp. s showed disease incidence of 10 to 16% and 6 to 20% respectively. A.flavus exhibited 20 to32% DI.

The metabolites of Trichococcus extracted using partially purified ammonium sulphate and alcohol showed maximum protection for peanut pathogens. 75% protection was offered for ground nut pathogen treated for 10 minutes with ammonium sulphate precipitated metabolites whereas 84-96% protection offered with alcohol precipitated metabolites treated for 20 to 30 minutes. Results suggest that c extract was more effective for peanut nut seed treatment.

The Trichococcus metabolites treated peanut showed increased radical length over the control where However 20 minutes treatment was more effective when compared to 10 minutes treatment for all the seeds. In all the samples both treated and untreated seeds root length was more when compared to shoot length. The result revealed that Trichococcus metabolite could be used as effective biocontrol agent for seed treatment. It also showed that it could influences growth of seedlings. The seeds treatment for 20 minutes would be more effective for seed treatment.

In peanut, the root length was increased with Biocontrol treatment when compared to untreated seeds. In Trichococcus treated seeds shoed increased Vigour Index. over the control. Maximum Vigor Index was recorded in case of 20 minutes treatment. The
treatment given for 20 minutes was more effective for all the seed samples. The present result indicating that Trichococcus metabolites could be used as an effective biocontrol agent for seed treatment which would increase yield also by means of increasing root and shoot length (Table 1).

**Table 1**: Vigour Index of Trichococcus metabolites.

<table>
<thead>
<tr>
<th>Name of the seed sample</th>
<th>Time of treatment</th>
<th>Vigour Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peanut</td>
<td>0</td>
<td>124.8</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>208.0</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>478.4</td>
</tr>
</tbody>
</table>

In the inhibition assay, crude extract of *Trichococcus* metabolites showed inhibition against only for 30 minutes with ammonium sulphate sample (Table 2).

**Table 2**: Inhibition assay for different samples using Trichococcus metabolite against *Penicillium* spp.

<table>
<thead>
<tr>
<th>Partial Purification Methods</th>
<th>Zone of Inhibition (in cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time of Treatment (min)</td>
</tr>
<tr>
<td></td>
<td>10 min</td>
</tr>
<tr>
<td>Crude</td>
<td></td>
</tr>
<tr>
<td>Ammonium sulphate</td>
<td></td>
</tr>
<tr>
<td>Alcohol</td>
<td></td>
</tr>
</tbody>
</table>

4. Conclusion

*Trichococcus* spp., metabolites showed considerable reduction in pathogen development. In partially purified, metabolite treatment showed considerable increase in root and shoot length when compared to untreated. Vigour Index was also increased as the time treatment increased with up to 20 minutes. *Trichococcus* spp., could be served as a better Biocontrol agent in control of seed–borne pathogens of peanut.

**References**


Biocontrol Efficiency of Trichococcus spp., Against Seed-borne Fungal Pathogen 669


xxxxx xxxxxxxxxx